Cancer of the Prostate and Kidney

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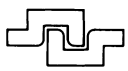

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PREFACE

This book is the record of the proceedings of a NATO Advanced Study Institute held in Erice, Sicily, from the 2nd - 12th June 1981, during which scientists and clinicians interested in the problems presented by cancer of the kidney and the prostate were encouraged to present, to discuss and to challenge the opinions expressed and the beliefs held by the different contributors.

It is uncommon for scientist, physician, and surgeon to meet with great regularity or for prolonged periods of time and it must be exceedingly rare for such people to immerse themselves in each other's work and company for a period of almost two weeks. For this to occur in a situation of total isolation such as that provided by the marvellous Ettore Majorana Centre in Erice, Sicily must be unique.

The fact that differences of opinion remain will be evident to all who read the book, as will the wealth of scientific and clinical work being undertaken within and beyond the NATO countries.

We are very much indebted to the Science Committee of NATO for their recommendation for support for this meeting and to the Ente Fiuggi and to several pharmaceutical firms.

We should also like to acknowledge our gratitude to Miss Pinola Savalli and Dr. Alberto Gabriele, who played such a large part in making our meeting both pleasant and successful, to the Department of Medical Illustration at St. James's University Hospital, Leeds, and to Mrs. S. Conyers, Miss M. Calder, Mrs. S. Purdie, and Miss S. Stevenson of the Departments of Urology and Oncology of St. James's University Hospital, Leeds, for the enormous amount of work which they have undertaken in preparing and typing this volume.

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RENAL CANCER

In spite of advances in all fields of medical science, the prognosis of renal cell carcinoma has not improved during the last 25 years. The development of more sophisticated diagnostic tools such as arteriography, cavography, ultrasound and C.T. scan facilitate a more accurate assessment of the extent of the primary growth and its spread; however this knowledge has not yet contributed to therapeutic adaptations which result in a better prognosis (Table 1).

Simple nephrectomy and radical nephrectomy yield about the same cure rate. Preoperative irradiation increases local operability and hence local cure rate, but has not contributed to a better prognosis. The same applies to postoperative irradiation (1,2). Apparently prognosis is usually already determined by subclinical metastasis at the time of operation. In a patient with a solitary distant metastasis it remains doubtful whether removal of the primary has any beneficial influence on the course of the disease. Embolisation of the primary, followed by nephrectomy, might improve prognosis in case of limited metastasis (3).

In patients with multiple metastases either at the time of diagnosis or after previous nephrectomy, hormone therapy which seemed to be promising - 17% success up to 1971 - has turned out to be disappointing - 1.6% success after 1971. Chemotherapy, though sometimes producing partial regression or stabilisation has not improved prognosis. Immunotherapy, as the sole type of treatment or in combination with hormone treatment or chemotherapy, seems to be promising but still no proven improvement has yet been demonstrated (4). Radiotherapy delivered to painful or threatening metastases (threatening

Table 1. Renal cell carcinoma - survival rates in literature

Author	Type of tumour	No. of patients treated	% 5 year survival
Riches 1964 (5)	Well differentiated	42	70
	Poorly differentiated	42	30
	Renal vein -	60	58
	Renal vein +	26	27
Skinner et al 1972 (6)	T1 T2		
	Renal vein -	102	65
	Renal vein +	59	64
Murphy 1973 (7)	T1 T2	46	57
	T3 T4	40	49
Werf-Messing et al 1978 (2)	Renal vein -	104	65
	Renal vein +	62	28
Syrjänen & Hjelt 1978 (8)	Papillary	53	53
	Glandular/Tubular	31	58
	Undifferentiated	27	37
Sullivan et al 1974 (9)	Simple nephrectomy T1 T2	24	74
	Simple nephrectomy T3	9	66
	Simple nephrectomy Renal vein + or N1 N2	10	20
	Radical nephrectomy T1 T2	13	31
	Radical nephrectomy T3	7	86
	Radical nephrectomy Renal vein + or N1 N2	14	29

fracture or paralysis) usually results at least in "partial regression" and - if not given too late - very often in "local control" (Table 2). Half Body Irradiation (lower half or upper half depending on the site of the most distressing metastases) can also relieve pain (10).

As from a prospective study at the Rotterdam Radio-Therapy Institute could be concluded that invasion of the renal vein is the most important prognostic criterium (2), elective adjuvant therapy seems justified in these high risk pateints. Adjuvant chemotherapy, immunotherapy or hormone therapy could be considered; however, in view of their poor results in patients with proven clinical metastases, a beneficial effect is doubtful but could still be investigated. Elective irradiation of high risk regions could theoretically improve

prognosis (2). Another approach, elective total body hyperthermia with or without low dose total body irradiation in patients with proven renal vein involvement could be rewarding; this approach is now being investigated at the Rotterdam Radio-Therapy Institute.

PROSTATIC CANCER

Prostatic cancer has been a challenge since the beginning of this century. In 1911 Jean Cauhapé (11) published the results of the first radium implants in prostatic cancer. About three decades later Huggins et al (12) discovered by serendipity the beneficial influence of oestrogens on prostatic cancer. Since that break-through most prostatic cancer patients in the world have been treated endocrinologically and other approaches were only pursued in a few centres. Only after the publication of the Veterans' Administration Study (13,14) which showed that hormone treatment as the initial approach in non-metastatic prostatic cancer was not the ideal type of treatment, was interest again focussed on new possibilities.

Accurate staging, using for instance the TNM classification of the UICC, made it possible to compare treatment results in comparable groups of patients. Improved diagnostic procedures facilitated more accurate staging. However, careful rectal palpation still remains the most accurate diagnostic procedure (15).

In patients with no evidence of distant metastases (M0) radical surgery in the lower T categories yields excellent results. The same can be achieved by interstitial implants of radio-active material. A comparable prognosis is offered by external megavoltage irradiation (Table 3,4). Though results in the same T categories are compared, selection of the cases according to general condition and age does influence the choice of therapy. The main advantage of radiation therapy is the low risk of loss of sexual potency (about 14% after external irradiation and less than 5% after interstitial implant).

There are nearly no complications after interstitial implant in combination with lymphadenectomy; after external irradiation they are acceptable, though multiple transurethral resections or other surgical interventions in the irradiation area do increase the risk of radiation complications (16 - 21).

Survival is largely influenced by the histological grade of the primary. If distant metastases develop hormone therapy can still prolong life considerably in patients with highly and moderately differentiated growths. However, in patients with poorly differentiated lesions the endocrine approach usually fails.

In those with distant metastases and failure of hormone therapy, chemotherapy also appears to be of no substantial value, though some partial responses have been reported (18). Also immunotherapy has been disappointing (22).

Table 2. Palliative radiotherapy for vertebral metastases of renal cell cancer in the Rotterdam Radio-Therapy Institute

	After irradiation				
	No pain	Less pain	Same pain	No neurological pathological signs	Same neurological signs
Pain only (28 patients)	19	5	4*	-	-
Pain and neurological signs (32 patients)	19	7	6*+	16	16..
	38 (63%)	12 (20%)	10 (17%)	16 (50%)	16 (50%)

* 1 died during treatment
\+ ? pain in 4 patients due to other metastases
.. 9 paralysis > 2 weeks, 1 paralysis within 2 days, 1 progressive

Table 3. Prostatic cancer - prognosis after interstitial irradiation combined with lymph node dissection or with external irradiation of regional lymph nodes

Type of treatment	Type of growth	Prognosis			Ref. No.
		No. treated	5 yr. survival		
Radical surgery + interstitial therapy 100 mCi 198Au, 2cc diluent	Category T3M0	147	60%		23
Radical perineal surgery	T1,2M0	132	76		24
Radical Perineal surgery	T3,M0	213	64		
			3 yr. survival	Free of disease	
Gold grain implant (± 4000 rad) + external megavoltage to a total dose of ± 8000 rad)	Stage C (Category T3M0, T4?M0)				
	Normal serum acid phosph.	13	13	8	25
	Abnormal " " "	3	0	0	
	Total stage C (T3M0+T4?M0)	16	13/16	8/16	
		All controlled locally			

(continued)

Table 3 (cont.)

Type of treatment	Type of growth	No. treated	Prognosis	Ref. No.
Retropubic bilateral lymphadenectomy + prostate implant with 125I 16000 rad/1 year = 2023 rets			Crude 5 yr. survival	26
	Limited: Category T1M0	65	100%	
	Limited: " T2M0	40	100%	
	Limited: " T3M0	23	77%	
	Limited: " T4M0	80	87%	
			Act. 5 yr. survival	
	Lymph nodes negative histologically	102	92%	
	Lymph nodes positive histologically	84	46%	
See above (26)	Histological grade according to Gleason		5 yr. relapse-free survival	27
	Well differentiated	19	81%	
	Moderately well diff.	46	69%	
	Poorly differentiated	15	48%	
	Size of the primary			
	$< 2.5\ cm^3$	18	89%	
	$2.5 - 7.9\ cm^3$	50	65%	
	$8 - 18\ cm^3$	32	26%	

Table 4. Prognosis after external supervoltage irradiation in prostatic cancer

Type of treatment and selection	Type of growth	Prognosis			Ref. No.
		No. treated	5 yr. survival		
25 MeV Photons (± 40 pts: Co60 or 4 MeV) 4 fields or rotation to prostate + peri-prostatic tissue 60% of patients: 7000-7500 rad/7-7.5 wks	Category T3M0, T4M0 Well differentiated Poorly differentiated	160 71	60% 30%		28
6 MeV Photons 5500 rad/5-7 wks 2 ant. wedge fields, 1 post. field + Hormones Stilboestrol and/or Estradurin (all pts. received antibiotics)	Category T3M0, T4M0 Poorly differentiated	25	3 yr. survival 64% (2 died of other causes)		29
			5 yr. survival	10 yr. survival	
4 MeV Photons 7000-7600 rad/7-7½ wks to prostate and periprostatic tissue (rotation or R + L lat. arc therapy)	Limited to prostate (Category T1M0, T2M0) Extracapsular (Category T3M0, T4M0)	230 200	72% 51%	44% 38%	30

(cont.)

Table 4 (con't)

Type of treatment and selection	Type of growth	Prognosis					Ref. No.
		No. treated	5 yr. survival		10 yr. survival		
			Free of disease	With disease	Free of disease	With disease	
4 MeV Photons	Limited to prostate	81	72%	2%	-	-	19
Small field to prostate +	(Category T1M0, T2M0)	28	-	-	46%	4%	
periprostatic tissue	Extracapsular extension	82	42%	6%	-	-	
± 7000-7500 rad/7-7½ wks	(Category T3M0, T4M0)	23	-	-	26%	5%	
4 MeV Photons	Stage A, B, C						
Patients ≤ 70 yrs of age	(Category T1M0, T2M0,		Follow up period				
lymph node biopsy at lap-arotomy	T3M0, T4M0)		1-50 m. (median: 22 m.)				
A. Nodes histologically negative → Random.:							31
			Free of disease				
a. 7000 rad/7 wks to prostate		17	15				
b. a + pelvic nodes X 4000 rad/7 wks		18	16				
c. a + b + paraortic X		2	1				
		37	32	87%			
B. Pelvic nodes histologically pos. → Random.:							
b or		9	7				
c		5	3				
		14	10	72%			

C. Para-aortic nodes histologically positive - c.		10	3	30%	
		Total 61	45	74%	
22 MeV Photons	Stage C (Category T3M0, T4M0)		5 yr. survival		32
I 47 total 7000 rad to prostate total 6000 rad to true pelvis total 5000 rad to pelvis inc. L5			Free of disease	Alive with tumour	
		97	42%	0	
II 50 Idem + hormone therapy (orchidectomy + estrogens)			No diff. between I and II		
10 MeV Photons Whole pelvic fields + Booster to prostate	Stage C (Category T3M0, T4M0)	221	5 yr. survival 58%		33
			Additional hormone therapy had no bearing on prognosis		
22 MeV Photons Study I. 4 fields (10 x 10 cm small field to 12 x 15 cm large field) Trial 5000 rad/± 5 wks Prostate booster 1000-2000 rad via ant. + post. field Rand. < Additional hormones / DES 5mg/day / No hormones Study II. Previous series (no trial): 6000-7000 rad/ 6-7 wks to true pelvis with or without hormones.	Stage B1C1C2 Category T2M0: 4 Category T3M0: 97 Category T4M0: 53		Mean follow up: 4 y. 7 m. 5 yr. survival		34
		79	74.7% after X only		
		75	61.3% after X + Hormones		
			No diff. between 7000 rad and ± 6000-6500 rad.		
			No diff. between "small" and "large" X-fields in study I and II		

(continued)

Table 4. (con't)

Type of treatment and selection	Type of growth	Prognosis			Ref. No.
		No. treated	5 yr. survival	Free of disease	
60 Cobalt 6000 rad/6 wks, 5 fields daily Patients ≤ 75 yrs Only patients with negative lymphography are included in study	A (Category T1N0M0)	21	88%	84%	35
	B1 (Category T2N0M0)	43	90%	61%	
	B2 (Category T3N0M0)	30	66%	43%	
	C (Category T4N0M0)	13	39%	37%	
Trial:	Stage C (Category T3M0, T4M0)				36
A. 1 mg Ethinyloestradiol daily		26	65%		
B. 1 mg Ethinyloestradiol daily + 25 MeV Photons 7000/7 wks by 2 opposing fields covering prostate and surroundings		30	55%		

25 MeV Photons 4000 rad + 2 wks split + 3000 rad 4 portals to prostate and surrounding tissue			Act. uncorr. survival				21
			3 yr.	Free of disease	5 yr.	Free of disease	
	T3M0 well and mod'ly diff.	40	80%	65%	80%	65%	
	T3M0 poorly and undiff.	15	55%	35%	fol. up too short		
	T4M0 well and mod'ly diff.	22	65%	65%	65%	63%	
	T4M0 poorly and undiff.	7	50%	25%	fol. up too short		

		No. treated	5 yr. survival	10 yr. survival	
6 MeV Photons or Cobalt 60	Stage A (Category T1M0)	13	85%*	-	37
± 6000-6800 rad in ±6-7 wks	Stage B (Category T2M0)	21	85%*	-	
Rotational therapy	Stage C (Category T3M0+T4M0)	112	55%	± 30%	
or					
4 field technique					
or	T3M0, T4M0				
Combination of whole pelvis	Well + moderately diff.	84	65%*	-	
with additional irradiation	Undiff.	16	30%*	-	
With or without hormones.					
	IVP normal	128	70%*	-	
	IVP abnormal	18	25%*	-	
Dose > 6500 rad	All T-categories M0	55	85%*	-	
Dose < 6500 rad	All T-categories M0	91	55%*	-	
Radiotherapy started within 6 m after diagnosis	All T-categories M0	121	70%*	-	
Radiotherapy started after ≥7 m.	All T-categories M0	25	30%*	-	
10 MeV Photons or Cobalt 60					38
Pelvic irradiation	Stage B	36	59%	-	
3 or 4 fields	(Category T2M0)				
6500-7000 rad/7-8 wks					
with or without hormones	Stage C	123	58%	30%	
	(Category T3M0+T4M0)				

No differences between radiation only and X + hormones.

* approximate figures

Radiation therapy of metastases can prevent fractures, eliminate pain and prevent neurological disasters. Pain can also be relieved by pituitary ablation (radiation therapy, implant with radio-active material, injection with alcohol). In case of disseminated painful metastases half body irradiation can yield temporary pain relief (39).

Attempts to improve prognosis can be focussed on improved cure rate of the loco-regional malignancy and on preventing subclinical metastases becoming clinically manifest.

Improvement of loco-regional results by combining hormones and irradiation could not be achieved (Table 4). Increasing the field of irradiation, so as to include all potentially involved regional lymph nodes, has also been unsuccessful (Table 4). The application of multiple daily fractions might offer better results at the cost of more treatment inconvenience to the patient. Theoretically a Pi-meson booster to the prostatic region, after irradiation of the primary and the regional lymph nodes, could improve local cure rate and perhaps prognosis, without increasing the risk of complications. This concept will be implemented at the Pi-meson facility in Villigen. The combination of irradiation with local hyperthermia could theoretically also improve loco-regional results without enhancing the risk of damage.

In prostatic cancer the local cure rate is probably not the main problem though the meaning of persisting positive biopsy or positive cytology after external irradiation is still controversial; as prostatic cancer is a slowly growing malignancy and as after radiation therapy cancer cells only disappear at the time of mitosis, it is possible that non-viable cells will persist for a long time without having any prognostic meaning (17,21,37,40-45). It is most likely that at the time of treatment of the primary sub-clinical metastases already exist. The most reliable indicator for their becoming clinically manifest appears to be an elevated prostatic acid phosphatase in the serum (21). The problem remains whether systemic therapy should be given at the time of the first indicator or at the time of clinical evidence of metastases.

In patients with poorly differentiated lesions about 70% who initially present without clinical evidence of distant metastases will develop metastases within three years (21). As these metastases are usually unresponsive to hormone therapy (or respond only for a short period) and as no really effective chemotherapy or immunotherapy has yet been developed, a pilot study was started in Rotterdam to give elective lower and upper half body irradiation in these high risk cases. The results of the pilot study are presented in Table 5. The procedure is dangerous and requires, in view of the high risk of serious thrombocytopenia (Fig. 1), the facilities of sophisticated hematological support. In a prospective randomised

Table 5. Poorly differentiated prostatic cancer T3,4 MO treated at the Rotterdam Radio-Therapy Institute electively with 6 Gy lower HBI followed 9 weeks later by 6 Gy upper HBI

Alive 15/20		Dead 5/20	
6 - 12 months	: 7	1 month	: sepsis after lower HBI
13 - 18 months	: 6 (1 with mets after 18 mths)	6 months	: sepsis in another hospital
		3 months	: Guillain Barré syndrome (PM: no mets)
19 - 24 months	: 2	12 months	: lung cancer
		17 months	: mets (evident after 12 mths)

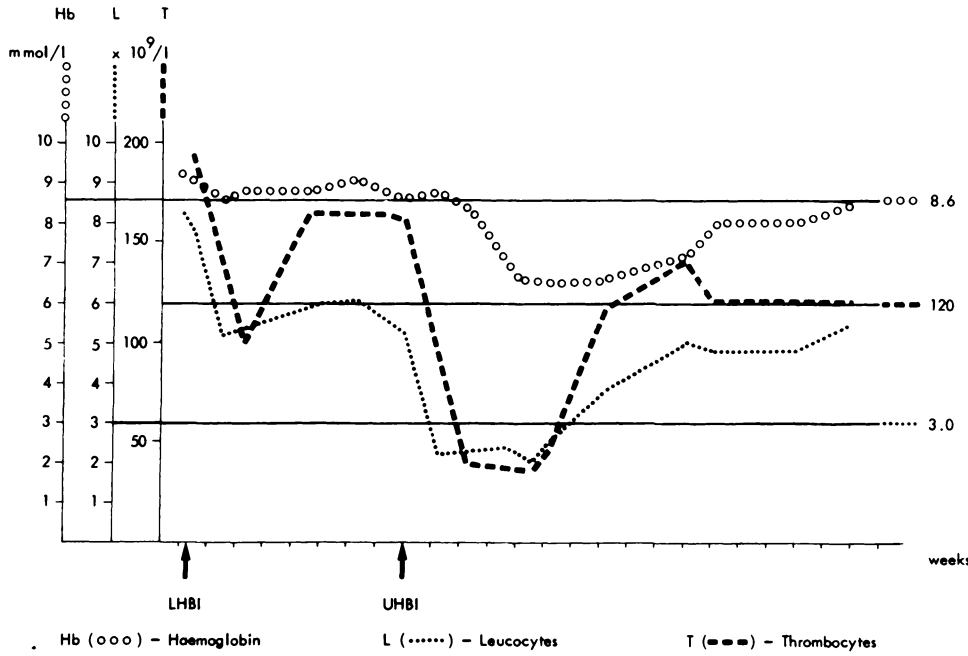

Fig 1. Hematological effects of half body irradiation in advanced prostatic cancer.

trial the value of this approach is now under investigation at the Rotterdam Radio-Therapy Institute. It is hoped that in some instances the existing subclinical metastases can be eradicated, and that in others the treatment may postpone the time at which they become clinically evident.

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CANCER OF THE PROSTATE

PROSTATIC CANCER: EPIDEMIOLOGY AND ETIOLOGY

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INTRODUCTION

Cancer of the prostate is a very common disease in western Europe and in the United States: it is one of the leading causes of death from cancer in man. However, the incidence of the disease is sometimes controversial due to a confusion in terminology and methods of statistical computation.

One must distinguish on the one hand between overt cancers with urinary or metastatic signs and symptoms and on the other hand latent cancers which do not manifest any symptomatology and are discovered by chance or by systematic pathological investigation.

The incidence of microscopic cancer, studied by the "step section technique" is shown in figure 1: as clearly demonstrated, it increases exponentially and over 50% of men over 80 have some microscopic areas of cancer in their prostate (1,2). A routine microscopic analysis (a few sections at random in the gland) ignores many microscopic cancers, as shown in figure 2. The striking difference between the curves suggests that the tumor has a long period of biological latency, about 20 years (1).

The clinical incidence of prostatic carcinoma is, happily, far below the microscopic incidence. This disproportion is due to many factors including the fact that prostatic cancer is a disease of the elderly man and that many such subjects with focal and unsuspected cancer die of other conditions such as cardio-vascular, respiratory and other diseases.

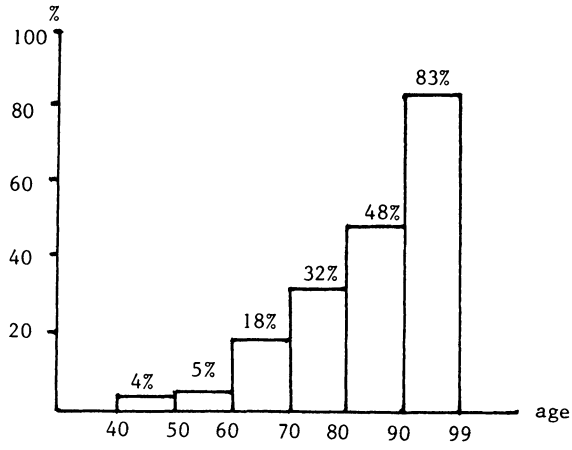

Fig. 1. Incidence of prostatic cancer, with the step section technique. From Hirst and Bergman (1).

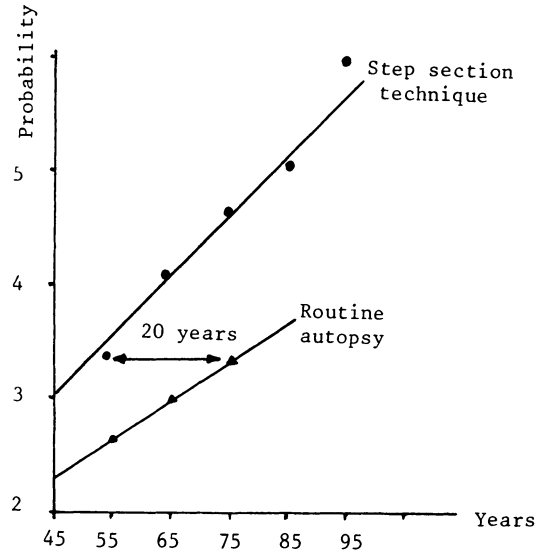

Fig. 2. Probability of discovering a P.C. with the step section technique and with routine autopsy examination.

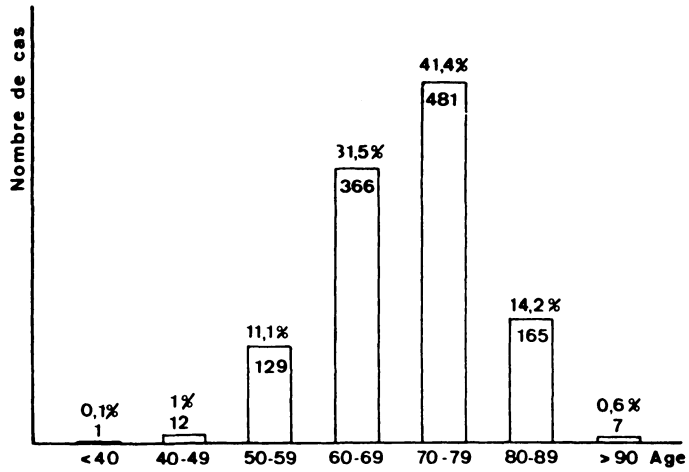

Fig. 3. Incidence of clinical prostatic cancer with age.

INFLUENCE OF AGE

Figure 3 shows the age distribution of patients in whom prostatic cancer (P.C.) was first discovered in our department (3). A quick glance leads one to suppose that the incidence of the disease decreases after 80 years. However, the percentage of distribution by age group is biased by a significant decrease of the population after 80 and probably also by less accurate diagnosis in these older patients.

If one considers the mortality rate by decade, relating the number of deaths in each decade to the living male population in that decade, one observes that the chance of dying of P.C. rises rapidly with age: above 80 years, it is nearly 500 times greater than at 50 years (figure 4).

RACIAL AND GEOGRAPHIC INFLUENCES

Figure 5 shows the mortality rate for P.C. in 16 countries in 1969. Great differences are observed in the distribution of the malignancy which appears to be very common in Western countries and much more uncommon in Eastern countries, especially Japan.

Such differences are also observed in the United States where the incidence of P.C. increases much faster among blacks than among whites and in the Hawaiian Islands where important discrepancies are noted among racial groups living in the same social conditions.

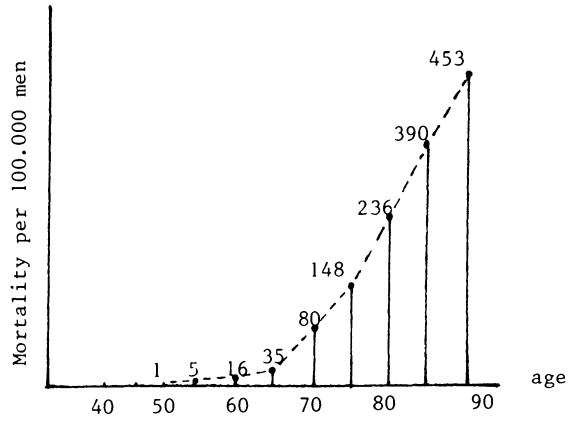

Fig. 4. Mortality rate for prostatic cancer at differing ages (U.S.A. 1958).

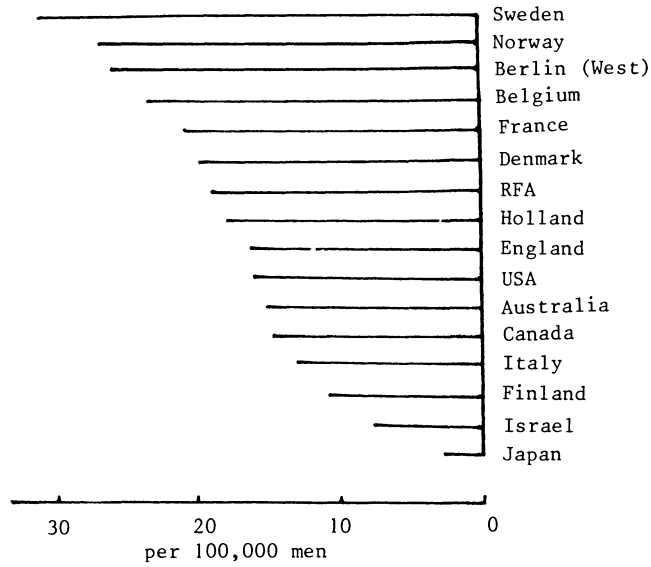

Fig. 5. Prostatic cancer mortality rate in 16 countries (1969).

Although the results can be biased by several factors, such as quality of medical care, median survival rate, and the organisation of a Cancer Registry, the great discrepancy between caucasians and Japanese is stressed by all epidemiologists (4-7). Therefore, a racial, genetic, perhaps hormonal, factor seems to favour the incidence of the disease.

However, this statement must be tempered by the following considerations:

1. Microscopic prostatic cancer, as demonstrated by autopsy data, is as common in Japanese as in caucasians (8,9). If this is so, the difference in the clinical incidence of the disease would result from a more rapid evolution of in situ cancers among whites, and not from a difference in the oncogenic transformation rate.

2. Racial differences partially disappear when one considers the incidence in immigrants coming from low-risk countries and living in high-risk countries; Haenzel and Kurihara (10) have drawn attention to these differences (Table 1). If substantiated, these data suggest that environmental factors also play a role in the growth of the disease.

TIME TRENDS

An increase in the P.C. mortality rates has been reported during the last decades as shown in figure 6.

It may be questioned whether or not the increase is real. As a matter of fact, we believe, like many others, that the longer life span, better diagnosis and better statistical enquiries can often account for the reported rise. However, in the United States, age-adjusted mortality rates have risen precipitously among blacks, and yet remained unchanged among whites (figure 7).

Table 1. Carcinoma of the Prostate. Percentage Mortality in Relation to Age (10).

	Mortality % with Age	
	65-74 y.	75 y. and over
Japanese	11.6	28
Issei	40.2	130
American (whites)	92.6	307.5

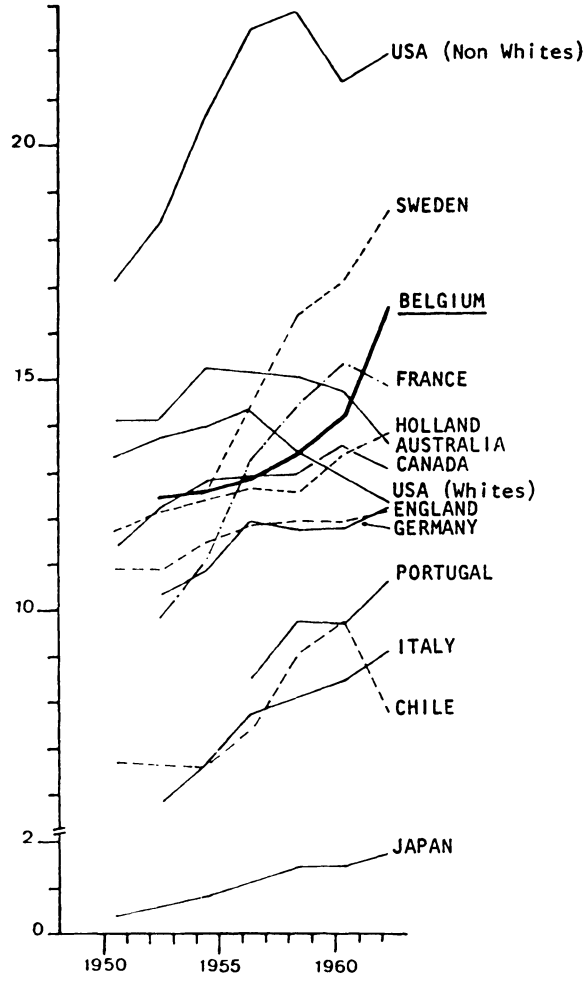

Fig. 6. P.C. mortality rates in 14 countries (1950-1963).

Ernster, Selvin and Wilkenstein (11) examined the mortality rates by age and birth cohort and observed that peak rates occured at every age in the cohort 1896-1900 and declined thereafter (figure 8). This presages a reversal of time trend, as more recent non-white cohorts reach the ages of maximum risk. If this observation is consistent, it would be of the greatest interest to research the historical experience of the 1896-1900 cohort in attempting to detect a common feature which could explain the higher risk in this cohort.

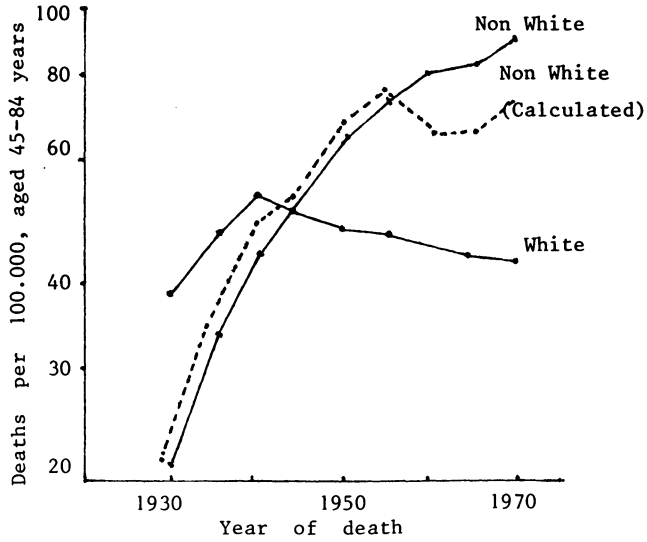

Fig. 7. Change in mortality of prostate cancer in males in the United States (11).

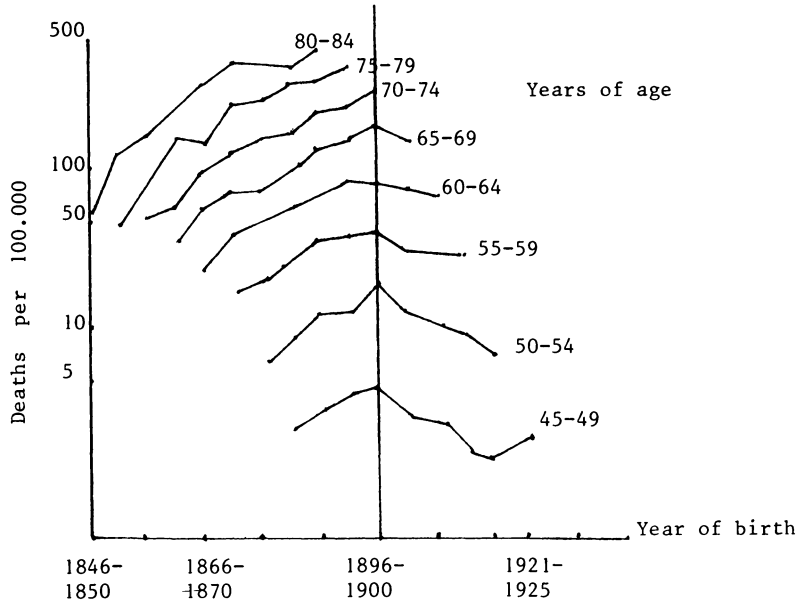

Fig. 8. Mortality rates for prostatic cancer by age and birth cohort (11).

SOCIAL, ECONOMIC AND RELIGIOUS FACTORS

Many investigations have been made in attempts to correlate P.C. and socio-economic status (S.E.S.). The results are in contradiction: Hakky, Chisholm and Skeet (12) found a higher risk with a lower S.E.S. but most of the other studies (4,5,13) failed to indicate any correlation.

King et al (5) reported a higher frequency among Protestants, lower among Jews and intermediate among Catholics for the population of New York City.

Wynder et al (4) established that the profile in each religion group reflects that of their place of origin (e.g. differences between Jews from Europe and Jews from Asia are larger than between European Jews and European Catholics) in such a way that ethnic factors alone can explain the discrepancy.

MORPHOLOGY, MARITAL STATUS, FERTILITY AND SEX HABITS

The hormonal dependence of P.C. has naturally led investigations in this direction.

Anthropometric studies including height, weight, somato-type and hirsutism failed to reveal any statistical difference between P.C. patients and controls (4,14).

A few authors have suggested that there is a higher rate of P.C. among married men and mainly among fertile men (5,15): a positive correlation between the number of children and the risk of P.C. seems to exist so that fertility and sexual activity were considered as risk factors.

Steel et al (16) reported greater sexual drive in P.C. patients as indicated by multiple sexual partners and greater extra-marital activity. A recent paper by Schuman et al (17) also came to the same conclusion and suggested a role for viral factors in the causation of disease in the urethra and prostate. However, the number of children is a very unreliable indicator of man's fertility and marital status and sexual behaviour depend on so many factors (social, cultural and educational) that these parameters are of little value in assessing the risks of development of P.C. Nevertheless, sexual activity derserves consideration because it offers a risk of urogenital contamination and many arguments suggest the possible role of viruses in the development of P.C.

VENEREAL DISEASE, PROSTATIC INFECTION

In 1942, on the basis of low incidence of P.C. among Jews, Ravich (18) presented the concept that it was a venereally trans-

mitted viral disease and that circumcision could protect against it. In a recent paper (19), the same author incriminates the method of statistical computation of P.C. in Japan, where early prophylactic circumcision is not performed and where P.C. is rare, to defend his opinion. In fact, circumcision does not seem to protect against P.C.: Kaplan et al (20) Wynder et al (4) found no statistical difference in the number of circumcisions between non-Jewish cancer patients and controls.

Chronic prostatic infection as a possible cause of P.C. led some authors to look for a relationship with a previous history of gonorrhea. Reports from Wynder (4), Kaplan (20), Steel (16) and Krain (21) resulted in contradictory conclusions and prompted Heshmat et al (22) to perform new studies. These authors presented a very interesting analysis of association of the incidence of gonorrhea and prostatic cancer mortality rates in Denmark. As shown in figure 9, both curves correlate quite well with a free period of 45 years, as if gonorrhea could induce local changes transforming prostatic cells after a long period of latency.

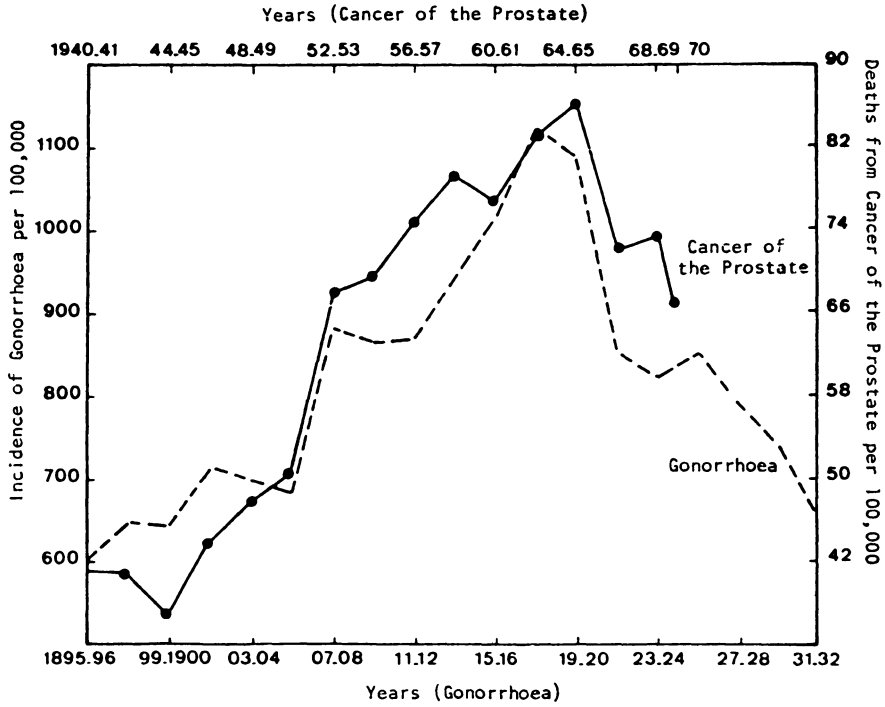

Fig. 9. Relationship between incidence of gonorrhea and mortality from prostatic cancer in Denmark (22).

Two hypotheses have been advanced to explain this relationship:

a) Venereal-viral hypothesis: gonorrhea allows an oncogenic virus to infect prostatic cells and induce potential changes leading to malignant transformation.

b) Chronic prostatitis hypothesis: bacterial chronic inflammation of the prostate induces atrophic and dystrophic pre-malignant lesions.

If this relationship proves to be true, we would expect a highly significant increase of P.C. around the end of this century, considering the dramatic rise of venereal disease since 1958. However, quick cure of gonorrhea since the advent of antibiotics in 1945 must, in our opinion, considerably limit the influence of this hypothetical factor.

HEREDITY, BLOOD GROUPS

Morganti et al (23) and Woolf (24) clearly demonstrated a higher rate of P.C. among the relatives of P.C. patients. If it is possible that a genetic component favours the development of the malignancy, then the problem is: what is the genetic cause of the disease?

We have studied the distribution of blood groups among our prostatic cancer patients and we have compared this distribution with that of the general population in our country. As shown in Table 2, there is no significant difference especially if we consider that some P.C. patients with blood group AB received transfusion with group A blood.

Table 2. Relationship Between Blood Group and Cancer of the Prostate

Group	Prostate Cancer (250 patients)	General Population
0	50.5 %	43 %
A	41.1 %	37 %
B	7.4 %	7 %
AB	1 %	3 %

ENVIRONMENTAL FACTORS, TOXICITIES, IRRADIATION

Enquiries on tobacco and alcohol consumption among P.C. patients and controls did not show any disproportion (4), and an interesting study performed by Bean et al (25) on routine autopsy material from Hiroshima and Nagasaki showed no relation between the presence of a P.C. and the dose radiation category.

Some workers postulating an association between the degree of urbanization and the incidence of P.C., suggested that the difference between urban and rural areas might be correlated to the level of suspended particulate air pollution (5,26). However, our own analyses on the relative frequency of P.C. by area in Belgium and similar analyses from England and Wales do not provide such evidence. It is possible that the unequal distribution of medical care and an uneven accuracy in the keeping of Cancer Registries in rural and urban areas may account for the differences.

Few data are available concerning the influence of industrial and domestic toxicities but Kippling and Waterhouse (27) drew attention to the high and statistically very significant incidence in P.C. among 248 workers exposed to cadmium dust. Such observations deserve further study.

PROSTATIC ADENOMA AND CARCINOMA

Widely diverging opinions have been put forward regarding the relation between benign prostatic hypertrophy (B.P.H.) and P.C.

Greenwald et al (28) compared the risk of developing a cancer among a group of adenomectomized patients and an age-matched group with prostatic enlargement: they found no differences but Armenian et al (29), in a prospective and retrospective study, found that the relative risk of developing a P.C. was three to five times higher among non-operated B.P.H. patients as compared to controls without prostatic enlargement. Further studies are required to establish a firm correlation but one must admit that the old-fashioned idea that adenoma protect against cancer must be discarded. On the other hand, considering that nearly 50% of the males present some degree of asymptomatic B.P.H. after the age of 60 and that prostatic cancer deaths for these age-groups represent about 1%, one realises that the relationship is far from obvious.

ASSOCIATED DISEASES

To our knowledge, there is no report of extra-genitourinary disease or physical condition significantly associated with P.C., except for the negative relationship with cirrhosis.

Liver cirrhosis provokes hyperestrogenism as a consequence of

decreased liver metabolism of estrogens. In order to determine the effect of hyperestrogenism on the incidence of P.C., Glantz (30) compared, by routine autopsies, 550 patients with cirrhosis and 650 patients without liver disease: he observed a 3.3% P.C. rate among the cirrhotic group and 9.0% in the control group. The age-group distribution is illustrated in figure 10.

This study confirms the prophylactic effect of estrogens on the growth of a clinical prostatic cancer but does not demonstrate any decrease in microscopical cancer as histological examination was not performed with the step section technique.

VIRUS AND CANCER

Paulson et al (31,32) induced, by viral manipulation (SV-40), in vitro, the transformation of normal hamster prostatic tissue into neoplastic tissue, exhibiting the characteristics of an undifferentiated carcinoma. Transformed cells injected into appropriate hosts gave rise to cancers showing some analogy with P.C.

Virus-like particles have been identified with electron microscopy in various specimens of P.C. (33). In addition, many recent experimental studies provide arguments in favour of the viral-oncogenic hypothesis of Todaro and Huebner (34). Immunological investigations have detected antibodies against known oncogenic viruses like SV-40, Herpes 2, Cytomegalovirus (35,36,37) but these antibodies are often not cancer specific and can be discovered in people without malignant disease (38).

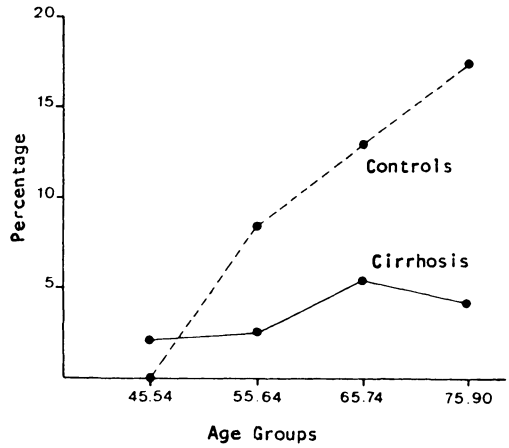

Fig. 10. Relationship between incidence of prostatic cancer in 550 patients with cirrhosis and 650 patients without liver disease (30).

The role of sexual activity, in particular with partners having genital infections, also provides arguments for the possible influence of viruses.

Although there is no evidence yet of a viral origin of human prostatic cancer we cannot ignore these studies relating prostatic cancer and viruses; the hypothesis of a viral origin of Human Prostate Cancer modulated by genetic, individual and environmental factors cannot be neglected.

ENDOCRINE STATUS

There is no doubt that changes in the hormonal environment influence the evolution of prostatic cancer. As a reminder, prostatic cancer does not exist among true eunuchoids; hyperestrogenism and post-pubertal castration greatly reduce the incidence of the disease: there is a very close relationship between the geographical distribution of prostate cancer in males and breast cancer in females.

Many workers have attempted to stress changes in the hormonal status of prostate cancer patients (39-44). We have determined the levels of testosterone, LH and FSH in men with P.C., B.P.H. and in men free of prostatic disease. The relevant data are given in Table 3. Like many others, we could not find any statistical differences among the three groups.

Similarly, hormonal levels found in low-risk populations like the Japanese and high-risk populations did not reveal any difference.

Table 3. Hormone Levels in Prostatic Cancer, Benign Prostatic Hypertrophy and Patients Free of Prostatic Disease

Patients	Testosterone (ng/100 ml)	LH (MUI/ml)	FSH (MUI/ml)	No. of Cases	Mean Age
No prostatic disease	414+/-251	16.3+/-9.1	14.4+/-7.2	20	71.1
B.P.H.	439+/-279	11 +/-7.6	13.2+/-5.8	20	70.3
P.C.	444+/-261	12.1+/-4.8	13.8+/-9.7	20	68.8

At the present time, the study of the endocrine status does not provide arguments explaining the relative risk of P.C. Of course the intra-cellular metabolism of hormones in prostatic cancer cells shows quantitative, if not qualitative, differences with normal prostatic cells but these changes reflect the modifications induced by the neoplastic state rather than the cause of the transformation.

CONCLUSION

Except for age, race and possible but as yet obscure environmental and genetic factors, little is known about the causes favouring the development of carcinoma of the prostate. It is most probable that the disease, like most other malignancies, does not depend on a single etiology and that we are dealing with complex interactions, some facilitating and other inhibiting any potential oncogenic transformation.

Much work remains to be done to obtain a better understanding of the origin of the disease, so that a more adequate measure can be taken to prevent and detect this common cancer.

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SOME OBSERVATIONS ON INCIDENCE AND NATURAL HISTORY OF PROSTATIC CARCINOMA

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The natural history of prostatic cancer is incompletely known, especially its preclinical and early clinical phases. According to the Swedish Cancer Registry the incidence is increasing but the mortality rate is not rising in a similar manner (1,2). Thus the biological activity of the cancer often seems to be so low that clinical symptoms do not appear.

The frequency of prostatic cancer in the population has previously been evaluated from autopsy studies in which the prostatic glands from all patients, whatever the cause of death, were serially sectioned for histological studies. Liavåg in a Norwegian study in 1968 found 26.5% of histologically detectable cancer in men older than 50 years (3) and in material from Malmö in Sweden from 1970 Lundberg and Berge (4) found a frequency of 39.7%.

During the last decades we have had a fairly constant population in Örebro as a basis for our clinical material of prostatic diseases. For many years all prostatic patients in the district have been sent to us rather than taken care of by other doctors or institutions. It can be calculated that from our population of 170,000 we should have around 26,500 men over the age of 50. Thus from the previous autopsy frequencies of histologically verifiable prostatic carcinoma it can be calculated that we have something between 6,000 and 9,000 cases of prostatic cancer to detect.

In an earlier study from our department (5), it was shown that during the late fifties the annual detection rate was around 40 new cases per 100,000 men. More recent studies show a figure of 70/100,000 men during the late sixties. At present we have almost doubled the detection rate to 130 cases/100,000 men per year within

the last decade. This means that with unchanged diagnostic effort we are now able to detect approximately 1/3 of all calculated cases of histologically detectable prostatic carcinoma in our population while the patients are still alive.

During the three year period March 1977 - April 1980 301 new cases of prostatic carcinoma were detected and staged according to the VACURG criteria (6) (Table 1).

Compared to most earlier series, e.g. the VACURG material from 1967 (6) with 13% early stages, the patients with early stage disease now dominate the picture. More than half the tumours in our material are in stage I and II. Tumours of low grade malignancy dominate stage I and are fairly common in stage II. Because of this change and the fact that the mortality rate for prostatic carcinoma had not increased to the same amount as the morbidity rate, we decided to apply a detailed program of observation without treatment to patients in stage I and those with well differentiated carcinomas in stage II.

Till now, during a mean observation time of three years, 14 of 100 untreated patients have died (none of prostatic cancer). Most of them (9 cases) died from cardiovascular diseases. If some kind of treatment had been given the deaths would erroneously have been attributed to the therapy. However, even selected by the above criteria, prostatic cancer is by no means a harmless disease as 9 of the patients progressed - one patient in stage I and two patients in stage II developed osseous metastases after an observation time of 1 - 3 years, while the others had local progression of tumour growth. So far however, all these patients have had a good and prompt response to therapy instituted at the time when progression was discovered.

Table 1. Prostatic Cancer, Örebro, March 1977 - April 1980

	Patients	Histological Grade		
		Low	Medium	High
Stage I	53	39	13	1
Stage II	105	49	43	13
Stage III	62	10	38	14
Stage IV	81	13	36	32
Total	301	111	130	60

CONCLUSIONS

An increasing number of prostatic carcinomas, mainly in the early stages, are diagnosed due to increased watchfulness and easier and more accessible diagnostic methods, for example fine needle biopsy. A shift to early detection of the cancer has occurred. Thus, more than 50% of the patients are now in an early stage of the disease at diagnosis and this fact may have changed the clinical picture of the prostatic carcinoma patients taken as a group. Some of the cancers are detected by chance while the patients are investigated for some other disease such as cardiovascular or other serious illness. This will unfavourably influence the patients' life expectancy as compared to the general population of comparable age. This fact should be considered in therapeutic trials. Moreover the serious drawbacks of most kinds of treatment should be carefully weighed against possible advantages for the patients' quality of life in a group like this, in which many patients are fragile and others probably will never have trouble with their cancer during their life time. However, some untreated patients progress rapidly and ordinary histological and cytological methods are not sufficient to discover those in which such rapid progress will occur. Other methods designed to determine the biological activity of prostatic carcinoma are urgently needed to indicate which patients can be safely left without treatment.

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ANIMAL MODELS IN PROSTATIC CANCER

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INTRODUCTION

Many aspects of human cancer can only be investigated properly by the use of suitable experimental model systems. In the field of prostatic cancer such model systems are relatively scarce. For any model system, it is essential that its properties reflect those of the human situation as closely as possible. Isaacs and Coffey (1) have published a list of requirements that should be fulfilled by an ideal animal model for prostatic cancer. Some of the most important properties of the ideal prostatic cancer model are:

(i) histology similar to human prostatic cancer
(ii) metastatic patterns to lymph nodes and bone
(iii) slow growth rate
(iv) initial response to hormonal manipulation
(v) ultimate relapse to hormone insensitive state

It is not likely that the ideal model does exist. However, each model should be characterized extensively, especially with respect to the points just mentioned, before treatment studies are performed.

In this paper three types of model systems will be considered:

(a) models derived from spontaneous animal tumours
(b) models dervied from induced animal tumors; and
(c) transplantable human tumors.

SPONTANEOUS ANIMAL TUMORS

In contrast to its occurrence in man, the incidence of prostatic cancer in laboratory animals is relatively low (2). Most of the spontaneous animal tumors have been found in aged rats.

Dunning Tumors: In 1961 Dunning (3) discovered a prostatic adenocarcinoma in an aged Copenhagen rat. This tumor has been passaged by subcutaneous transplantation since its original discovery and gave rise to more than ten distinct sublines (4-6). One of these sublines, R3327-H, appeared to be composed of both androgen-sensitive and androgen-insensitive cells (5,7,8). From this tumor line an androgen-insensitive subline, R3327-HI, has been isolated by long-term passage in castrated male rats (5). Another androgen-independent subline, the rapidly growing anaplastic tumor, R3327-AT, did arise spontaneously from the R3327-H tumor (8). Several studies, especially on the -H, -HI and -AT sublines, have been published in which the histological appearance, the hormonal dependence (5), the tumor kinetics (9), the immunological parameters (9,10) and the biochemical properties (11) were described (for recent reviews, see refs. 1 and 12).

The three related sublines, R3327H, R3327HI and R3327AT are especially suited to search for possible markers for hormonal dependence. As expected, the highest levels of androgen receptor was found in the hormone sensitive subline (1). Also Coffey and co-workers have elaborated a method that employs enzyme activities, rather than receptor concentrations. A so-called relative enzyme index, R.E.I., has been defined as follows:

$$R.E.I. = \frac{(3\alpha\text{ HSD}) \times (\text{LAP}) \times (\text{LDH})}{(5\alpha\text{ reductase})(7\alpha\ 6\beta\text{ hydroxylase})\ (\text{Alk P})} \quad (11)$$

(HSD = hydroxysteroid dehydrogenase; LAP = leucin amino peptidase; LDH = lactate dehydrogenase; Alk P = alkaline phosphatase).

The enzyme activities were normalized to those measured in the normal dorsolateral prostate of the rat. As shown in Table 1, the values of the R.E.I. are widely different for the three different sublines. It is worthwhile to check whether a similar method might be applied to human tumors as well.

The heterogeneity of the Dunning tumor sublines may well reflect the variability observed with human prostatic cancer, e.g., with respect to its biological behavior or to its response to therapy. The natural history of the H and the HI sublines demonstrates that the well known phenomenon of change to the hormone insensitive state could be the result of a selection process.

Table 1: Relative enzyme index in Dunning tumor sublines (after ref. 11)

	range	average R.E.I.
R3327H	0.7-4	0.89
R3327HI	26-110	41.4
R3327AT	2337-9260	2533

Pollard Tumors: Other interesting prostatic tumor lines have been developed from three spontaneous tumors detected by Pollard in aged germ-free Lobund-Wistar rats (13). The tumor lines, that were designated as PA-I, PA-II and PA-III, differ on the basis of morphology, histology and the pattern of metastasis (14). Male rats appear to be somewhat more susceptible to tumor transplants than females, but a detailed endocrinological characterization has not yet been accomplished. In all lines there is a partial response to DES. An important feature of the Pollard lines is their ability to metastasize to the lymph nodes and the lungs (15,16). Some studies on the modulation of metastasis have been carried out (16).

INDUCED ANIMAL TUMORS

In addition to the spontaneous tumors, a few models based on induced prostatic tumors have been described. Most important of these are the tumors, described by Noble, initiated by prolonged exposure of Nb rats to steroid hormone implants. Such treatment has resulted in a number of transplantable tumor lines originating from the dorsolateral prostate (17,18,19). The tumors were either androgen-dependent, estrogen-dependent, or autonomously growing (20). The growth rate is not much different for the three types of tumor (doubling time around five days). The autonomous lines tend to metastasize more readily than the hormone-dependent lines. The tumors have also been grown in nude mice (21) where the hormone dependency is similar to that in the rat.

A very interesting observation with the hormone-dependent Nb tumors is the prevention of autonomous growth by a low dose of hormone. Complete withdrawal of hormones (by removal of the steroid implant) did ultimately result in the recurrence of a hormone unresponsive tumor. However, if the original steroid implant was replaced by an implant containing a lower dose of the same steroid (20%), the tumor that recurred appeared to have retained its hormone responsiveness (22). Thus, the change to autonomy could be prevented by means of a low maintenance dose of steroid. This finding could imply that hormone dependence is a property that might be influenced by the endocrine environment. This is in contrast with the view, supported by the establishment

of the Dunning R3327H sublines, that the change to autonomy is due to a selection process. It is obviously of greatest interest to find out whether either of these mechanisms, or perhaps both, is operative in human prostatic tumors.

TRANSPLANTABLE HUMAN TUMORS

Although valuable information about the biological and biochemical properties of prostatic cancer will be obtained from the studies using the models mentioned above, these models all share a common disadvantage, their non-human origin. For that reason several attempts have been made to propagate human tumors in immunodeficient animals, such as the athymic nude mouse. So far, however, very few successes have been reported in this line of research.

In vitro cell lines that originate from the human prostate, such as EB33 (23), DU145 (24), PC-3 (25) and LNCaP (26), will in most cases induce tumors in nude mice. With the possible exception of the recently established LNCaP cell line, there are serious doubts whether these cell lines can be considered as meaningful representatives of prostatic carcinoma.

Only a few reports exist on the successful heterotransplantation of prostatic tissue in nude mice. Shimosako et al (27) and, very recently, Jones et al (28) have reported the establishment of rather undifferentiated, hormone-independent tumor lines. Reid et al (29) have described a transplantable line derived from a moderately differentiated adenocarcinoma of the prostate. This tumor has a slow growth rate and is at least partly dependent on androgens (29). In our laboratory the transplantable line PC82 was developed in 1977 from a moderately differentiated carcinoma (30). Some of the characteristics of this tumor line are summarized in Table 2.

Table 2: Characteristics of the PC82 Prostatic Cancer (30)

Histology:	adenocarcinoma; cribriform
Function:	secretion present human prostatic acid phosphatase human LDH-isoenzymes
Growth:	doubling time 15.8 $\pm$ 2.5 days
Endocrinology:	no growth in females no growth in castrated males regression after castration androgen receptor present

Table 3: Some Important Properties of Tumor Model Systems

	Dunning R3327 H	Dunning R3327 HI	Dunning R3327 AT	Pollard PA I-III	Nb tumors 2Pr	Nb tumors 13Pr	Nude mouse PC82
ORIGIN	rat probably dorsal lobe			rat ?	rat dorsal lobe		human primary prostatic tumor
ADENOCARCINOMA	yes			yes	yes		yes
RATE OF METASTASIS (lymph nodes, lungs)	low			high	moderate high		absent
GROWTH RATE (doubling time in days)	15-20	15-20	2	1	5.3	4.3	16
HORMONE SENSITIVITY Androgen required	yes	no	no	?	yes	no	yes
Response to castration	partial	-	-	?	partial	-	yes
Recurrence after regression	yes				yes		?

The histology of the tumor shows that it is an adenocarcinoma with a cribriform pattern that resembles the structure of the original tumor quite well. The tumor contains a large amount of human prostatic acid phosphatase as demonstrated by immunohistochemistry (30). Serum levels of acid phosphatase are significantly increased in mice bearing the PC82 tumor. Also the presence of human LDH-isoenzymes could be demonstrated in the tumor as well as in the mouse serum.

The growth rate is relatively slow and has, until now, not changed in subsequent passages. The PC82 tumor is, so far, completely dependent upon the presence of androgens since it did in no case grow on female nude mice, nor on castrated male mice. Castration of a male nude mouse bearing a PC82 tumor did result in regression of the tumor. So far, recurrence after regression has not been observed.

CONCLUSIONS

Some of the most important properties of the tumor models discussed above, are compared in Table 3. This table shows that there is a variety of models that differ with respect to rate of metastasis, growth rate and hormonal dependence. This situation does well reflect the differences in human prostatic tumors clinically presented. It is therefore advisable to use different appropriate models to study different aspects of human prostatic cancer. The question of which of the presented models should be considered as a realistic model for human cancer is very difficult to answer at the present time. In this regard it should be noticed that the big advantage of the PC82 model over all other models is its human origin. Therefore the PC82 model might be considered as a suitable model for human prostatic cancer, especially for studies on the effect of hormonal manipulations.

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TISSUE CULTURE IN PROSTATIC CANCER

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INTRODUCTION

The aim of this presentation is to give an overview of the studies performed in vitro with prostatic tissue and also to give a summary of our own work in this field.

"In vitro" culture of prostatic tissue is not a new technique since Burrows et al (1) reported in 1917 the growth of cells from an explant of human prostatic adenocarcinoma. After sinking into oblivion for some decades, the method has been steadily developed with the progress of technology. The increasing interest of tissue culture techniques as a model for studying human prostatic adenocarcinomas is partially due to the lack of suitable animal models.

The theoretical advantages of "in vitro" techniques are numerous:

- many experiments can be made with a minimum of tissue material;
- the medium surrounding the cells can be more or less precisely defined and cells are not submitted to systemic influences;
- numerous drugs can be tested alone or in association;
- the tissue can be endocrinologically manipulated and direct cellular influences can be studied;
- relations between epithelial cells and stroma can be studied according to the type of the culture (cell or organotypic culture);
- the growth can be monitored by rather simple cell counts;
- culture is the only way to study simunltaneously or repeatedly the influences of drugs or hormones on human prostatic tissue.

On the other hand, culture of prostatic tissue suffers many

shortcomings and limitations resulting from its highly artificial nature and despite the abundance of in vitro studies, little knowledge of practical value has so far been obtained.

CULTURE OF ANIMAL PROSTATIC TISSUE

Culture of Normal Rodent Prostatic Tissue

In the 1950's, Lasnitzki, Franks, and Bengmark et al (2-4) were among the first who succeeded in culturing prostatic tissue from rats and mice. Following this pioneer work, many studies have defined the influence of the medium, hormones, and vitamins on growth, maintenance of the morphology and metabolism of prostatic tissue (3,5-17). Although some discrepancy in the response was noticed in relation to the origin (ventral or dorsal prostate) or the age of the donor, general conclusions can be drawn from those studies: in vitro, animal prostate usually exhibits an androgen dependence; different metabolites of testosterone may produce different effect; the serum diminishes the effect of the androgens, probably due to binding of the hormones by the globulins; prolactin increases the effect of testosterone; vitamin A is necessary to the maintenance of the secretory activity of the epithelium; estrogens have variable effects, sometimes inhibiting the growth of the cells but sometimes enhancing it; insulin and hydrocortisone have a stimulating effect. Potentially therapeutic agents have also been tested on organ cultures of rodent prostates with various results (18-20).

Influence of Carcinogens

As early as 1950, Lasnitzki studied the influence of methylcholanthrene on explants of young rat prostates. She noticed that the carcinogen induced epithelial hyperplasia with squamous metaplasia and increased mitotic activity. Estrogens increased the effect of the carcinogen but cortisone, testosterone and vitamin A had a protecting influence (21-26). Thereafter, some authors produced malignant transformation "in vitro" by culturing normal prostatic tissue of rats with carcinogenic hydrocarbons (27-31); but the tumors induced were fibrosarcomas or epidermoid epitheliomas without any hormonal dependence.

Effect of Oncogenic Viruses

Paulson, Fraley and Ecker (32) produced "in vitro" malignant transformation of normal hamster prostate by introduction of a DNA oncogenic virus (SV-40) within the culture. Transformed cells injected into homologous hamsters produced epithelial tumors

secreting acid phosphatases and metastasizing.

This experience opened new fields in the study of the role of viruses in the occurrence of prostatic cancers but produced an imperfect model for human prostatic cancer as the hormonal dependence was variable and different from that of the human cancer (33).

Spontaneous Transformation "In Vitro"

After many subcultures of epithelial cells from adult C3H rat prostates, Chen and Heidelberg (34) observed a spontaneous malignant transformation (cells growing quickly, in multiple layers). Injected into autologous hosts, these cells, although having an epithelial appearance, produced fibrosarcomas.

Once more, these experiences, though interesting for the study of some oncogenic mechanisms, gave models of very limited interest for the study of human prostatic adenocarcinoma.

Cultures of Natural Animal Adenocarcinomas

In some instances, spontaneous rodent prostatic tumors have been discovered and transplanted to successive generations of syngenetic hosts: Dunning's tumors, Noble's tumors and Pollard's tumors. Many studies have been performed on these animal models (35-37).

In some cases, in vitro sutdies were made with these tumours in order to identify morphological or biochemical markers of cancerous transformation (38,39). They were not very conclusive.

Cultures of Prostatic Tissue from Dogs and Primates

More recently, studies "in vitro" used prostatic tissue from the dog and the Rhesus monkey (40,41). This material was chosen because of its closer relation to human prostate. Indeed rodent prostates have anatomical, histological and biochemical differences from the human prostate and their behaviour "in vitro" shows some striking differences in such a way that an extrapolation of the experimental results to the human situation can be misleading. So far these recent studies have not led to any development of great significance or practical relevance.

CULTURE OF HUMAN PROSTATIC TISSUE

After the pioneer work of Burrows et al in 1917 (1) Röhl published his experience of the culture of adenomatous and cancerous

human prostatic tissue (42). Since then, culture of human prostatic tissue (normal, adenomatous or carcinomatous) has elicited a sustained interest in many centers, mainly in the United States, in Scandinavia, in Great Britain and in Germany. A great deal of information has been collected, often scattered, sometimes contradictory, always optimistic; but the objective analysis of the data is rather disappointing and inspires a pessimistic view as to their practical application.

A review of the literature on human prostatic models in vitro reveals a broad variety of techniques, each center trying to resolve a specific question and conceiving an artificial system fitted to its preoccupations.

a) At the beginning of the 1970's, efforts were made to define the optimal conditions for reproducing short-term cultures of human adenoma and carcinoma (43-51). The nutritional needs, the influence of the serum and the role of hormones have been widely investigated (52-61). The results were often contradictory: the stimulating effects of androgens and the inhibiting effects of estrogens on the growth were not the rule and the response of the explants varied considerably in relation to the age of the donor, the origin of the tissue (normal, adenomatous or cancerous) and the type of experimentation.

b) Studies in electron microscopy tried to define some morphological differences between normal, adenomatous and cancerous cells "in vitro"; these works were not convincing (62), but it was clearly demonstrated that submicroscopic alteration occurred with the ageing of the cultures (lysosmal, mitochondrial alterations, and increase of cytoplasmic microfilaments ...) (63-65).

c) The metabolism of androgens has been widely studied "in vitro" (66,67). The major role of DHT has been established; but the results of hormonal influences on the human prostate "in vitro" were inconsistent and sometimes contradictory.

d) The prostate is constituted of smooth muscle, a vascular stroma and epithelial acini. The latter are responsible for the malignant transformation, adenocarcinoma representing more than 95% of all prostate cancers. Prostatic adenocarcinoma is very heterogeneous: stromal areas, normal epithelium, adenomatous foci and adenocarcinoma are often intimately mixed.

Although preliminary work from Franks et al (45) indicated that epithelial cells separated from their stroma cannot grow "in vitro", many authors, considering that epithelial cells alone were necessary for the study of the cancer, used technical artifices (mechanical separation, trypsin, collagenase or viokase enzymatic digestion or gradient sedimentation) to isolate epithelial cells and

to obtain pure epithelial cell cultures (68,77). As stressed by Schroeder (74), most of these techniques proved to be ineffective.

e) Because of the heterogeneity of the material, one of the major problems with culture of human prostatic adenocarcinoma is the identification of the growing cells: are they stromal cells or epithelial cells and if the latter, are they normal or cancerous cells? Indeed, if one aims to draw practical conclusions from the behaviour of human prostate cancer "in vitro", it is fundamental to make sure that the material studied is actually cancerous. In this respect, electron microscopy (62), histochemical and immunochemical studies can distinguish epithelial cells and fibroblasts (78,79), but up to now, there is no specific marker to distinguish normal epithelial cells from cancer cells.

Furthermore, some works seem to prove that, paradoxically, when explants of cancerous prostates are grown "in vitro", the growth around the explant is due to the proliferation of basal cells from normal glands while cancer cells degenerate (64,80,81). Chromosome counts (82) on cells surrounding the explant give uniform diploid values. Two explantations can be advanced: either these cells correspond to a well-differentiated carcinoma in which diploidy is usual; or we have another argument in favor of the benign origin of cells growing in monolayer around the original explant of prostatic cancer tissue.

f) An "in vitro" model can give practical information if it reflects the characteristics of the cancer in vivo. As proved by the above considerations, the culture of human prostate cancer meets major problems in this regard and the uncertainty of the cancerous nature of the growing cells is surely not one of the least!

g) Recent work from Romijn and Schroeder (83) and Cowan et al (84) using separated pure epithelial and pure stromal cells originating from prostatic tissue, showed that 5-α-reductase (transforming testosterone into DHT) is essentially localized within the stroma where the transformation of active hormones, necessary to the epithelial cells, occurs. These investigations illustrate the highly artificial condition of cell cultures which nevertheless have been widely used to try to determine the hormonal response of prostatic cancer to various hormonal compounds.

h) As in animals, human prostatic tissue has been submitted "in vitro" to oncogenic compounds and oncogenic viruses (85-87). Cellular alterations and transformations suggesting cancerous potentialities were observed but these cancers were, once more, very different from human prostatic adenocarcinoma and could not be used for clinical applications.

i) Permanent cell lines have also been widely studied. Many

permanent cell lines derived from primary cultures of normal or cancerous human prostates are available and are often considered useful models for the investigation of potential therapeutic agents. The better known are:

Line MA-160 (Fraley and Ecker) (88-90)
Line EB-33 (Schroeder et al) (91-93)
Line HPC-36 (Lubaroff) (94)
Line NP-2 (Webber) (95)
Line DU-145 (Stone et al) (96)
Line PC-3 (Kaighn et al) (97)
Line LNCaP (Horoszewick et al) (98)

Colleagues interested in the origin and the properties of these lines will find all the details in the original papers of the authors. Although many authors claim the usefulness of those lines, we believe that the greatest care is necessary when they are manipulated. Some of these lines are of uncertain origin (line MA-160 and

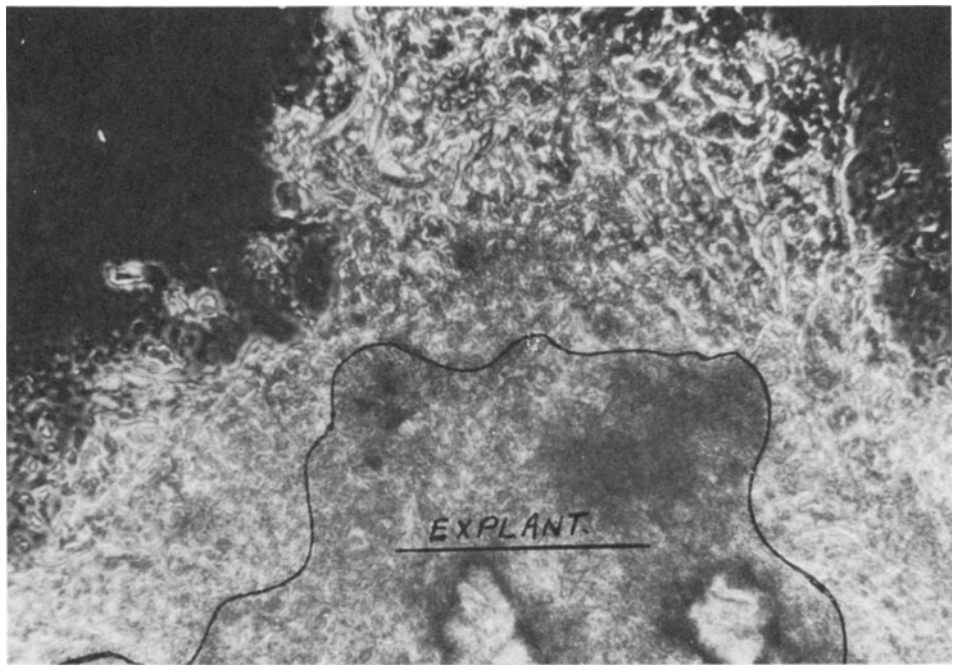

Fig. 1. Human prostatic adenoma.
7th day of culture. Area of growth surrounding an explant.
(phase contrast).

perhaps line EB-33 should not be prostatic cell lines but the result of a contamination by HeLa cells!); there is no doubt that these lines have modified their nutritional needs and their enzymatic system with prolonged culture and that most of them do not have a clear hormonal dependency. It would be dangerous, in our view, to extrapolate the therapeutic results obtained with these highly selected lines to human prostatic cancers, even to those which are hormone resistant, in vivo.

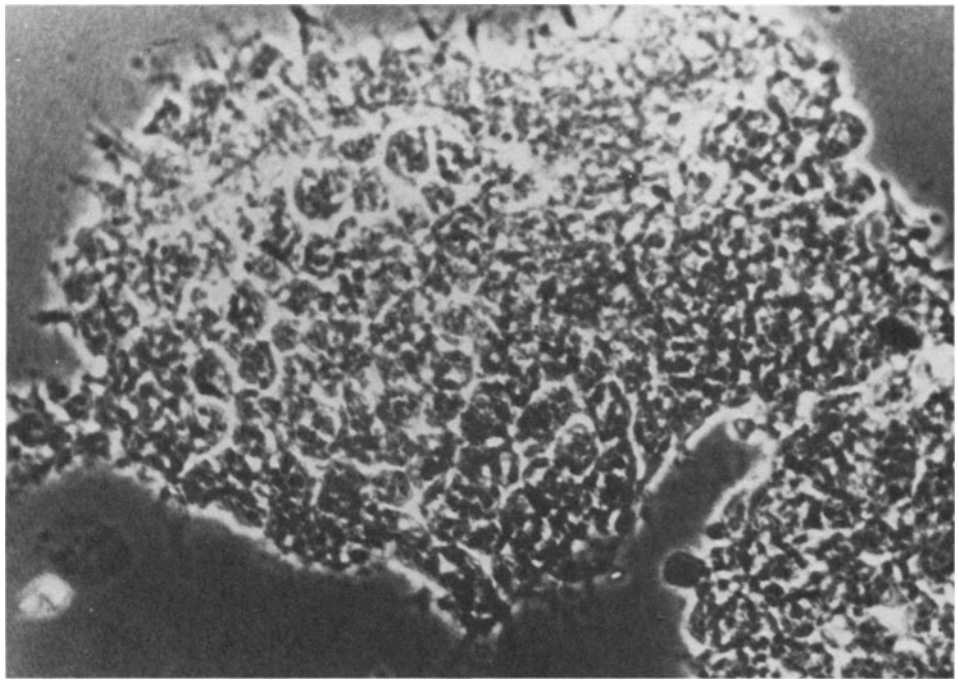

Fig. 2. Culture of human prostatic adenoma. 23rd day of culture. One notices a diffuse cellular necrosis in this islet at the periphery of one explant (phase contrast x 400).

Our Experience of the Culture of the Human Prostate

Preliminary studies were performed with human prostatic adenoma obtained either by suprapubic surgery, or by trans-urethral resection (TUR). Cultures were performed at 37°C, in a closed atmosphere (without adding carbon dioxide) and a synthetic buffer (HEPES) was added to the medium to prevent changes of the pH.

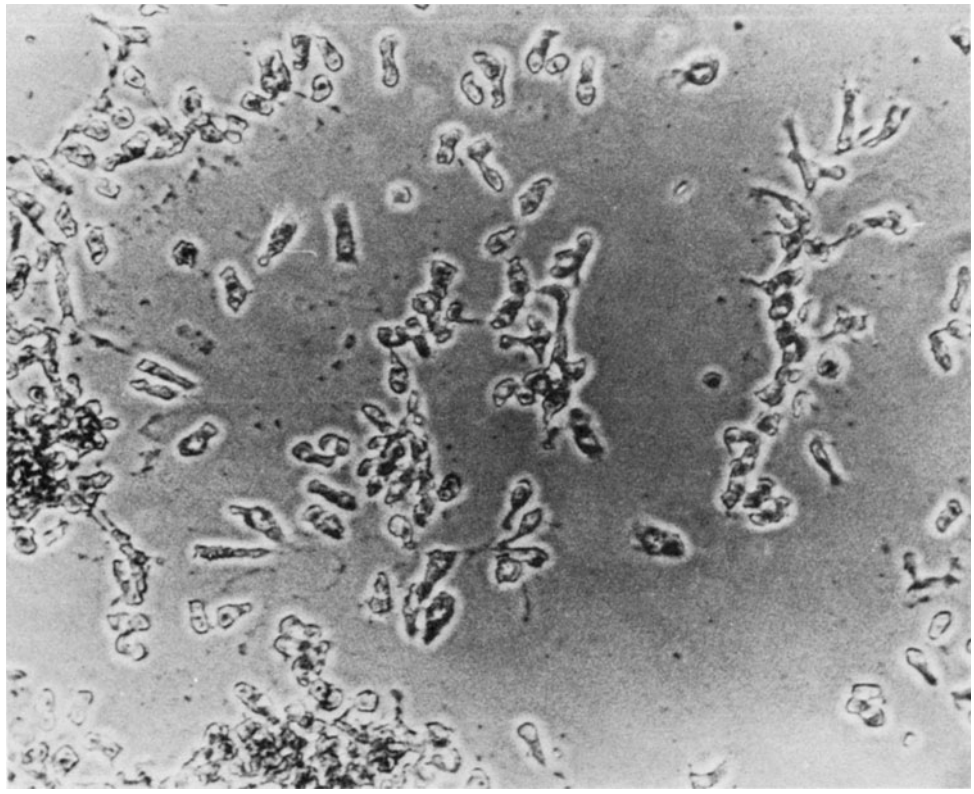

Fig. 3. Epithelial prostatic cells isolated according to our mechanical method (phase contrast: G x 200)

In these preliminary studies, we have tried to define the optimal nutritional needs, the patterns of growth of organ cultures and the behaviour of isolated epithelial cells.

a) Organ Cultures. The medium was composed of BME (Basal Eagle's medium) or MEM (Minimum Essential Medium) or Medium 199 (Flow Laboratories) to which was added glutamine, serum (HS or FCS, at concentrations of 5 to 20%), and antibiotics (Gentamycin, 50 µg/ml). We observed a peripheral growth in nearly 40% of the explants, as is shown in Figure 1.

The growth was usually epithelial and not fibroblastic but the cell population surrounding the explants was not pure. Growth

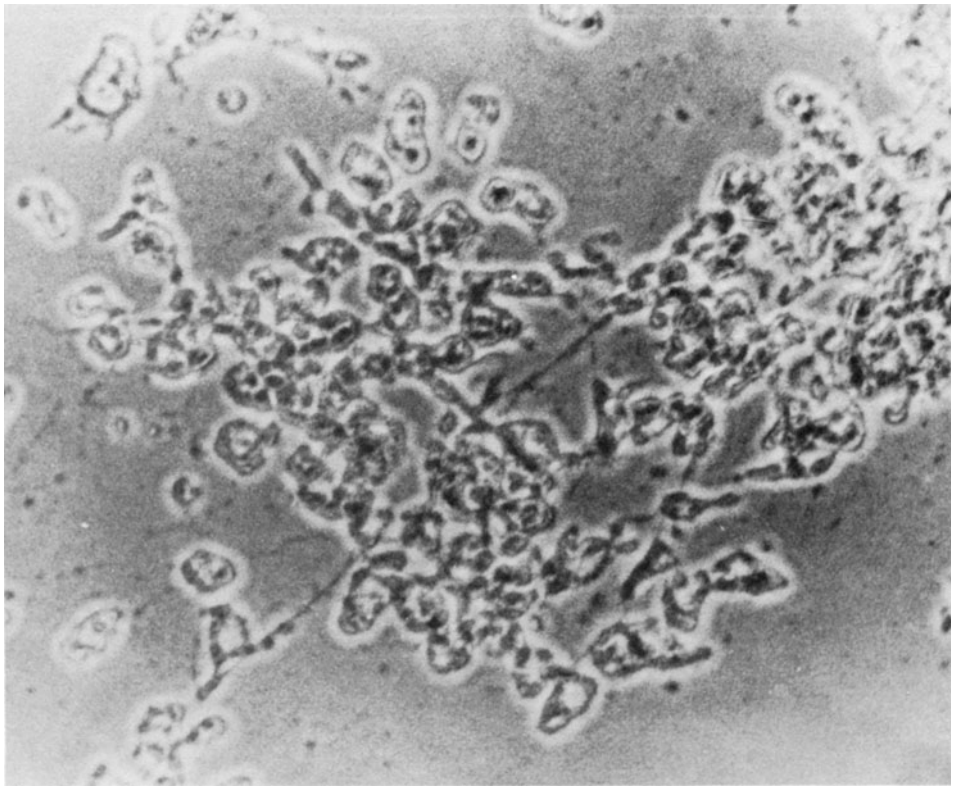

Fig. 4. Cell culture: 5th day. Most cells appear viable (contrast x 400)

usually started after 48 to 72 hours. It was always slow and we never observed a confluence of the cell sheets in the Petri dishes. In most cases, after two to three weeks, toxic signs appeared with pyknosis of the nuclei, accumulation of cytoplasmic granulations and death of the cells. We never succeeded in obtaining subcultures (Fig. 2).

Significant bacterial contamination occurred in many explants obtained by TUR in spite of aseptic and antiseptic measures.

The type of serum (HS or FCS) and its concentration (5 to 20%) or the type of medium (BME, MEM or 199) had no very significant influence on the growth. However insulin (at a concentration of

0.08 U/ml) and vitamin C (150 g/ml) seemed to have a stimulating effect on the growth.

b) Cell Cultures. Epithelial cells were obtained by applying pressure and irrigation between two slides of prostatic tissue samples. The cell suspension was then centrifuged for ten minutes at 800 r/min. and the pellet was put in culture (Fig. 3). During

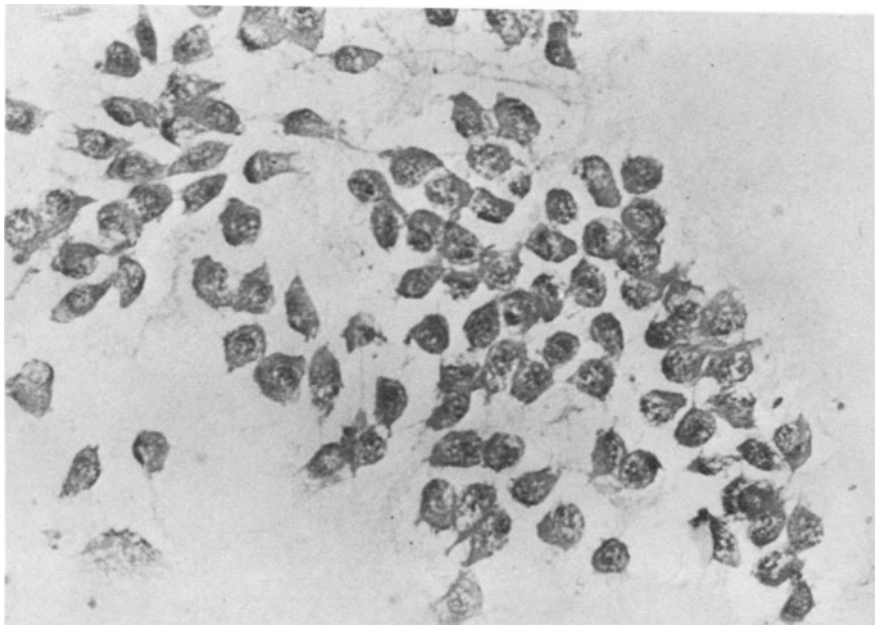

Fig. 5. Prostatic adenoma cell culture fixed and stained after 23 days of culture. Many cells exhibit necrosis with vacuolization and rupture of the cytoplasm (G x 375)

the first days, the cells seemed viable (Fig. 4), but after eight to ten days, they showed degenerative alterations and a large percentage of cells exhibited necrosis after 20 days of culture (Fig. 5).

We never observed significant cell multiplication and the addition of colcemid (between the 6th and 12th day) to the culture very rarely showed evidence of blocked metaphase. Therefore, our

work seems to indicate that it is difficult if not impossible to grow epithelial cells "in vitro" after mechanical separation from their stroma.

These conclusions are in accord with those of Franks et al (45) but in contradiction with other experiments (69-71, 76) in which epithelial cell cultures were successful. It is probable that our mechanical method used to separate the epithelial cells, only releases superficial secreting cells with high exfoliative capacity from the acini - and these cells have lost the property to divide - while other methods (such as the enzymatic) may release basal cells of the acini which can divide actively and are probably responsible of the growth.

In a second phase, we repeated organ cultures of adenoma and carcinoma of the prostate, in more classical conditions, using a gas incubator. In these conditions, nearly 50% of the explants showed a peripheral growth but, as previously, growth was always slow and limited and after two or three weeks, most cultures died; our attempts to produce subcultures were unsuccessful.

Studying the influence of the medium on the growth, we did not notice significant differences between BME, MEM and medium 199 as shown in Fig. 6. The influence of antibiotics added to the medium was also investigated. As shown in Table 1, the best results were achieved with Gentamycin at the concentration of 50 µg/ml. At this concentration, no cytotoxicity was noticed although Gentamycin at 200 µg/ml slightly reduced the growth.

It was also clear, in these experiments, that the method of collecting the tissue played an important role in the capacity for growth; only 30% of tissue samples provided by TUR against 55% of the samples obtained by retropubic surgery grew "in vitro".

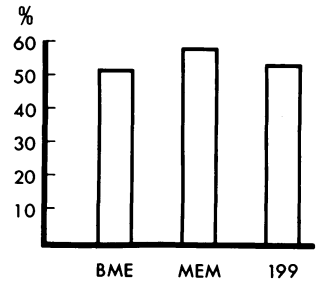

Fig. 6. Percentage of growing explants from prostate adenomas in relation to type of medium used.

Table 1. Influence of Antibiotics Added to the Medium on the Growth (P. = Penicillin, S. = Streptomycin, G = Gentamycin)

Antibiotic	Number of Cultures	Number of Infections	Percentage of growing Explants	Percentage of Growing Explants in Non Infected Medium
None	24	12	19%	38%
P. 10 U/ml S. 10 µg/ml	24	10	23%	40%
P. 100 U/ml S. 100 µg/ml	24	6	29%	39%
G. 50 µg/ml	24	5	33%	42%
G. 200 µg/ml	24	5	24%	30%

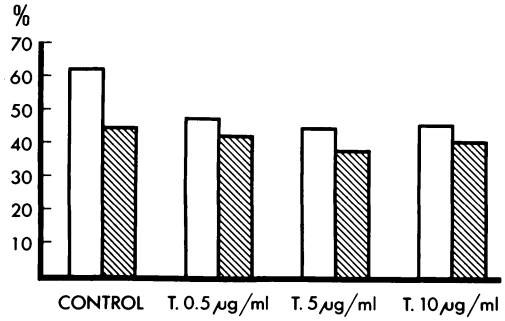

Fig. 7. Percentage of explants with peripheral growth in our experimental conditions in relation to concentration of testosterone in the medium.
(Blank columns = adenomas. Hatched columns = carcinomas)

The influence of testosterone added to the medium was investigated. At concentrations of 0.5, 5 and 10µg/ml, testosterone had an inhibiting effect on the growth of the adenomas while it did not influence the carcinoma (Fig. 7). However the relevance of the hormonal influence assessed by counting the number of growing explants as we did, is subject to criticism on two points: firstly the hormonal concentrations were 50 to 1000 times higher than physiological levels; secondly we did not take into account the percentage of initially nongrowing explants, due to the quality of the tissue.

In a second experiment, we used testosterone at lower concentrations (0.01, 0.1 and 0.5 µg/ml) and we measured the area of growth surrounding explants of adenoma and carcinoma, at the seventh day of culture, on fixed material. We observed no effect, either stimulating, or inhibiting as compared with controls.

Two explanations can be suggested:

1. In vitro, human prostatic tissue loses its hormonal dependency, which seems unlikely in short term organ culture.
2. The determination of the androgen dependency was not correctly appreciated, i.e. the medium without addition of hormones can indeed possess enough testosterone to allow a normal metabolic activity of the cells.

To verify this latter hypothesis, we measured the level of testosterone, dihydrotestosterone (DHT) and androstenedione ($\Delta 4$) in the culture medium before and after two, six and nine days of culture in two different conditions: in a control medium (no testosterone) and in a medium with added testosterone (0.1 ug/ml). The results are given in Table 2.

Therefore it appears that the explants contain hormonal reserves and that a medium not supplemented with hormones is rich enough, during the first few days, to allow a normal metabolism of the prostatic tissue, and that addition of testosterone to the medium is not a valuable method for the study of hormonal influence on the growth in short term organ culture.

DETERMINATION OF THE INDIVIDUAL RESPONSE OF PROSTATIC CARCINOMA TO THERAPEUTIC AGENTS BY IN VITRO ASSAYS

Whilst randomized clinical trials are fundamental to determine the overall value of different compounds, one knows that individual response is sometimes very different from one patient to another. The purpose of our "in vitro" experiments with human prostatic carcinoma was to investigate some of these variations.

Table 2. Levels of Testosterone, DHT, and 4 in Horse Serum and Control Medium With and Without Added Testosterone (Results in Picogram/ml).

Medium	Testosterone	ΔDHT	Δ4
Horse serum	100	< 40	60
Control medium before culture	< 40	< 30	< 30
after 2 days culture	4.100	360	< 50
6 days culture	5.200	720	65
9 days culture	2.200	320	< 50
Control + Testosterone, 0.1 µg/ml			
after 2 days culture	57.000	4.250	620
6 days culture	86.000	4.415	560
9 days culture	77.000	5.390	720

This shows clearly that:
- Horse serum and control were very poor in T. and DHT.
- After incubation of the explants, the control medium becomes very rich in T. and DHT.

Table 3. Response of Tissue Cultures From Patients with Prostatic Cancer Refractory to Stilboestrol to the Addition of Hormonal Agents.

Drug Added to the Medium	Number of Explants Evaluated	Number of Growing Explants	Percentage Growing
Control	48	21	43%
Estracyt (100 µg/ml)	52	24	46%
DES (1 µg/ml)	61	14	23%
MPA (10 µg/ml)	55	20	36%
RO-6-1963 (10 µg/ml)	43	17	39%

Whilst undertaking these "in vitro" studies, we became aware of the severe practical limitations of the technique:

1. The collection of prostate cancer tissue suitable for culture raises problems: in cases suitable for hormonal treatment or chemotherapy, the only way to obtain tissue samples for culture is by needle biopsy or TUR which provide great risks of infected or non viable tissue.

2. Independently of any therapeutic effect, nearly 60% of the explants do not grow in vitro and it is difficult to draw the distinction between the inhibiting effect of the drug and the initial alteration of the tissue.
3. In vitro, systemic influences are suppressed: the hypothalamic control, so important in the hormonal effect is no longer present, and the influence of serum binding globulins and the possible potentiation of the effect by substances like insulin, corticosteroids, growth hormone, and prolactin are also absent.
4. Last but not least, prostate cancer is very heterogeneous and one does not know whether the cells growing are true cancer cells or normal basal cells present in the explant. The lack of specific markers for prostatic cancer cells is another important shortcoming.

In spite of these limitations, but aware of them, we tried to evaluate "in vitro" the therapeutic value of different compounds on explants of individual prostatic carcinomas. The following drugs were tested: Estracyt, Stilbestrol (DES), Medroxyprogesterone acetate (MPA), and RO-6-1963 (an inhibitor of 5-α-reductase).

Unfortunately, the detailed results of the experiments performed on nearly 600 explants from 10 prostatic carcinomas did not allow us to draw any practical conclusions. Perhaps it is reasonable to give one example:-

M.L., 71 years old was treated for prostatic cancer with DES (5 mg/day) for two years. The patient deteriorated despite treatment and bone metastases appeared together with a right hydronephrosis.

A TUR was performed and tissue was put in culture to evaluate the response to the previously mentioned drugs. The results appear in Table 3.

The inhibition of growth was the greatest with DES "in vitro", but in vivo the disease still progressed aggressively. In vitro, the least response was observed with Estracyt, but with Estracyt, the patient showed an excellent objective response for 18 months.

CONCLUSION

We do not believe that "in vitro" models can, at the present time, provide us with information of practical value in the clinical management of prostatic cancer. We do not claim that culture of normal or cancerous tissue is useless; indeed, it has contributed to the elucidation of some important problems in normal and cancer cell biology. However we caution against the premature extrapolations of experimentors who, using specific systems in specific situations, believe that they can draw direct clinical conclusions, ignoring the fundamental practical problems.

Thus, our conclusions are the same as those of Schroeder and Oishi who stated, in a recent review of the problem (74), that "On the whole, attempts to use tissue culture techniques in research related to prostatic cancer have been disappointing. They have contributed minimally to the understanding of this tumor and to its better management".

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HISTOPATHOLOGY OF THE HUMAN PROSTATE GLAND

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INTRODUCTION

In the past, the modality of treatment, whether it be surgical, hormonal or chemotherapeutic, depended on the histological type and organ system from which the tumor cells were derived. Unfortunately, at the present time, we have no clear-cut definitive explanations as to why some prostatic carcinomas, in many institutions, are not usually defined as to histological type, extent or grade. Also, why some tumors act in a more benign clinical fashion in one patient and in a more malignant manner in another. Many of these prostatic carcinomatous patterns should be diagnosed as to their type and also their extent both in terms of volume and percentage of the involved prostate gland. Perhaps, some time in the near future, the various histological types and grades as well as their extent will also be correlated with the regions of the world in which the patient dwells and with other epidemiological factors. Without these multispectral analyses, the pathobiology of prostate carcinoma will remain an enigma as it has for the last 40 years.

HISTOPATHOLOGICAL PROCESSING OF TISSUE WITH INCIDENCE OF CARCINOMA OF THE PROSTATE IN SURGICAL SPECIMENS

The histopathology of prostate carcinoma is extremely frustrating as compared to that of many other organ systems, such as the cervix, endometrium, and breast. Equally frustrating in many institutions is the manner in which the prostatic tissue has been processed whether from a whole surgical specimen obtained by suprapubic prostatectomy or by means of a transurethral resection (TUR).

There is a high incidence of cancer, from 16 to 30% depending

on the age group. It is the dictum of our institution [Columbia-Presbyterian Medical Center (CPMC)] that one section be taken for every 5 gms. of prostatic tissue obtained from either a suprapubic or retropubic prostatectomy specimen. In the case of a TUR of the prostate, every gram of prostatic tissue must be put through for processing. When this is done, the incidence of carcinoma of the prostate at CPMC varies between 23 and 28% depending on the surgical procedure. The question then arises: what does the urologist do next? It is easily demonstrated that there is tremendous variation in histological pattern and involvement of the different forms of prostatic carcinoma. In many institutions at the present time the exact histological type of prostate carcinoma and/or the extent to which it is present within the prostatic tissue unfortunately is not determined. The urologist needs to know the extent of the carcinoma as well as the morphological type. These factors can be expressed in different grading systems (1-3). Many pathologists have demonstrated that the degree of cellular grading may also indicate to the urologist that he or she is dealing with a more biologically active form of prostate carcinoma.

DIAGNOSTIC CRITERIA FOR HISTOPATHOLOGICAL EVALUATION OF PROSTATIC TISSUE FOR CANCER

Most pathologists and urologists who examine histological sections see many different patterns of carcinoma of the prostate. These patterns, if unfamiliar, are often dismissed as being non-carcinomatous. This is not only disastrous to the patient, but also allows many false therapeutic interpretations to be perpetuated. Many pathologists and urologists are now aware of the time-proven pattern recognitions of prostate carcinoma (1,4). At the time of surgery or autopsy, these patterns are seen in the histological sections of the prostate gland. In many instances it may be extremely difficult to define the grade or neoplastic potential of the prostate tissue because of the large numbers of patterns displayed even within one microscopic field in a TUR specimen. Also, the diagnosis is further complicated if the TUR material is severely traumatized, i.e., either crushed or cauterized. The patterns seen may include carcinoma-in-situ of the small or large glandular type, Indian file and a cribriform pattern. Recently three other forms of prostatic carcinoma have been clinically recognized, the periurethral ductal transitional cell tumors, endometrioid tumors and carcinoid tumors of the prostate gland (5-7). They are now being defined by their biological activity and by immunological determinations for acid phosphatase.

Numerous atlases and texts present in detail the various patterns and histological types seen within most carcinomas of the prostate (1,4). For the urologist or prostatic specialist evaluating a biopsy or tissue section taken from a surgically removed prostate, the following histological structural changes are indica-

tive of prostatic malignancy: (a) prostatic acini are back-to-back, (b) the cells lining the acini are often in a single layer. The basal layer is usually not present, and if present, there is a great tendency on the part of many pathologists to consider these glands as non-neoplastic, (c) large prominent eosinophilic nucleoli are usually present in carcinomatous cells. To observe these nucleoli the tissue sections must be quickly and properly fixed either in formalin or in Bouin's solution, (d) prostatic acini are seen in a linear infiltrate entering the surrounding fibromuscular tissue. This streaming pattern resembles rowboats racing up and down a river, (e) nuclear hyperchromatism may or may not be seen depending on the quality of tissue fixation. For this reason, we do not believe that nuclear hyperchromatism can be evaluated from laboratory to laboratory unless there is uniform fixation. Cautery artifact is often seen in the TUR specimen and quite often may lead to misdiagnosis when hyperchromatism is entertained as a diagnostic feature, (f) perineural invasion may or may not be discernible. When detected, especially in a frozen section, it is usually indicative of the malignant potential of a well-differentiated prostatic glandular pattern. All of these patterns may vary from one microscopic field to another. The tumor may also be seen as a solid mass resembling a granuloma. Fortunately, the Indian file and the signet ring patterns are usually not the predominant cell pattern (4). These two patterns of prostatic carcinoma are easily obscured by too much electrical cutting current within the resectoscope and are obliterated by cautery artifact. Extreme caution must also be used because some of the features mentioned above may be present and still the diagnosis may be that of benign disease (4). On the other hand, in many instances, not all the criteria need to be present for the diagnosis of malignancy.

Microacini are often present in the posterior lobe of the prostate and yet the gland is benign. These microacini are also frequently associated with prostatitis. When carefully examined, many of these microacini are not back-to-back and under the high-power objective in the microscope, they are usually seen to be lined by a double layer of cells (4).

In many instances, where there is adequate tissue sampling as described previously, the periacinar ducts of the prostate as well as the periurethral ducts may be filled with bizarre cells that are very active mitotically and yet, in many institutions, when they are seen in a TUR specimen, they are signed out as carcinomas of the prostate. This lesion which is of a transitional cell nature in many cases is often misdiagnosed and treated as a regular carcinoma of the prostate. The periurethral ducts as well as the glands around an area of infarction may show extensive squamous cell metaplasia and nuclei with prominent nucleoli. When care is not taken, this is often misdiagnosed as a malignancy (4). Many times diethylstilbestrol may induce squamous metaplasia as well as obscure

the carcinomatous cells in the fibromuscular tissue sections (4). Thus great care must be taken as a consortium of many factors influence the diagnosis of carcinoma of the prostate.

A group of bizarre cells with pleomorphic nuclei will most likely be seminal vesicular epithelium. The pathologist should look for the brownish refractile cytoplasmic pigment that is characteristic of seminal vesicular epithelial cells (4).

The mechanical distortion or compression of nuclei that makes them appear hyperchromatic, is the one consistent problem that most pathologists see in evaluating prostatic cancer in needle biopsies, especially in those patients who have elevations of acid phosphatase (4). This effect will usually confuse the diagnosis in either direction, i.e., malignancy or benignity. If there is a question as to diagnosis, the surgical pathologist should request additional tissue levels from the biopsy or ask for another biopsy.

PROSTATIC CARCINOMA-IN-SITU

The term "carcinoma-in-situ" denotes an incidental carcinoma with an intraacinar neoplasm. We tend to favor this term of carcinoma-in-situ although others do not (1). Usually there is no apparent light microscopic invasion of the surrounding fibromuscular tissue. However, when these microscopic foci of carcinoma-in-situ are seen, the surgical pathologist should do extensive sectioning of the remaining tissue that might be left over from the suprapubic or retropubic prostatectomy. When serial sections are taken, the incidence of prostate carcinoma increases from 14 to 34%. Therefore, it is important that all of the prostatic tissue be embedded, cut properly and each section examined. If this examination is carefully executed then at least 24% of the tissue so examined will reveal different patterns of carcinoma. In many instances, there is only carcinoma-in-situ and no other form of prostate cancer. In these cases, the surgical pathologist should indicate in the diagnosis that there is a carcinoma-in-situ present only in one chip out of whatever number of chips were placed on the microscope slide.

TRANSITIONAL CELL CARCINOMA OF THE PROSTATE GLAND

Until very recently, periurethral ductal or transitional cell carcinomas of the prostate were seldom recognized or categorized as a special classification in the pathology section of the Urology boards. However, this classification is justified since these types of prostatic carcinomas are much more biologically active than the regular acinar forms. The initial reports in the literature stated that out of 200 consecutive carcinomas of the prostate, seven arose in the periurethral prostatic duct (6). The percentage at our institution is almost double because of our awareness of the lesion and the numerous histological levels that are taken. In

the original report, these lesions were not associated with chemical elevations of serum acid phosphatase (4). Although the data were scant at that time, these lesions did not appear to have an innocuous course. Ende et al (6) noted that the lesions paradoxically extended into the retroperitoneal lymph nodes with subsequent obstructive uropathy and ensuing uremia. In numerous cases, they did not find any associated urothelial neoplasm elsewhere in the urinary tract.

ENDOMETRIAL TUMORS OF THE PROSTATE GLAND

Endometrial neoplasms of the prostate arise not only in the verumontanum of the prostate but also in the periurethral prostatic ducts. Periurethral lesions may also be of an adenocarcinomatous variety and as such may be papillary in form and spread intraductally as some carcinomas of the breast do. This is another variety which is the subject of much controversy (1, 4, 7). In many instances, it can arise from the prostatic utricle which is the colliculus seminalis and is derived from the müllerian duct. These tumors and those that originate in the periurethral prostatic ducts may demonstrate a pattern that is very similar histologically to the endometrium of the uterus. When they arise from the müllerian duct, the histology is like that of the endometrium and is indicative of a non-metastasing neoplasm. In the majority of these types of tumors, there is an associated carcinoma of the prostate which is contiguous but not continuous with the endometrial tumors. There was no metastasizing potential with the exception of two out of 100 cases of endometrial tumors seen at this institution. When they do arise in the periurethral prostatic ducts and not in the prostatic utricle, they may have an endometrioid pattern in conjunction with a regular transitional carcinomatous pattern. In these malignant lesions, there is usually a wide variation in patterns in the papillary portions of the periurethral prostatic ducts which may be clinically exophytic into the prostatic urethra. In such cases, these lesions may also demonstrate, in some focal areas, a regular adenocarcinomatous pattern of the cribriform or transitional cell type as well as an endometrioid pattern. In these lesions, it is not uncommon to find an associated elevation of serum acid phosphatase as determined by immunological methods.

MIXED ADENOCARCINOMA-CARCINOID TUMORS (THE ENDOCRINE COMPONENT OF PROSTATIC CARCINOMAS)

In normal and hyperplastic prostatic epithelium, the occurrences of argyrophil and argentaffin cells have been long recognized. Recently Capella et al. have published an extensive report dealing both with the ultrastructure and histochemical identification of these endocrine cells in prostatic carcinomas (5). They have identified two different types of endocrine paracrine cells in normal and hyperplastic prostate cells. The type 1 cells resemble

enterochromaffin cells (EC) and the type 2 cells are similar to urethral endocrine-paracrine cells previously reported by Casanova et al (8). Capella et al (5) noted that about one third of the 40 prostatic carcinomas contained EP cells and four tumors showed a very extensive number of these cells. Two of the tumors were composite tumors exhibiting both adenocarcinomatous and carcinoid patterns. The tumors were studied ultrastructurally and histochemically and the presence of ACTH and beta-endorphin immunoreactive cells ultrastructurally resembling pituitary corticotrophic cells were noted.

SUMMARY

With the advent of immunohistochemical light histology and ultrastructural techniques applied to the prostate, the future may reveal that there are certain tumors that are immunoreactive for certain chemical constituents like acid phosphatase and other substances such as beta-ACTH. At the present time, there have been no histological pattern and grade correlations with these immunological histochemical and ultrastructural findings. If these correlations are forthcoming along with a better understanding of tumor biology, then the future seems very promising.

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CYTOLOGY OF THE PROSTATE

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As early as 1930 Ferguson described a method of needle puncture and aspiration of the prostate for diagnostic purposes (1). From his work mainly originated the so-called thick needle biopsy for which several instruments were designed. The best known are the Vim-Silverman needle, the Veenema needle and that most commonly used now, the Tru-cut needle. All give punch-biopsies which can be used for histological examination as well as for histochemical reactions (acid phosphatase, peroxidase, etc.). For several decades this was the recognized method for pathological diagnosis of prostatic carcinoma, the diagnosis being based mainly on the structure and architecture of the malignant epithelial cells in relation to the stroma and on some cytologic criteria.

However there were a number of disadvantages. The puncture, whether transperineal or transrectal, is usually painful and cannot be done without some kind of anaesthesia. Local anaesthesia is sufficient in most cases but there are urologists who demand general anaesthesia, which in turn carries a certain risk factor. Also one needle puncture could accidentally miss the cancer nodule and as pathologists sometimes have great difficulty in making a diagnosis on a small piece of tissue, they often require more than one puncture biopsy, preferably from different sites which of course makes it more disagreeable for the patient. Finally the procedure is not convenient for follow up histological observations of a certain treatment.

It is not surprising therefore, that aspiration biopsy cytology as it was first presented by Franzén et al in 1960 (2) has attracted more and more attention in the last 10 years. The method is simple and can be done on a routine out-patient basis. The instrument was

designed by Franzén. It consists of a syringe with a special holder to apply a vacuum with one hand whilst a thin needle, in which the cells are aspirated is guided by a needle guide that fits the index finger of the other hand, transrectally, to the cancer nodule in the prostate. The needle can be directed very accurately to the right place, i.e. to the nodule and to any other part of the prostate from which it is felt necessary to get material from different sites of a nodule or of the prostate. The patient feels no more than a little unpleasantness and anesthesia is not required.

By applying a vacuum and making back and forth movements, the epithelial cells and some prostatic fluid with erthrocytes are drawn into the needle. Stromal cells are never aspirated. Care must be taken to equalize the pressure before withdrawing the needle to prevent the aspiration of rectal contents and epithelium. It is wise to use a sterilized instrument to prevent the introduction of hospital bacteria into the patient. By a careful technique and by avoiding the use of the Franzén needle for diagnosing prostatitis the risk of infection for the patient is extremely small as is the risk of transplanting cancer cells. Infections and transplant metastases have been reported in thick needle biopsies, but in large series of aspiration cytology they are virtually absent (3).

The cellular material is expressed from the needle onto glass slides and smears are made of it. They can be fixed by air-drying and then stained using the M.G. Giemsa technique which is used universally and gives satisfactory results. However some cytologists prefer the Papanicolaou-stain.

The cytologic patterns of normal, hyperplastic and carcinomatous epithelium are described in detail by Esposti (4) and Staehler (5). They stated that grading according to cytological criteria is more accurate than can be done on tissue-sections. The main criteria for malignancy are:

1. decreasing cellular adhesiveness.
2. decreasing cytoplasmic/nuclear ratio.
3. nuclear atypia and prominent nucleoli.

As we will hear more about grading in other lectures of this course the subject will not be pursued here. However the question remains whether cytology and histology are adequate for diagnosis. To that end in Leiden in 1979 the results of histology and cytology of 294 patients were reviewed and compared. They are given in Table 1. Our conclusion from this study was that on a routine basis histology and cytology are nearly aequal, but from the academic standpoint the two are complementary.

At the time of initial diagnosis both methods are desirable to get a complete picture and give a reliable grading and staging

Table 1. Comparison of Diagnoses by Histology (Needle Biopsy) and by Cytology (Thin Needle Aspirates) of 294 Patients, Suspected of Prostate Cancer

	Histology				
Cytology	negative	positive	dysplasia	not enough material	number of patients
Negative	125	3	-	-	128
Positive	6	140	8	-	154
Not enough material	-	-	-	12	12
Total	131	143	8	12	294

In 10% of cases disagreement

4% by not enough or no proper material

3% by dysplasia

3% by false-negative, c.q. false positive

(6,7,8). However for follow-up purposes we should resort to and confine ourselves to cytology, as it has the advantage of being tolerated well by the patient and of indicating whether a tumour is responding to a given treatment or not.

Finally the cellular material, obtained by aspiration, can be used for different kinds of sophisticated research, such as cytomorphometry and karyometry, flow-cytometry and cell-sorting. In these methods cells are stained with one or more fluorescent stains, which can then be measured by fluorescence microscopy. Also other methods of cytochemistry can be applied of which the method of using fluorescent steroids (fluoresceine-steroid conjugates) to locate steroid receptors is very interesting. Last but not least cell-kinetic techniques can be employed. Some of these methods will be considered further in this course.

In conclusion, it is my opinion that cytology of the prostate will become more and more important, because of the:

1. Simplicity of the procedure, which is well tolerated by the patient.

2. Diagnostic reliability and possibility of accurate grading of prostatic cancer.
3. Usefulness for follow-up evaluation of different forms of therapy, and
4. Possibilities for more sophisticated research.

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THE ESTIMATION OF DNA BY ABSORPTION CYTOPHOTOMETRY ON SMEARS OF TRANS-RECTAL FINE NEEDLE ASPIRATION OF THE PROSTATE

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INTRODUCTION

Mitosis, the predominant step of cell multiplication, occurs when a cell has doubled its metabolic and genetic contents.

Among the synthesis mechanisms preceding mitosis, the synthesis of DNA, a major component of the chromosomes, plays an important role.

Normal human tissue, not actively dividing, consists predominantly of cells in the pre-synthesis phase or "resting"-phase (G1) with diploid chromosomes (2 c = 46) and a corresponding content of DNA (about seven picograms in human cells). During the phase of synthesis (S), the DNA content increases up to a tetraploid value corresponding to a double set of chromosomes (4c). When the cell has reached this tetraploid value, it does not divide immediately: there is a post-synthesis phase (G2) during which other protein formation occurs.

During mitosis (M), the DNA content is equally distributed between both daughter cells and returns to diploid values, and the cell-cycle continues (1,2).

Fig. 1. shows that, in normal tissues, most of the cells are in G1 and have a diploid content of DNA. Few cells are in S-phase or G2 with an intermediate or a tetraploid value of DNA.

In actively growing cancer tissues the percentage of cells in the S- and G2-phase is increased and, due to pathologic division of the nuclei, heteroploid or aneuploid cells can be encountered.

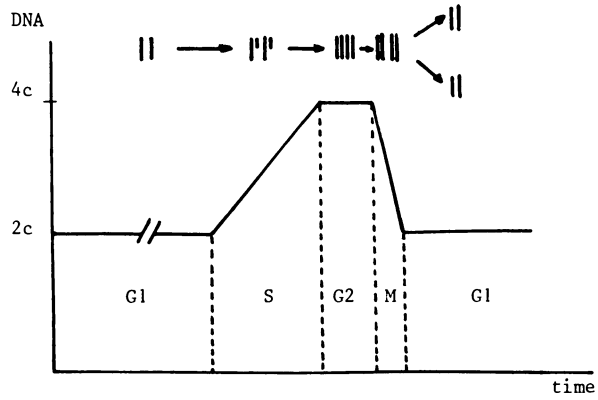

Fig. 1. Schematic representation of the modifications in DNA content during the cell cycle.

Therefore, the number of cells with intermediate DNA content (between 2 c and 4 c) increases (aneuploid cells or cells in S-phase) and the number of cells with tetraploid DNA content also increases (basal tetraploid cells or diploid cells in phase G2) and hexa- or octoploid cells (e.g. tetraploid cells in phase G2) or even hexadecaploid cells can be encountered (3-7).

Generally speaking, the lesser differentiated the cancer the more aggressive it is; the percentage of non diploid cells is increased accordingly. Therefore, the measurement of DNA is of value in the evaluation of the initial aggressiveness of a tumor and in the study of its response to treatment.

MATERIAL AND METHODS

Material provided by trans-rectal fine-needle aspiration of the prostate was divided into two slides to allow a comparison. One of the slides was air-dried and stained with May-Gruenwald-Giemsa, while the other was fixed in an equal volume of acetone and absolute ethanol at $4^{\circ}C$ for 24 hours. Thereafter, the slide was plunged into absolute alcohol at $4^{\circ}C$ for 24 hours minimum; after drying, it was stored in darkness until staining with Feulgen reaction (8,9). The measurement of DNA in the individual cells of the smear was performed on a Vickers M86 Scanning Microdensitometer. Completely automated, this apparatus determines the DNA content of the examined nucleus in five seconds.

The value is expressed in abitrary units, but the presence of segmented white blood cells, which are always diploid, in the smear allows an easy conversion of these arbitrary units. At the same time, the densitometer provides a measure of the area of the nucleus. DNA histograms were performed in each case, on a minimum of 100 nuclei.

RESULTS

We have analyzed:

1. The DNA histograms in adenomas, prostatitis and adenocarcinomas of the prostate.
2. The relation, in prostate adenocarcinomas, between the cytological grade and the corresponding histogram.
3. The changes occurring in the histograms following the treatment.
4. The practical significance of DNA cytophotometry for the diagnosis, grading, and prognosis of prostatic cancer.

DNA Histograms in Adenomas, Prostatitis and Untreated Carcinomas

Adenomas. Sixteen cases of benign prostatic hypertrophy were examined. The histograms always show an obvious peak corresponding to the diploid value of DNA. Less than 5% of the nuclei have aneuploid values deviating more than 20% from the diploid value (Figs. 2 and 3).

This finding means that in prostatic adenoma, very few cells are dividing and that the disease is more likely a dysplasic phenomenon than a true benign tumor in which an increase of the tumor mass should be correlated with an increased mitotic activity.

Prostatitis. Chronic prostatitis was investigated in 10 patients. In some cases, the histograms are the same as in the adenomas, exhibiting a great majority of cells with diploid values of DNA (Fig.4). In others however, the DNA content spreads beyond the diploid value with a few nuclei exhibiting tetraploid values (Fig. 5). This appearance is identical to that observed in well-differentiated carcinoma and results from an increased mitotic activity in an attempt to restore the epithelium destroyed by the microbial infection. In this situation, the DNA histogram cannot provide a differential diagnosis between well differentiated carcinoma and prostatitis but it can help when the histogram returns to normal after a few weeks of treatment with antibiotics (Fig. 6).

The following case illustrates the situation:

M.B. is 76 years old. Rectal examination reveals a limited induration of the prostate. Cytology is performed and cannot exclude a well differentiated cancer although the presence of

numerous segmented neutrophils and lymphocytes suggests a prostatitis.

The DNA histogram shows many aneuploid nuclei close to the tetraploid value (Fig. 5) but after six weeks of treatment with tetracyclines, a further DNA histogram shows uniformly diploid values (Fig. 6) and virtually excludes a diagnosis of carcinoma.

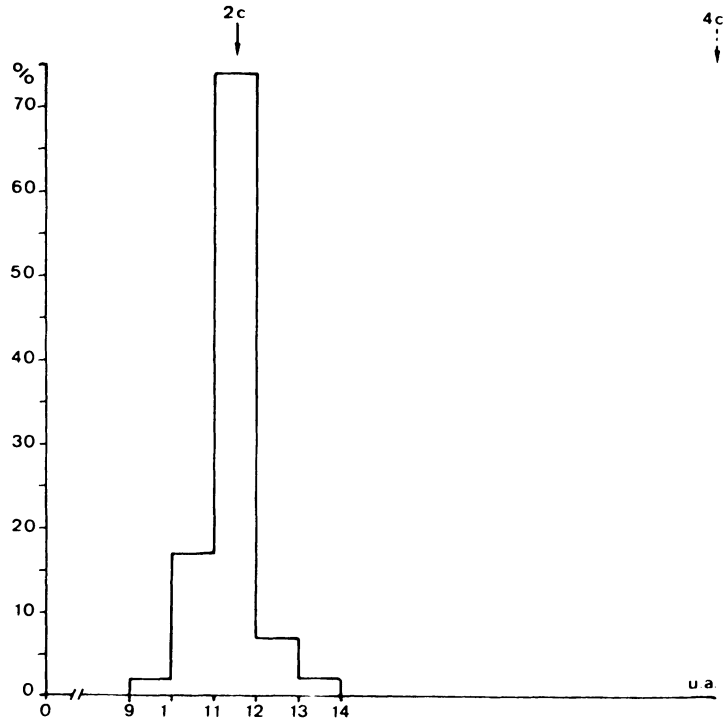

Fig. 2. DNA content in a smear of epithelial cells from prostatic adenoma, obtained by transrectal aspiration, 102 nuclei were successively studied by absorption cytophotometry after Feulgen reaction.
Abscissa, the value in DNA expressed in arbitrary units (u.a.).
Ordinate, the percentage of nuclei.
The full line above the histogram indicates the mean diploid value calculated (2 c = 2 DNA). The broken line indicates the mean tetraploid value (4 c) obtained by multiplication of the diploid value by two.

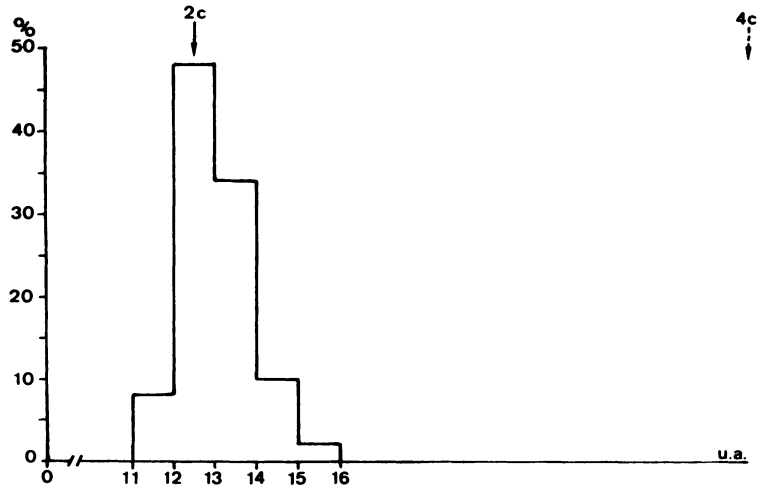

Fig. 3. DNA content in a smear of epithelial cells from a prostatic adenoma (n = 100) For details, cf legend Fig. 2)

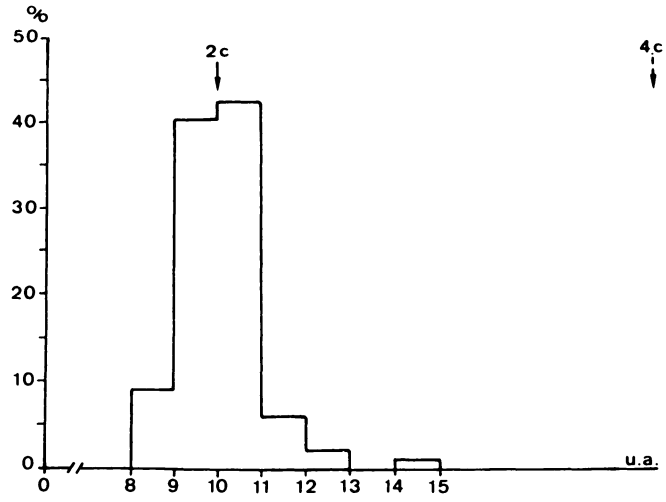

Fig. 4. DNA content in a smear of epithelial cells in a case of chronic prostatitis (n = 102) For details, cf legend Fig.2).

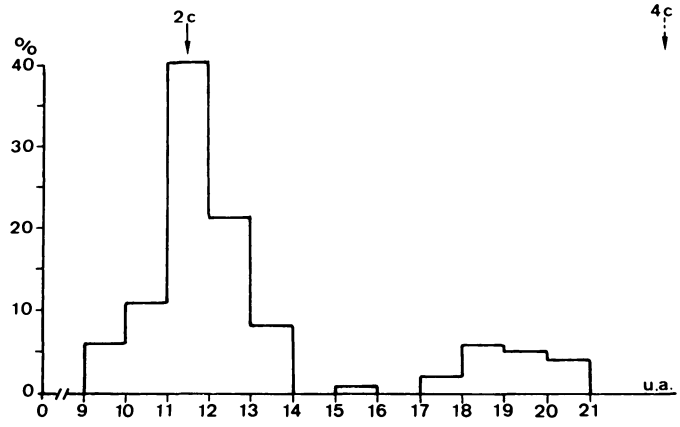

Fig. 5. DNA content in a smear of prostatic aspiration in a patient with an untreated chronic prostatitis (n = 104) For details, cf legend Fig. 2).

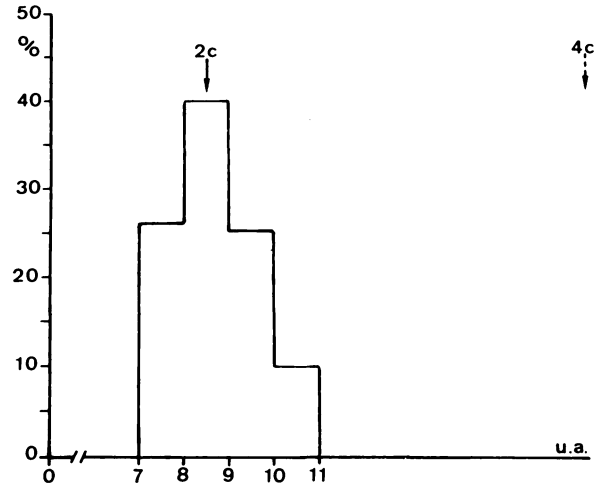

Fig. 6. DNA content in the smear of prostatic aspiration in the same patient (cf Fig. 5) after antibiotic therapy (n = 102) For details, cf legend Fig. 2.

Carcinomas. Untreated carcinomas were investigated in 32 patients. The DNA histogram allows a classification into three categories:

Category 1 - cancers in which at least 80% of the cells display diploid values of DNA (Fig. 7).

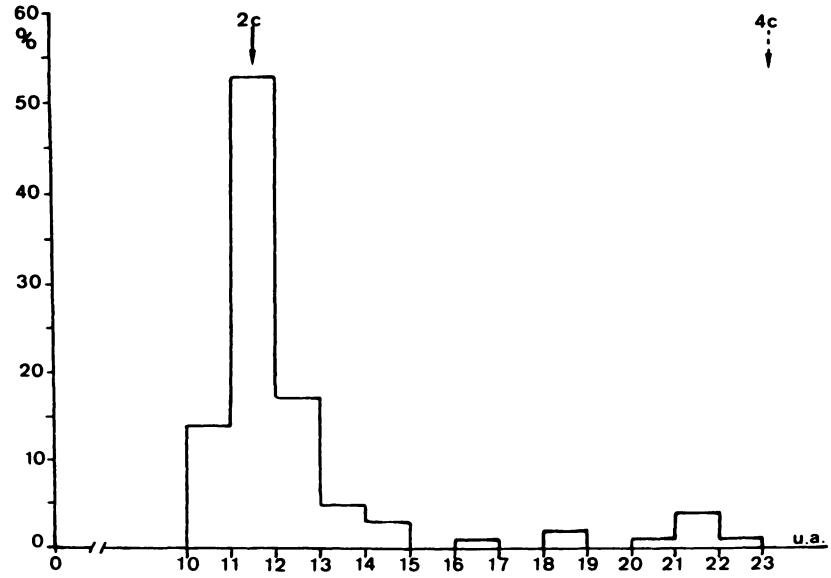

Fig. 7. DNA-histogram in a smear of prostatic aspiration performed in a patient with a well differentiated prostatic cancer (grade 1), stage T2 NX MO (n = 11). For details, cf legend Fig. 2.

Category 2 - cancers in which less than 80% but more than 50% of the nuclei display diploid values of DNA (Fig. 8).

Category 3 - cancers in which less than 50% of the nuclei show diploid values of DNA. Many cells are aneuploid or tetraploid(Fig. 9).

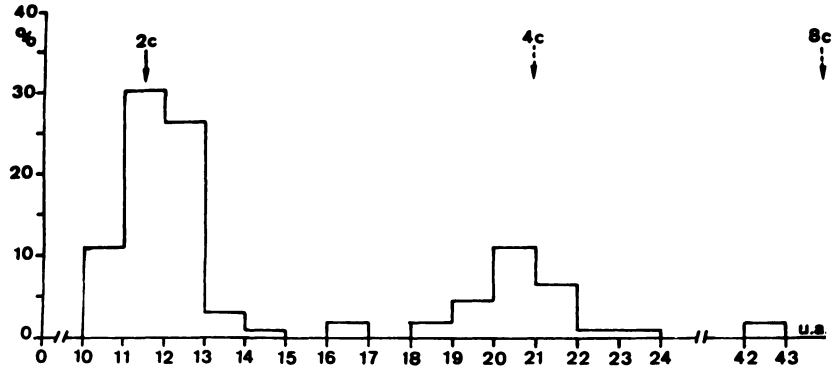

Fig. 8. DNA-histogram in cells of a prostatic aspiration performed in a patient with moderately differentiated prostatic cancer (grade 2), stage T3NXM0 (n = 110).
For details, cf legend Fig. 2.

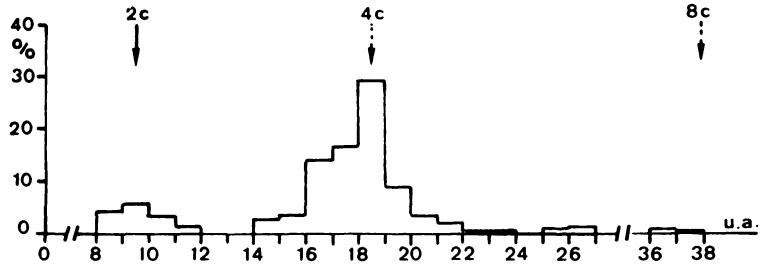

Fig. 9. DNA-histogram in a smear of prostatic aspiration performed in a patient with a poorly differentiated prostatic cancer (grade 3) T3NXM1 (n = 184).
For details, cf legend Fig. 2.

Relation Between the Cytological Grade and the DNA Histogram

In the 32 cases of untreated carcinomas we have compared the cytological grade and the histogram category, the aspirate having been distributed on two slides, one for cytological examination and one for DNA cytophotometry. The results are expressed in Table 1.

In 21 of the 32 cases (66%), the cytological grade and the histogram category are in agreement. Also none of the grade 1 cancer enters the category 3 of ploidy and none of the poorly differentiated cancers enters the category 1 of ploidy. It appears therefore that cytology is a good parameter for the study of tumor aggression.

One must particularly notice that among cytological grade 2 cancers, the DNA histograms allow stratification which distinguishes actively, moderately and poorly dividing cancers. Zetterberg and Esposti (10) have already shown an excellent correlation between ploidy, therapeutic response and survival.

When studying the prognosis of prostatic cancer in correlation with the histological or the cytological grade (11), correlation is excellent for poorly or well differentiated carcinomas but is less evident for the moderately differentiated tumors. The use of cytophotometric dosage of DNA, which distinguishes, in this group, cancers with different evolutive patterns, adds an important prognostic refinement.

Table 1: Relationship between cytological grade and DNA-histogram on 32 patients with untreated prostatic cancer

Cytological grade	Well diffd.	Moderately diffd.	Poorly diffd.	Total
	G1	G2	G3	
DNA histogram				
Category 1	5	7	-	12
Category 2	1	13	1	15
Category 3	-	2	3	5
Total	6	22	4	32

Changes Occurring in DNA Histograms during Treatment

In 24 patients with a prostatic carcinoma we have carried out cytophotometric studies during treatment. In 12 other patients we have obtained DNA histograms before and during treatment.

In 19 of the 24 patients examined during treatment, DNA histograms displayed normal patterns, all the nuclei showing DNA values ranging around the diploid value. In all these patients, the clinical response to treatment was very good.

In five of the 24 patients, histograms showed marked aneuploidy and polyploidy. In three of them, clinical signs of progression were obvious. In two, the clinical response seemed to be good at the time of examination but one progressed three months later.

In the 12 patients in whom we performed DNA-histograms at the time of diagnosis and during the treatment, an objective clinical response was observed in eight. Comparison of DNA-histograms before and during treatment shows a striking reduction of poly- and aneuploidy during therapy (Figs. 10 and 11).

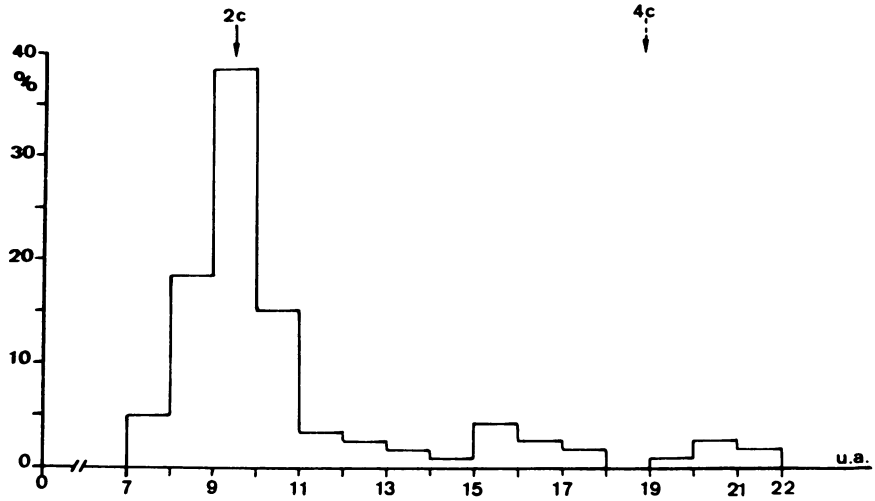

Fig. 10. DNA-histogram is a smear of prostatic aspiration performed in a patient with a moderately differentiated prostatic cancer (G2), stage T2N+M0, before treatment (n = 118). For details, cf legend of Fig. 2.

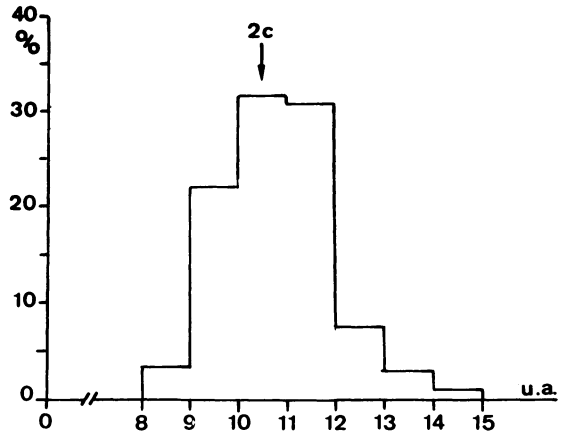

Fig. 11. DNA-histogram in the same patient (n = 108), six months after orchiectomy. One notices a normalisation of the histogram; the clinical response is excellent.

In three patients, the DNA-histogram showed a partial reduction of aneuploidy and polyploidy . Although the immediate clinical evolution was good in two patients, progression occurred within six months. In the third patient in this group, who did not exhibit response to the estrogenic treatment, the DNA histogram remained unchanged, revealing an important percentage of non diploid cells.

DISCUSSION

There have been a few reports of measurement of DNA in prostatic cancer (10,12,13,14,15). Our results are in agreement with them and indicate that the use of absorption cytophotometry for the measurement of DNA in prostatic cancer cells yields information which is of value in the classification and the prognosis of the disease.

Modifications of the DNA-histograms during treatment constitute, in our experience, one of the earliest and the most reliable signs of objective response to the treatment: a normalization of the histogram is always a sign of a good therapeutic response while the persistence or the reappearance of abnormalities in the DNA histogram is proof of an incomplete response or a premonitory sign of impending progression which invites a change of the treatment. Kjaer et al (16) and Leistenschneider and Nagel (17) draw the same conclusions but consider that the return of the histogram to the

normal pattern is observed, in cases of favorable response, only after three to six months of treatment. In our experience, this evolution is quicker and significant changes in the histograms are observed after four to six weeks of treatment.

In recent years several authors have also advocated pulse cytophotometry (14,16,18,19,20) which is a form of emission cytophotometry in which the cells, treated with a fluorochromic compound, fluoresce when stimulated by ultra violet light. These cells are aspirated in a flux which is analyzed through the lens of a photometric microscope. The results are directly expressed by computer on a histogram. The advantage of this completely automated technique is its rapidity; thousands of nuclei can be analyzed in a few minutes. However the method also has the disadvantage in that all nuclei (cancer cells, normal cells, white blood cells ...) are analyzed without any distinction, aggregates or fragments of cells can alter the results, and lastly, this technique gives no information on the morphological aspect of the cells. On the other hand, our technique of absorption-cytophotometry on single cells after Feulgen staining gives important morphological information.

CONCLUSION

The measurement of DNA by microdensitometry is a technique providing important information for the diagnosis, classification and prognosis of prostatic cancer. It is a relatively simple and quick technique, which is easily performed in centers equipped with scanning microdensitometers. It has the advantage over pulse cytophotometry of providing morphological information.

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ASPIRATION BIOPSY CYTOLOGY IN THE MANAGEMENT OF PROSTATIC CARCINOMA AND IN MONITORING RESPONSE TO TREATMENT

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INTRODUCTION

Transrectal fine needle aspiration biopsy of the prostate (1) is a fast, safe and accurate method of identifying malignant disease and also of assessing the grade of malignancy (2). At the Karolinska sjukhuset an annual average of 800 aspiration biopsies of the prostate have been performed for the last twenty years. The fine needle of the Franzén instrument gives minimal trauma and the procedure can be performed repeatedly without causing undue discomfort to the patient. This allows the response to therapy to be monitored by repeated biopsies.

In patients not given active treatment for various reasons, but checked regularly, biopsies have given identical findings over a long period of time in the majority of the cases, indicating that the method is reproducible. In some cases, however, there was an increase of malignancy grade with time; a well-differentiated carcinoma becoming more polymorphous and less well differentiated (Fig. 1). In others, in patients with reduction of the local tumour following therapy, a gradual disappearance of the malignant cell population occurred.

MONITORING OF RESPONSE TO THERAPY

After Radical Surgery

In patients subjected to radical prostatectomy where pelvic nodules were palpable per rectum, aspiration biopsy verified local recurrence of the tumour.

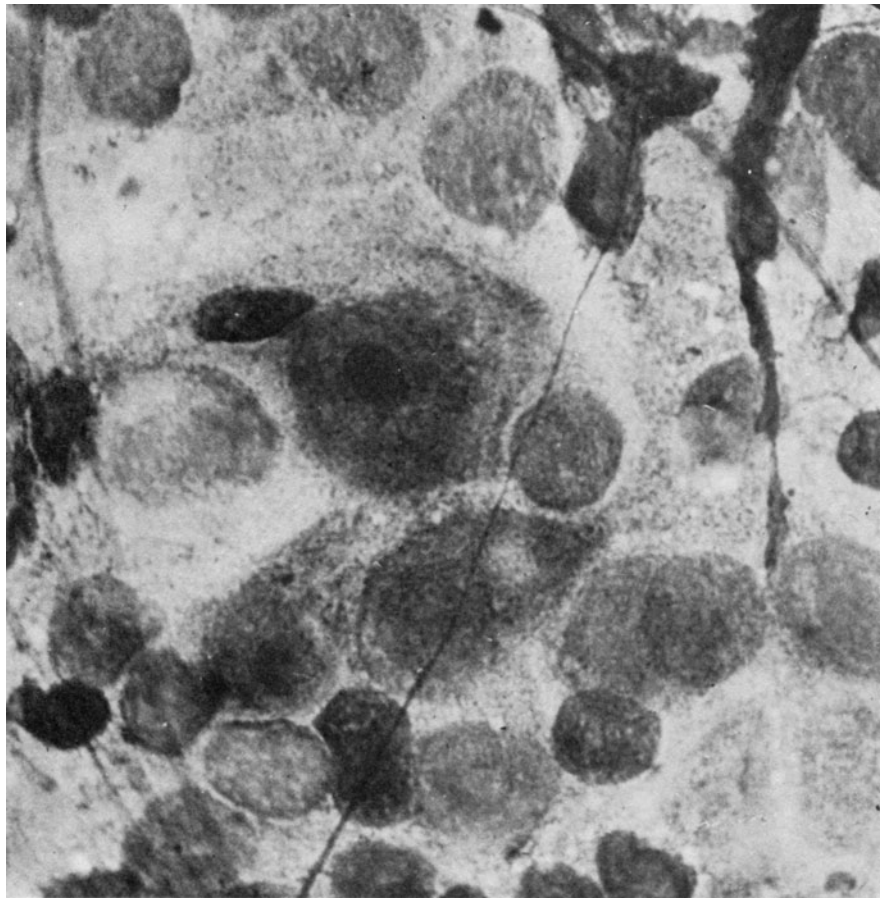

Fig. 1. Poorly differentiated prostatic carcinoma, with polymorphous nuclei and evident nucleoli. Recurrence after estrogen therapy. MGG-stained smear from prostatic aspirate.

Hormone Therapy

The cellular changes in histologic sections of prostate carcinoma following estrogen therapy were first demonstrated by Kahle, Schenken and Burns in 1943 (3). These changes can easily be studied by transrectal aspiration biopsy of the malignant gland. The nuclei become uniformly dark and lose their chromatin details. Large squamous cells with pyknotic nuclei appear together with the so-called glycogenic cells (Fig. 2). The latter are probably the most characteristic cells of a prostatic smear after estrogen therapy. They are round or oval, appear single or in small groups and in special staining prove to contain glycogen. They are never seen in the prostatic aspirate before therapy and they disappear if the patient stops his medication.

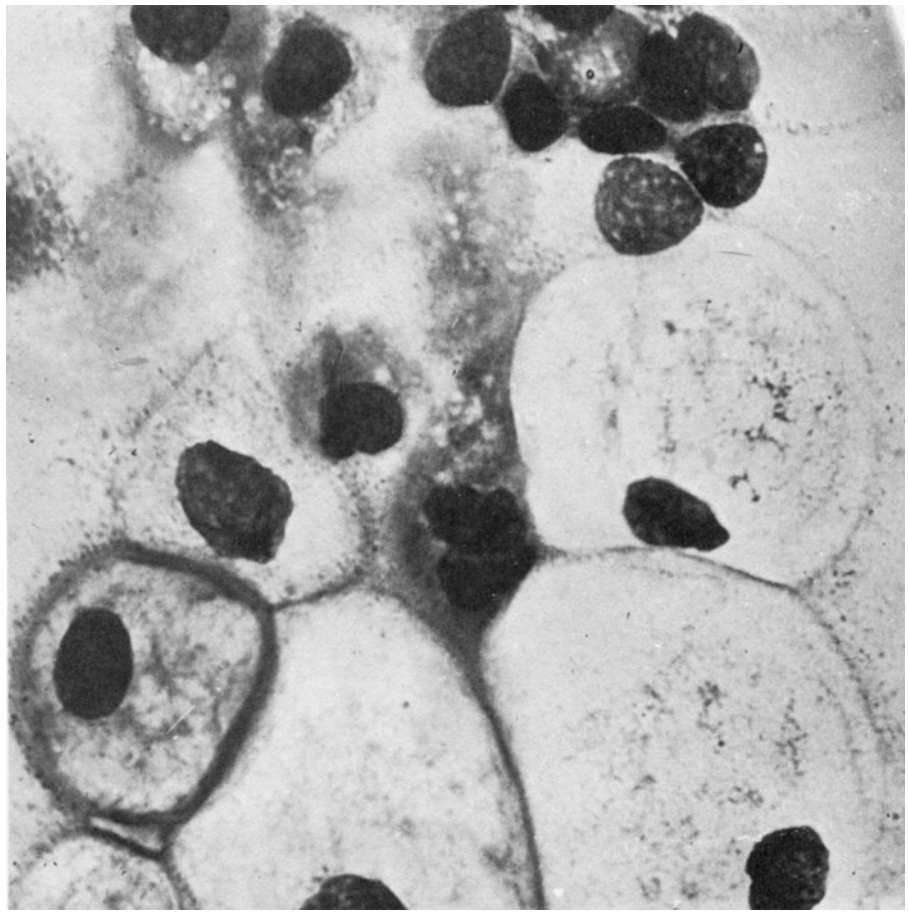

Fig. 2. Cytologic changes in prostatic carcinoma after estrogen therapy: altered carcinoma cells with pyknotic nuclei and glycogenic cells. MGG-stained smear from prostatic aspirate.

In patients in whom long term hormonal therapy is successful it is increasingly more difficult to identify obvious carcinoma cells, while squamous metaplastic and glycogenic cells will dominate in the smear. On the other hand, the response to therapy is classified as poor when the smears on check-up are dominated by unmodified cancer cells.

Radiation Therapy

Morphological changes in the malignant cell population occur slowly after radiotherapy and in our experience a control biopsy of the prostate should not be carried out earlier than four months

after radiotherapy for two reasons - (a) the gland is still oedematous, bleeds easily and there is a danger of complications, and (b) several atypical cells are still present, morphologically resembling carcinoma cells but not viable. In fact they disappear later on. When performing a control biopsy too soon there is a risk of a false positive diagnosis. The ideal time for a check biopsy after radiotherapy is probably between four and six months.

If the therapy has been successful, unmodified carcinoma cells will later on become less numerous while radiation changes become evident: cytoplasmic vacuolization, and markedly enlarged nuclei of

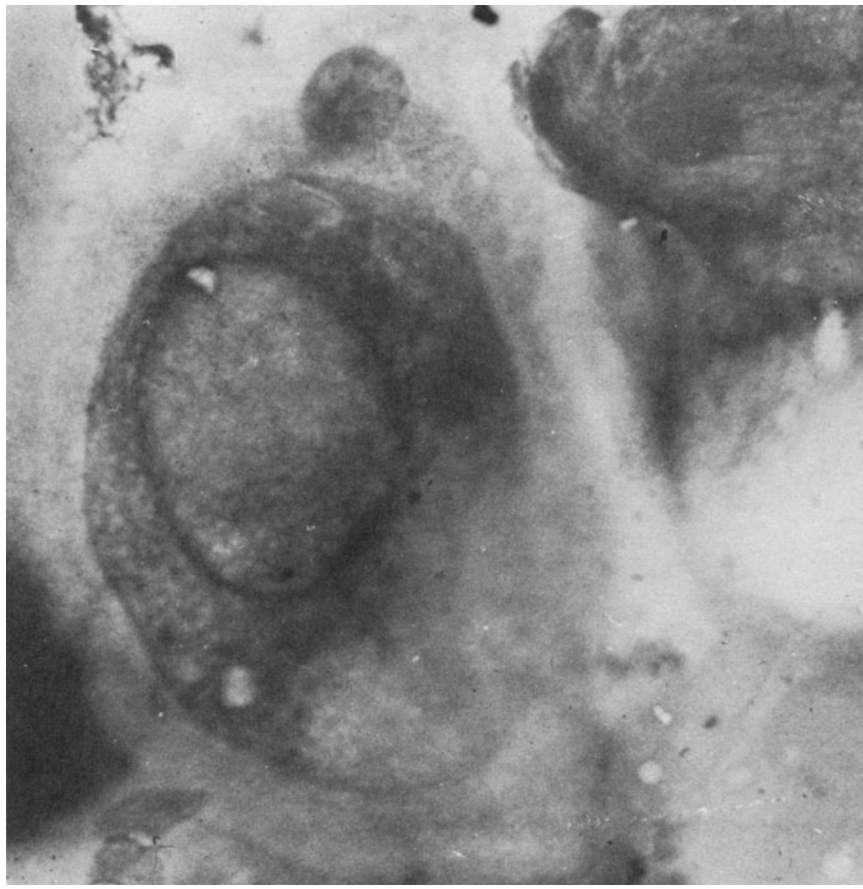

Fig. 3. Radiation changes in prostatic carcinoma cells: enlarged nuclei with vacuolisation. MGG-stained smear from prostatic aspirate.

bizarre shape, exhibiting signs of nuclear damage such as large vacuoles (Fig. 3). The cellularity of the irradiated gland decreases gradually and, with full effect, a control biopsy will yield scant material and no carcinoma cells.

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THE ROLE OF ASPIRATION BIOPSY IN FOLLOWING PATIENTS TREATED WITH CHEMOTHERAPY AND RADIATION

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INTRODUCTION

The value of fine needle aspiration biopsy (FNA) in the diagnosis of prostate cancer has been confirmed repeatedly from numerous studies (1,2). The technique has a high degree of efficiency in that the sensitivity (true positive) and specificity (true negative) correlate well with the standard needle biopsy. The FNA is not recommended as a general screening test for prostate cancer but to help establish the diagnosis in patients whose prostates are suspicious for carcinoma. An additional use of the test may be to help determine response to therapy.

The method has several advantages as a diagnostic study. Anaesthesia is not required, the complication rate is low, the study can be repeated at frequent intervals, and very small areas of the prostate can be examined. As experience with the technique has increased, cytologic grading has improved and can be correlated with over 90% of the histologic specimens (1,3).

Based on cytomorphologic criteria, Esposti and others have described the characteristics associated with tumor grade (3,4). Cytologic aspiration of well differentiated tumors (Grade 1) tends to demonstrate clusters of cells, nuclear polymorphism of a moderate degree and nucleoli which are distinct and not enlarged. The cells are often grouped into an adenomatous complex with the nuclei on the periphery.

Moderately differentiated (Grade 2) lesions show an increase in nuclear polymorphism and less cellular adherence. Poorly differentiated lesions (Grade 3) are characterized by extreme nuclear

polymorphism, enlarged nucleoli and cellular dissociation.

RESULTS

We have evaluated serial FNA biopsies in patients who have been treated with external beam irradiation, implantation of I^{125} seeds, hormonal therapy and combination chemotherapy.

Changes following hormonal therapy (5,6) have been adequately reported and this discussion will be confined to a small series of patients who were treated with irradiation or chemotherapy.

The patients were biopsied prior to the initiation of treatment and at regular intervals (3 - 6 months) depending on the type of treatment. Four to six slides were obtained from each patient. The slides were labelled according to the site of the aspiration and compared to previous biopsies.

RADIATION THERAPY

There have been 39 patients with localised prostate cancer treated with some form of irradiation. Twelve received external beam irradiation 6500r and 27 patients were treated with interstitial irradiation using I^{125}. The dose varied between 28,000 and 42,000r. These patients were biopsied every six months following completion of their therapy. Grade 1 tumor, as determined by a combination of FNA and histologic biopsy, was the predominant grade in 18 patients, Grade II in 14 patients and Grade III in 7 patients.

The cytologic effect of irradiation was the same in patients receiving external beam or interstitial irradiation. The predominant effects characteristic of radiation change are the 'smudging' or indistinctiveness of cellular fractures and boundaries, pyknotic nuclei and small or absent nucleoli (7).

All twelve patients treated with external beam irradiation demonstrated malignant appearing cells six months following therapy (Table 1). Ten patients (87%) had a definite radiation response with altered cytology. The remaining two patients showed no apparent change from their original biopsy. At 12 months all of the patients had biopsies disclosing abnormal, irradiated cells. Eighteen months following therapy, no malignant or radiation altered cells could be found in five (40%) patients. The remaining patients demonstrated radiation altered cells. There were changes in the predominant cell pattern which were attributed to a beneficial therapeutic response. There were six patients originally considered to have Grade 2 tumors. Following irradiation, three of these patients had a predominant Grade 1 tumor. One of the three patients with a Grade 3 tumor had a change in the predominant cell pattern in Grade 1. After twenty-four months, seven patients (58%) had no evidence of radiation

altered cells or neoplastic cells. Two of the remaining five patients demonstrated neoplastic cells which did not seem to be altered by radiation, while the last three had bizarre cells due to radiation effect. None of the 12 patients in this group has yet shown evidence of metastatic disease.

The group of 27 patients treated with interstitial irradiation were biopsied six months following surgery. The biopsies revealed radiation effect in all the patients (Table 2). At 12 months, 9 patients (35%) had no evidence of carcinoma, 8 patients had only scattered cells showing severe radiation effect, and 10 patients demonstrated numbers of irradiated cells, of whom three also had neoplastic appearing cells which did not have a radiation effect.

At eighteen months, 14 patients showed no evidence of carcinoma in their biopsies, eight patients had neoplastic cells showing radiation effect and five patients demonstrated 'healthy' neoplastic cells in addition to cells with radiation effect.

Twenty-four months following surgery, no tumor could be identified in 18 (66%) patients and nine continued to show neoplastic cells with radiation effect. Five of these patients also demonstrated unaffected cancer cells.

The presence of apparently unaltered cancer cells in addition to the continued presence of irradiated cells has led us to believe that these patients may not have had a good distribution of the I^{125} seeds. None of the patients has received additional therapy and there has been no evidence of recurrence.

Table 1. Results of fine needle aspiration biopsy (FNA) in 12 patients receiving radiotherapy (6500r)

Result of FNA	Months after radiotherapy			
	6	12	18	24
Positive with XR response	10	12	7	3
Positive without XR response	2	-	-	2
No tumor	-	-	5	7
Decrease in grade (8)	-	-	4	2

Table 2. Results of FNA in 27 patients treated with I^{125}

Result of FNA	Months after I^{125} therapy			
	6	12	18	24
Positive with XR response	27	18	8	9
Positive without XR response	0	3	5	5
No tumor	0	9(33%)	14(52%)	18(66%)
Decrease in grade (13)	0	5	2	2

Tumor grade could be evaluated in 13 patients. Twelve months following therapy, an alteration in the predominant cell grade could be seen in five patients and an additional two patients had an alteration in their cell grade at 18 months. These instances of downgrading are not likely to reflect an actual decrease in the grade of the malignancy, but a change in the predominant cell pattern. This change may be due to an absence of higher grade tumor cells or a reflection of sampling.

CHEMOTHERAPY

Fifty-six patients were treated with a combination of cisplatinum (100 mg/m^2), cytoxan (0.8 gm/m^2) and stilphostrol (0.8 gm/m^2) (Table 3). Full dose therapy was given monthly for 2 - 4 months, followed by lesser doses of maintenance therapy at monthly intervals. Forty-two patients had received prior irradiation or hormonal therapy. Fourteen patients had newly diagnosed disease and had received no prior therapy. Grade 1 tumor was present in 21, Grade 2 in 23, and Grade 3 in 12 patients. Serial FNA was performed every three months to evaluate the effect of therapy.

The cytologic changes related to chemotherapy have been difficult to interpret because of the combination of therapies. The major cytologic changes were a change in the predominant tumor grade and an absence of cancer cells on biopsy. Hormonal changes were noted in some cells. Although the cytologic morphology appears to be altered by chemotherapy in the majority of patients, we have not been able to identify uniform or specific changes. Many patients showed no change in their cellular morphology.

The alterations in the tumor located within the prostate did not always correlate with those in tumor located elsewhere. In some instances there was a good response within the prostate as indicated

Table 3. Chemotherapy (56 patients)
DDP - CTX - Stilphostrol

Months	Patients	Morphological Change	Grade	No Tumor
3	56	31(55%)	9(16%)	0
6	48	35	18	4
12	37	22(59%)	18(48%)	8(21%)
18	25	16	15	7
24	16	11(68%)	11(68%)	5(31%)

by a reduction in prostate size, a softening of the gland, and a decrease in tumor grade or an absence of tumor whilst tumor in the bone or other locations was progressing. If we assume that prostate cancer is a polyclonal tumor, the therapy may be effective in eradicating some clones but ineffective in others.

A morphologic change attributed to the effects of chemotherapy was seen in 55% of patients at three months, 59% at 12 months and 68% at 24 months.

The predominant cell pattern had a decreased tumor grade in 16% of patients at three months, 48% at 12 months and 68% at 24 months. No tumor was seen on biopsy in 21% of patients at 21 months and 31% at 24 months. This table does not reflect patient survival but only changes in the results of FNA. The patients are heterogeneous - some had prior therapy and others had not been treated previously.

DISCUSSION

The value of FNA in monitoring the results of therapy is dependent upon the use of the data and the implication of residual cellular material in the prostate. If the presence of altered cells in an irradiated prostate has no clinical meaning, there is no need to perform biopsies. The presence of such cells does not necessarily imply that they are active or will contribute to metastatic disease. However, if unaltered cancer cells are identified two years following radiation, consideration could be given to the institution of additional therapy. A decrease in the predominant cell grade may suggest only a partial benefit from therapy.

FNA in patients receiving chemotherapy may be considered in the same manner. A variety of techniques are available to assess thera-

peutic response. An absence of tumor cells or a decrease in the predominant cell grade may reflect a benefit of treatment particularly if this corresponds to changes in other parameters.

There is not enough data to determine the specificity, sensitivity and efficiency of FNA as a sole diagnostic aid. Therefore caution must be exercised before one uses the results of FNA in making therapeutic decisions. At the present time the use of FNA should be as an adjunct in determining response to therapy.

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Editorial Note (M.P.M.)

Stilphostrol is a trade name for phospho-oestrol or stilboestrol diphosphate (honvan in most european countries), whereas cytoxan (endoxan in Europe) corresponds to cyclophosphamide. It is a pity that this study does not correlate the cytological response with the clinical response or the survival rate.

EPITHELIUM AND STROMA IN PROSTATIC CANCER AND HYPERPLASIA

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The normal morphogenesis and cytodifferentiation of rodent prostatic epithelium is strongly dependent on the continuous association with mesenchymal cells of proper (i.e. urogenital) origin (1,2). The inductive capacity of urogenital stroma has been tested on different epithelia, and it was found that, in the presence of androgens, integumental epithelium was transformed by urogenital stroma into a glandular epithelium characteristic of the source of the stroma (1,3). Moreover, adult bladder epithelium could be induced to form prostate-like acini by embryonic stroma of urogenital origin (3). From these observations McNeal (4) hypothesized that the formation of prostatic acini during the development of benign prostatic hyperplasia may be the result of a re-expression of embryonic inductive capacity. Cunha et al. (5) summarized the pre-requisites for prostatic development to be i) the presence of androgens; ii) the presence of "inductive" stroma and iii) the ability of the epithelium to respond to the inductive influences.

According to the generally accepted mechanism of steroid hormone action, androgens exert their effect(s) on target tissues through specific receptors present in the cytoplasm, which translocate to the nucleus after binding the androgen. In the nucleus, mRNA and protein synthesis are stimulated, leading ultimately to the observed hormonal effects (6). Prior to receptor binding, testosterone is reduced to 5α-dihydrotestosterone (7), which has a higher affinity for the androgen receptor. Androgen receptors (8-11) and 5α-reductase (11-14) are indeed present in human prostatic tissue.

CELL SEPARATION

For the study of possible biochemical interactions between stroma and epithelium from human prostatic tissue, the availability of a method to separate the two tissue compartments is essential. Mechanical (15,16) and enzymatic (17,18) methods have been used for the separation of human prostatic epithelium and stroma. Generally, however, the yield was low and the cells obtained lacked viability. In our laboratory a method was developed for the isolation of human prostatic epithelial cells, which is based on squeezing of finely minced tissue and purification of the epithelium by a series of sedimentation steps at unit gravity (19). Although 95% of the cells excluded trypan blue and about 45% of the cells incorporated uridine, it has not yet been possible to maintain the cells in culture. As an alternative to separation of cells, selective cultivation of fibroblasts, which are assumed to represent the stroma, and epithelium (20,21) have been used to obtain separated cell types. A possible disadvantage of these methods is that the cells obtained may not properly reflect the cells present in the original prostatic tissue sample.

MARKERS

The prostate is known to be rich in acid phosphatase and ornithine decarboxylase (22). Acid phosphatase is generally accepted to be a marker for prostatic epithelial cells (23-25) and its activity was found to be 25 times higher in epithelial cells than in the remaining stroma (19). Although the activity of prostatic acid phosphatase in the serum of patients with advanced prostatic carcinoma decreases after castration, the dependency of the enzyme activity on hormones has not yet been established unequivocally. Using the prostatic cell line MA 160, Ban et al, (26) showed that both androgens and oestrogens decreased acid phosphatase activity. There are, however,serious doubts whether this cell line is still to be considered as being prostatic (for review, see 27).

In 25% of patients with prostatic cancer and 8% of patients with benign prostatic hyperplasia elevated serum levels of spermidine have been detected (28). Because of the abundancy of ornithine decarboxylase in the prostate, this enzyme, which catalyses the first step in polyamine biosynthesis, may be useful for the study of androgen dependency of prostatic tumours. Carcinoembryonic antigen secretion has also been used as a marker for prostatic epithelial cells in culture (29). In the same study, the polyamine concentration of the culture medium was found to be of no value as a marker for prostatic epithelial cells.

BIOCHEMICAL PROPERTIES

Andogen receptors appear to be distributed evenly over epithelium and stroma, as does dihydrotestosterone (11). Sex hormone binding globulin (SHBG) was found to be associated exclusively with the stroma (16). These results could be explained by assuming that the SHBG provides a high testosterone concentration to the stromal 5α-reductase and that the DHT formed in the stroma moves to the epithelium where it interacts with the androgen receptor. Recent findings (14,30,31) that 5α-reductase is almost exclusively confined to the stroma are in agreement with such a model. The observation reported by Habib et al. (32) that stromal and epithelial 5α-reductase activites were of a similar order of magnitude, is in sharp contrast to these findings. Differences in the methods used for the separation of the cells and estimation of the enzyme activity may well be responsible for the differences in the reported results. In prostatic carcinoma tissue the 5α-reductase activity is lower than in BPH-tissue (33,34). This difference appears to be due to changes in the enzyme activity in the stroma, since the activity was much higher in fibroblasts and stroma from hyperplastic tissue than in fibroblasts and stroma from carcinoma tissue (35,36). The distribution of steroid receptors over the tissue compartments has also been studied by Pertschuk et al. (37,38). Using incubation of tissue slices with a fluorescent testosterone derivative, they found most fluorescence to be localized in the epithelium. There is, however, still serious doubt about the nature of the protein(s) which bind fluorescent steroid derivatives (39) and a proper biochemical study still needs to be carried out. Oestrogen (40), progesterone (41) and prolactin (42) receptors have been identified in human prostatic tissue. The concentration of oestrogen receptors appears to be higher in carcinoma tissue than in hyperplastic tissue (40). Krieg et al. (31) reported that oestrogen receptors could be identified more often in isolated stroma than in epithelium or in whole tissue preparations.

The distribution of all receptors over the prostatic tissue compartments as well as their possible role in stromal-epthelial interactions needs to be studied further.

CONCLUSION

In order to determine if stromal-epithelial interactions play a major role in the development of benign prostatic hyperplasia and/or prostatic cancer, future research must be directed towards:

1. improvement of techniques for the separation of prostatic epithelium and stroma, with special emphasis on the viability of the cells obtained;
2. the identification of proper markers for the stroma;

3. the study of possible effects of isolated stroma on the biochemical properties of the isolated epithelium, and, if such effects should indeed be observed;
4. identification of the factor(s) which may be responsible for these effects.

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GRADING OF PROSTATIC CARCINOMA – EVALUATION OF SINGLE PARAMETERS AND CYTOMORPHOMETRY

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INTRODUCTION

Since Broders' first tumor grading system in 1926 many attempts have been made to correlate various histologic features of a tumor with the prognosis of the patient. Also for prostatic carcinoma many grading systems have been developed (1), but only a few of them have found wide acceptance. One of the reasons for this is the tendency of many prostatic cancers to show varying degrees of differentiation and structure within a single section and often within a single microscopic field. Another reason may be the lack of reproducibility of most, if not all, grading systems. However, some grading systems seem to be very promising. Among these the most significant are those of Gleason (2) and of Mostofi (3).

Mostofi uses the term differentiation exclusively for the tendency of the tumor to form glands, and the term anaplasia for the variation from normal in size, shape, staining and chromatin distribution of the nuclei in the tumor cells. His grading system is built up with architectural criteria, such as the various tumor formations (small, intermediate or large glands, cribriform tumor and solid tumor), the amount of stroma and the amount of tumor, and with cytological criteria such as size of cell, the aspect of the cytoplasm, nuclear size, nuclear pleomorphism, presence of mitoses, presence of nucleoli and the presence of nuclear vacuoles. With these criteria he estimates an overall tumor grade.

Using Gleason's system Harada and associates (4) found a good reproducibility on repeat readings of this system, although there was less correlation between their readings and Gleason's readings of the same slides. They also found, using Mostofi's system, that nuclear anaplasia and glandular differentiation correlated well with death rates.

MATERIAL AND METHODS

In a series of 484 patients on whom the late Dr Elmer Belt performed a radical perineal prostatectomy for cancer the patient charts were reviewed retrospectively and in 346 cases histological slides from the prostatectomy specimens were available for review. These tumors were all regraded by Mostofi without knowledge of the follow-up of the patients. Most of the tumors consisted of a varying number of morphologically different formations. As Table 1 shows their number varied from one to four per patient and a total of 668 tumor formations have been matched with the clinical data of 346 patients.

The survival curves were estimated according to Kaplan and Meier and have been corrected for intercurrent, tumor unrelated and unknown death causes. For the calculations of P-values in the comparison of the survival curves the Logrank test was used.

In order to objectively evaluate nuclear variation in size and shape a semi-automatic computerized image analysing system was used (Videoplan, Kontron). This consists of a graphic tablet, connected to a desk-computer, and a microscope with a drawing tube. The graphic tablet is used for digitizing contour coordinates of figures drawn on the tablet with a cursor. A light-emitting diode is mounted in the centre of the cursor, which is visible as a small, red spot together with the normal visual field of the microscope, via the

Table 1. The Number of Tumor Formations and Their Relative Distribution in 346 Patients with Prostatic Carcinoma.

No. of Tumor Formations	No. of Patients	%	No. of Formations
1	113	32.6	113
2	152	44.0	304
3	73	21.1	219
4	8	2.3	32
Total	346	100	668

drawing tube. In this way contours of objects in the microscopic image can easily be traced manually under visual control. The digitized contours are fed into the computer, which calculates the preselected parameters. The area and perimeter was calculated in this case, together with two so-called form factors: a "form ellipse" which is derived from the longest diameter of a structure and the shortest diameter perpendicular to it in the following way:

$$\text{form}_{\text{ellipse}} = \frac{\text{shortest diameter}}{\text{longest diameter}}$$

As can easily be seen the largest value for "form ellipse" is one for a circle and less than one in the case of other structures. The second factor we estimated was: "form pe" which is derived from area and perimeter in the following way:

$$\text{form}_{\text{pe}} = \frac{4 \times \pi \times \text{area}}{(\text{perimeter})^2}$$

Here also the largest value for "form pe" is one in case of a circle and is less than one in all other structures. Reproducibility of contour tracing was within 5%. This is consistent with data from the literature (6,7).

RESULTS

Figure 1 shows the tumor related survival rates for the various tumor formations from tumors consisting of only one formation. The survival probability is plotted against the time in months. There is no difference in survival between patients with small glands, intermediate glands or large glands in their tumors, but these three differ significantly in survival from patients with cribriform or solid tumors. As Figure 2 shows, even the presence of cribriform or solid tumor together with small, intermediate or large glands makes prognosis significantly worse than in the case of small, intermediate or large glands alone. The amount of tumor (Figure 3) has prognostic importance insofar that patients with small amounts of tumor do better than those with medium or large amounts. However, one should not forget that all patients have had radical prostatectomy and that especially the patients with small amounts of tumor might have been cured by the operation.

The amount of stroma, the appearance of the cytoplasm, the presence of nucleoli, the size of cell and the presence of nuclear vacuoles, have all failed to identify groups of patients with different survival rates.

On the other hand nuclear pleomorphism, i.e. the variation in nuclear size and shape, identifies three groups of patients with

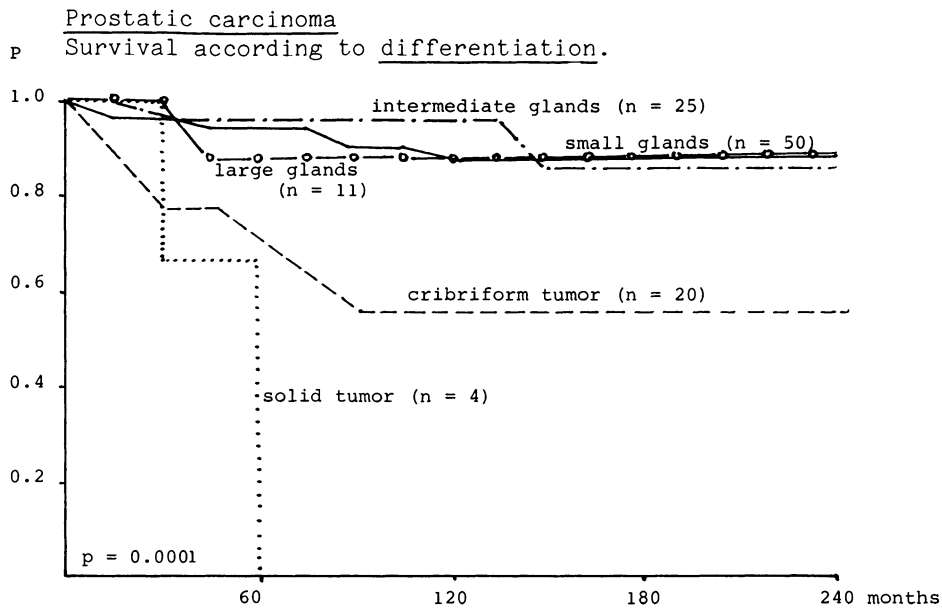

Fig. 1 Survival rates for 110 patients according to glandular differentiation. In each tumor only one tumor formation is present. (Mostofi's grading system).

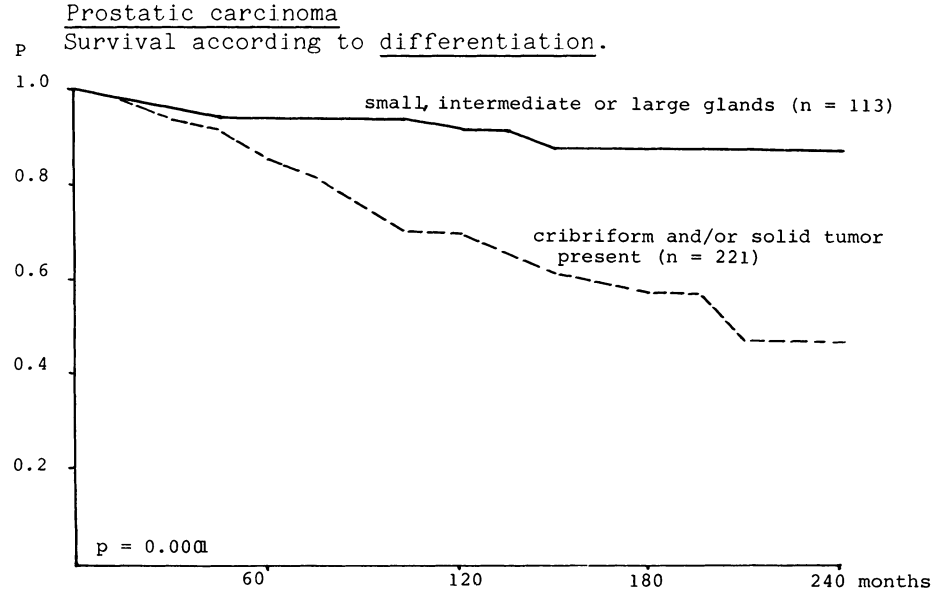

Fig. 2 Survival rates for 113 patients with only small, intermediate and/or large glands in their tumors vs. 221 patients who had glands and cribriform and/or solid parts in their tumors. (Mostofi's grading system).

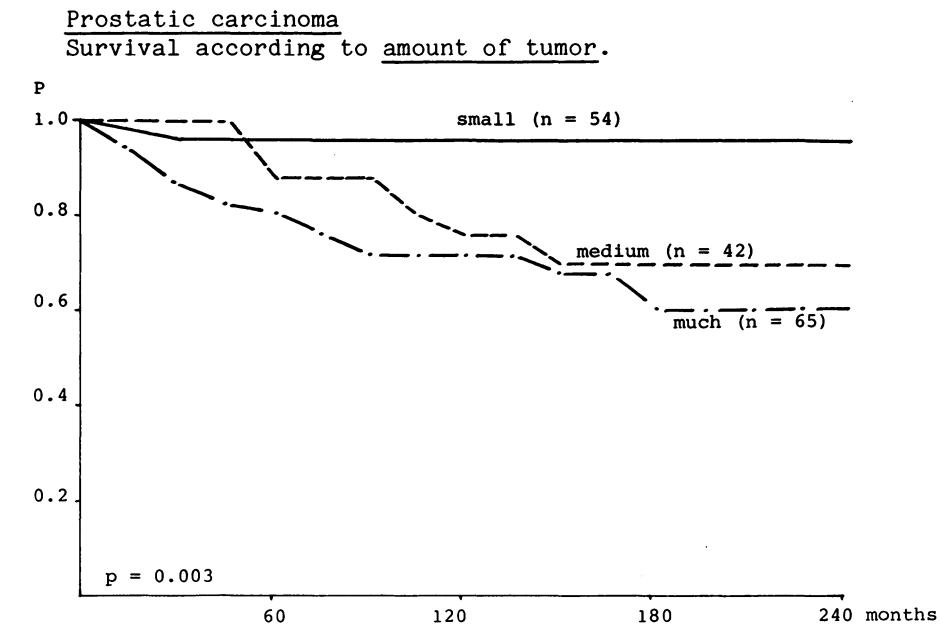

Fig. 3 Survival rates in 161 patients according to amount of tumor in their prostatectomy specimen (Mostofi's grading system).

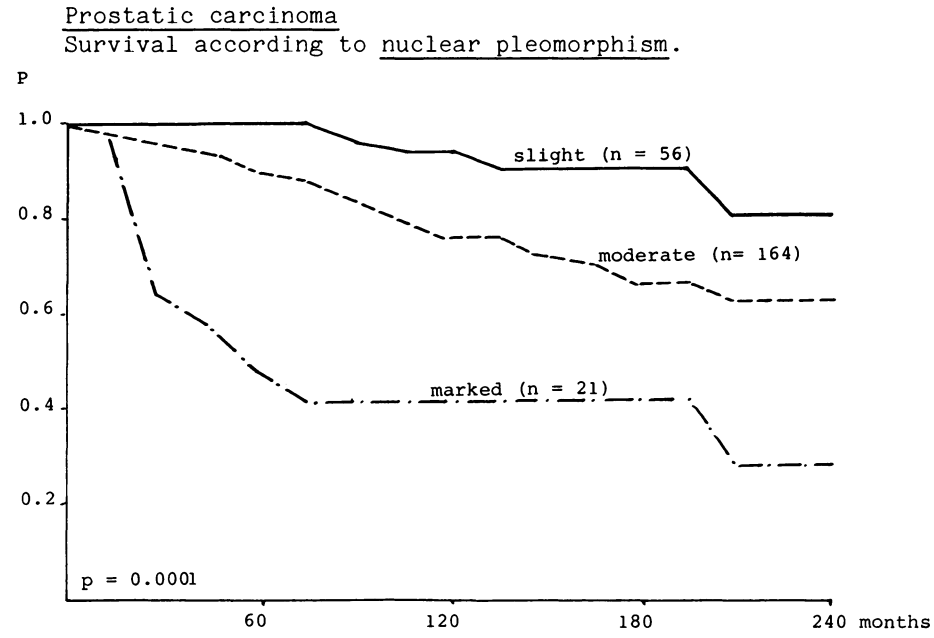

Fig. 4 Mostofi's grading system. Survival in 241 patients according to nuclear pleomorphism.

significantly differing survival rates (Figure 4). Patients with marked pleomorphism of the nuclei do considerably worse than those with moderate nuclear pleomorphism, while the group of patients with slight nuclear pleomorphism has a 20 years survival as good as 80%. Also the presence of mitoses identifies a group of patients with a worse prognosis, although few patients have many mitoses in their tumors.

As nuclear pleomorphism seems to have prognostic importance we tried to evaluate this objectively in order to obtain a reproducible and objectively estimated parameter.

With morphometry we measured 150 consecutive nuclei in each tumor formation and for each parameter we calculated the variation-co-efficient from the average value and the standard deviation. We used the variation coefficient as a standard for variation. Figure 5 shows the morphometrically estimated variation in nuclear size identifying two groups of patients with a significantly differing survival pattern. The value for V area (V stands for variation-coefficient) of 34% was found empirically.

The variation-co-efficient for "form ellipse" and for "form pe" did not identify significantly in differing prognostic groups.

Prostatic carcinoma
Survival according to V area.

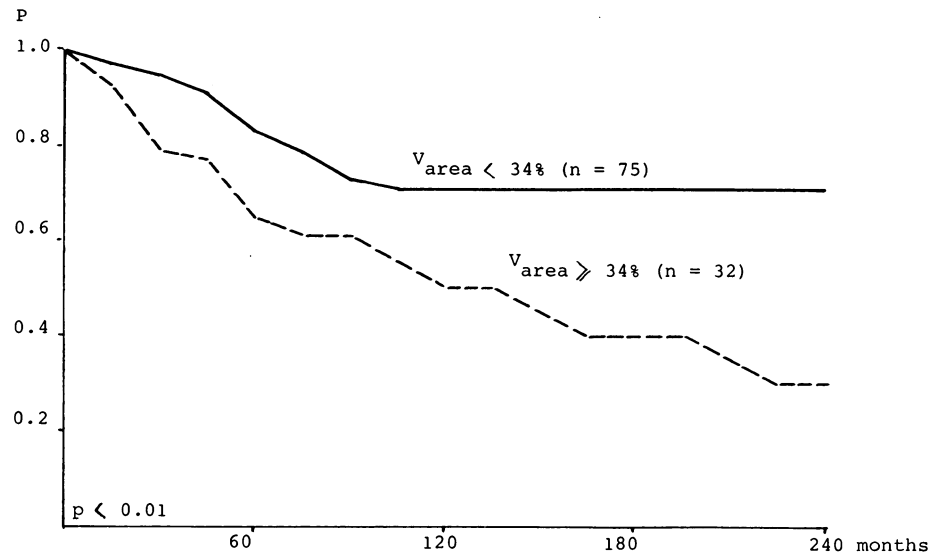

Fig. 5 Survival according to morphometrically estimated variation in nuclear size in 107 patients with prostate cancer.

DISCUSSION

As is shown, in Mostofi's grading system only a few parameters seem to have prognostic importance: glandular differentiation, whether the tumor forms only small, intermediate or large glands or whether it is growing in a cribriform or solid pattern. Secondly anaplasia, and especially the variation in nuclear size and shape has prognostic importance. The presence of mitoses may be of additional help, although most tumors show no mitoses. This suggests that the Mostofi grading system could be made simpler than it now is. With morphometry we were able to recognise two groups of patients with significantly differing survival patterns. It seems that only variation in size might be of importance, as variation in shape failed to show prognostic significance.

Although preliminary, this study shows that there may be a role for morphometry in grading prostatic carcinoma. It has the advantage of being objective and reproducible and is very easy to learn without special knowledge of grading. Maybe morphometry can cast some light on the complex problem of grading prostatic carcinoma. Until now we don't know the exact value of this technique in grading, but this will be a subject for further investigation.

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CARCINOMA OF THE PROSTATE: THE CLINICAL POTENTIAL OF BIOLOGICAL MARKERS OTHER THAN ACID PHOSPHATASE

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INTRODUCTION

Carcinoma of the prostate is a slowly progressive disease which may be managed in many different ways. Treatment alternatives include no treatment, surgery, irradiation, endocrine therapy and cytotoxic chemotherapy. Diagnosis is easily achieved by rectal examination and biopsy, and scanning techniques are readily available for accurate clinical staging. The common osteosclerotic metastases are not, however, easily assessed for progression or regression and other marker lesions suitable for accurate measurement do not often occur, even in advanced progressive disease. Therefore a good biological tumour marker would be invaluable to aid the clinician monitor disease progression and the response to treatment and to assess new hormonal and chemotherapeutic regimes in phase II clinical trials. Although Huggins and Hodges (1) used plasma acid phosphatase as a marker for this tumour it has the disadvantage that it is rarely elevated in non-metastatic cancer and that in metastatic disease its sensitivity (percentage true positive elevations) as a marker is low. In addition during hormonal therapy disease progression is often not accompanied by rising plasma levels of this enzyme.

Many alternative specific and non-specific biological markers of potential value in the management of prostatic cancer have been investigated during the last decade. These include enzymes, antigens and acute phase reactant proteins.

Table 1. Creatinine Kinase Levels in Patients with Carcinoma of the Prostate

STAGE	ELEVATED CK-BB (NO. ELEVATED/TOTAL PATIENTS)	
	FIELD et al 1980	SILVERMAN et al 1979
A	0/3	
B	1/26	3/4
C	0/35	2/2
D	11/46	10/11
D (with elevated acid phosphatase)	10/13	

POSSIBLE MARKERS

Creatinine Kinase

Creatinine Kinase is a foeto-placental enzyme. Its BB isoenzyme (SK-BB) is elevated in the serum of patients with metastatic carcinoma of the prostate but not in normal adults (2). Table 1 reports the experience of Silverman et al (3) and Field et al (4) in measuring this isoenzyme in different clinical stages of prostatic carcinoma. Homberger et al (2) found that it was elevated in 20 of 135 patients with benign prostatic hyperplasia and in 10 of 35 patients with bladder cancer. Therefore CK-BB does not have diagnostic value for carcinoma of the prostate but may yet be of value in predicting the development of metastases and in monitoring response to treatment.

Serum Lactic Dehydrogenase

There has been much interest in serum lactic dehydrogenase (SLDH) and its fifth isoenzyme SLDH-V as biological markers. Ishibi (5) demonstrated that in 22 patients with prostatic cancer, low pre-treatment plasma levels were associated with a 59% five year survival. Only 28% of 29 patients with high pretreatment levels survived five years. The prognostic significance of pre-treatment levels of total LDH and SLDH-V were the same. Changes during therapy did not correlate with survival.

Fructose 1.6 Diphosphate Aldolase

This enzyme is associated with anaerobic glycolysis. Maganto-Pavon et al (6) found that in 43 patients plasma levels were elevated in 37% compared with 32% who had elevated prostatic acid phosphatase and 27% with elevated SLDH.

Bone Alkaline Phosphatase Isoenzyme

Wajsman et al (7) have measured plasma total and bone isoenzyme alkaline phosphatases in 105 patients with metastatic disease. Bone enzyme levels were elevated in 91%. The patients with higher pre-treatment levels of both phosphatases did not respond well to treatment. In patients with a partial remission there was a greater than 25% decrease in bone alkaline phosphatase levels. Therefore these enzymes have potential value in predicting prognosis and response to treatment.

Plasma Carcinoembryonic Antigen

Carcinoembryonic antigen has been extensively investigated in the management of many tumours. Kane and Paulson (8) in the study of 27 patients with carcinoma of the prostate found decreasing plasma levels in 19 patients responding to treatment and in eight with progressive disease. Increasing levels were found in only four responders and in 20 patients with progressive disease.

Hydroxyproline

Hydroxyproline is an amino acid constituent of the polypeptide chain in collagen. As such it is a marker of bone matrix turnover (9). Bishop and Fellows (10) found that whilst 24 hour urinary hydroxyproline excretion was normal in all of 14 patients with non-metastatic disease it was significantly elevated in all nine of their untreated patients with metastases. Urinary concentrations were high in only seven of 12 treated metastatic cases and reduction in the concentration during treatment corresponded with symptomatic improvement with little or no bone scan changes.

Urinary Fibronectin

Fibronectin is a high molecular weight glycoprotein. Episodic elevations have been observed in the early morning urine of patients with carcinoma of the prostate. Webb and Lin (11) found that it was not elevated in benign prostatic hyperplasia. Using a single assay there were 42% elevations in 32 patients with carcinoma of the

prostate. In eight patients who had three sequential assays over a period of two to six months, elevations were found in 100%.

Serum Immunoglobulins

Serum Immunoglobulins reflect the host's humoral immune response to cancer and many other conditions. In 1972 Ablin and his colleagues (12) reported the remission of metastases after cryotherapy of the primary lesion in six patients. This phenomenon was associated with increased circulating antibodies. Gursel et al (13) confirmed that cryosurgery elevated circulating immunoglobulins IgG and IgM. They also found higher IgG levels in Stage II patients with untreated carcinoma of the prostate than in Stage IV patients. Lockwood et al (14) confirmed these results. In their series, in both treated and untreated patients, levels of IgG were higher in non-metastatic than in metastatic disease (Figure 1). In their series they were not able to show that oestrogen therapy like cryotherapy, produced elevations of immunoglobulins.

The immunoglobulin studies by Deture et al (15) give different results. They found significantly depressed levels of IgG in stage A and B disease compared with controls whilst the levels in patients with stages C and D disease were higher. The IgM levels were depressed in all stages of prostatic cancer and the IgA levels elevated in stages C and D disease.

The value of immunoglobulins in staging and monitoring prostatic cancer remains in doubt.

Acute Phase Reactant Proteins (APRP's)

APRP's are elevated in prostatic cancer as the total tumour mass increases (16,17). The levels of most APRP's are powerfully influenced by oestrogens making them unsuitable for monitoring Stilboestrol therapy (16,17,18). C-reactive protein (C-RP) and serum albumin concentrations, however remain independent of oestrogen control and Trautner and his colleagues (19) have shown that a chronic rise of C-RP, especially when preceded by normal levels for several months, strongly indicated disease progression. Albumin levels which fall with the development of metastases are a useful guide to overall biochemical status in a non-specific manner equivalent to performance status. Patients with high serum C-RP and low serum albumin levels have a poor prognosis.

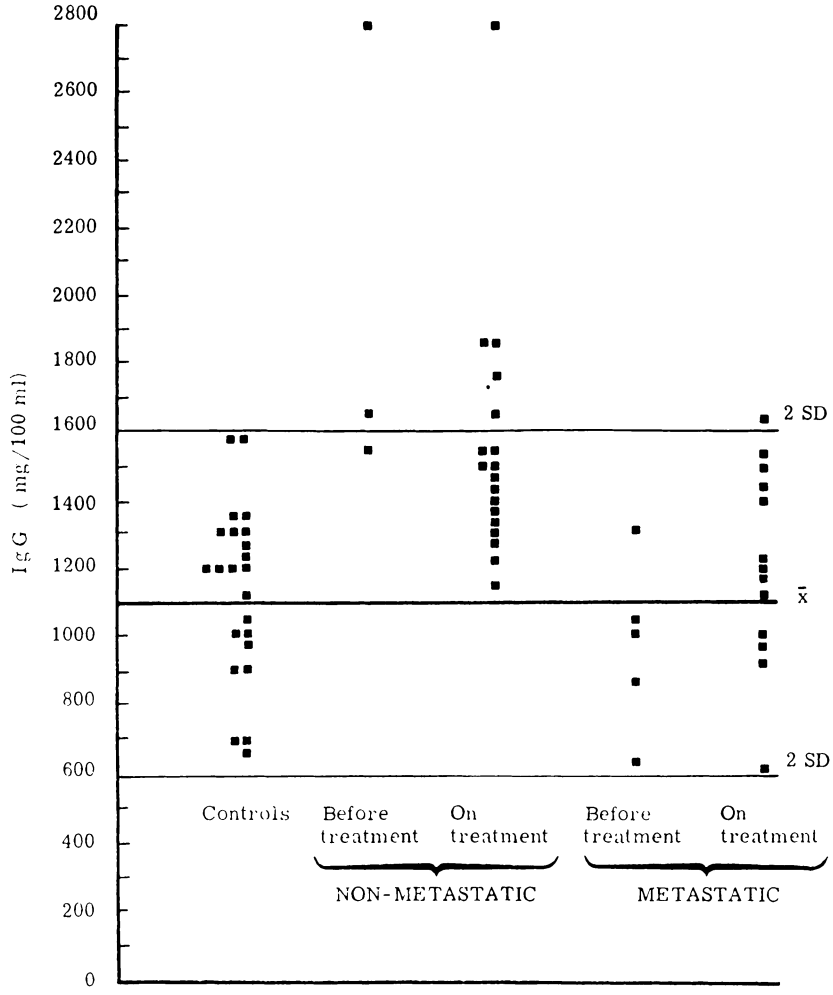

Fig. 1. Serum levels of IgG in patients with non-metastatic and with metastatic carcinoma of the prostate.

CONCLUSIONS

This paper has reviewed some of the possible biochemical markers of prostatic cancer alternative to acid phosphatase. An ideal marker has not yet been found. Controlled clinical and laboratory studies are required to identify and characterise all possible markers and to explore their potential clinical use.

SUMMARY

Acid phosphatase is not an ideal biological marker for prostatic cancer. Many other enzymes, antigens and proteins have been investigated in the management of this disease. Co-ordinated clinical and laboratory studies are needed to identify, characterise and clinically evaluate new markers.

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THE PREDICTIVE VALUE OF SERUM C-REACTIVE PROTEIN (CRP) LEVELS IN UNTREATED PROSTATIC CANCER

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C-reactive protein (CRP) is an acute phase reactant protein (APRP). The APRP's are a group of mainly glycoproteins which alter their plasma concentrations in response to a variety of stimuli including tissue injury, acute and chronic inflammation, connective tissue disease and cancer (1-3). Most of them are also influenced by oestrogens, which are often employed therapeutically in the management of prostatic cancer. CRP differs from the others in this respect (4). Plasma concentrations are, however, elevated as tumour burden increases (5). This present study evaluates the pre-treatment levels of CRP and serum acid phosphatase (SAP) in patients with benign prostatic hyperplasia and carcinoma of the prostate to determine the prognostic value of these tumour markers in the management of malignant prostatic disease.

METHOD

Fifty patients with category T3 prostatic cancer were studied in the prostatic clinics of St James's University Hospital, Leeds, and Pontefract General Infirmary. Of these 19 had M0 category cancer and 31 category M1 disease. An additional 15 patients with benign prostatic hyperplasia were also investigated. Pretreatment studies included clinical history and examination, digital examination of the prostatic gland, weight and haemoglobin measurements, histological confirmation of the diagnosis by transurethral resection or needle biopsy and isotopic bone scans for metastases with x-ray confirmation of hot spots. Minimal follow-up was at six-monthly intervals for the patients with malignant disease and included clinical history and examination, weight, haemoglobin estimation and bone scans.

Single radial immunodiffusion (6) was used to measure CRP (normal range 0-11 mg/l) and an enzymatic method (7) to estimate SAP (normal range 0-4 IU/l).

The 50 patients being treated for carcinoma of the prostate received different types of hormonal therapy. Twenty-four of them (48%) had clinical progression during the period of follow-up. Progression was defined as any increase in size of an existing marker lesion or the appearance of new lesions on a bone scan. Subjective factors taken into consideration were loss of weight, pain, performance status and anaemia.

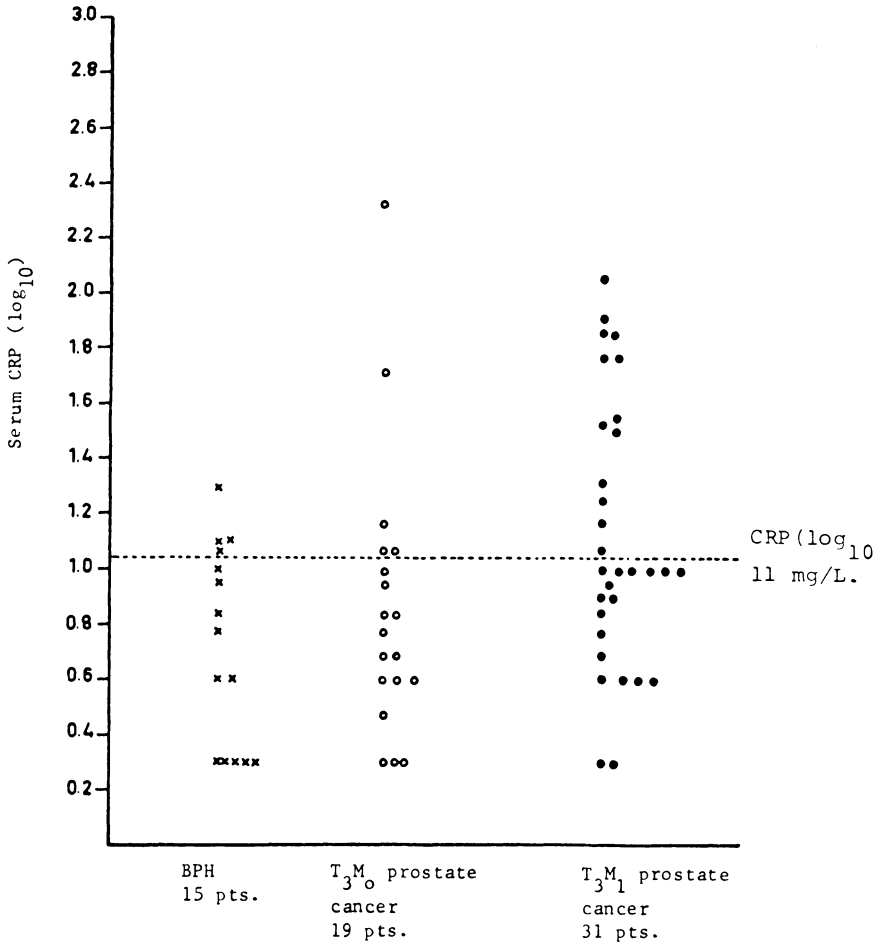

Fig 1. Pretreatment serum CRP (log_{10}) levels in BPH and prostate cancer.

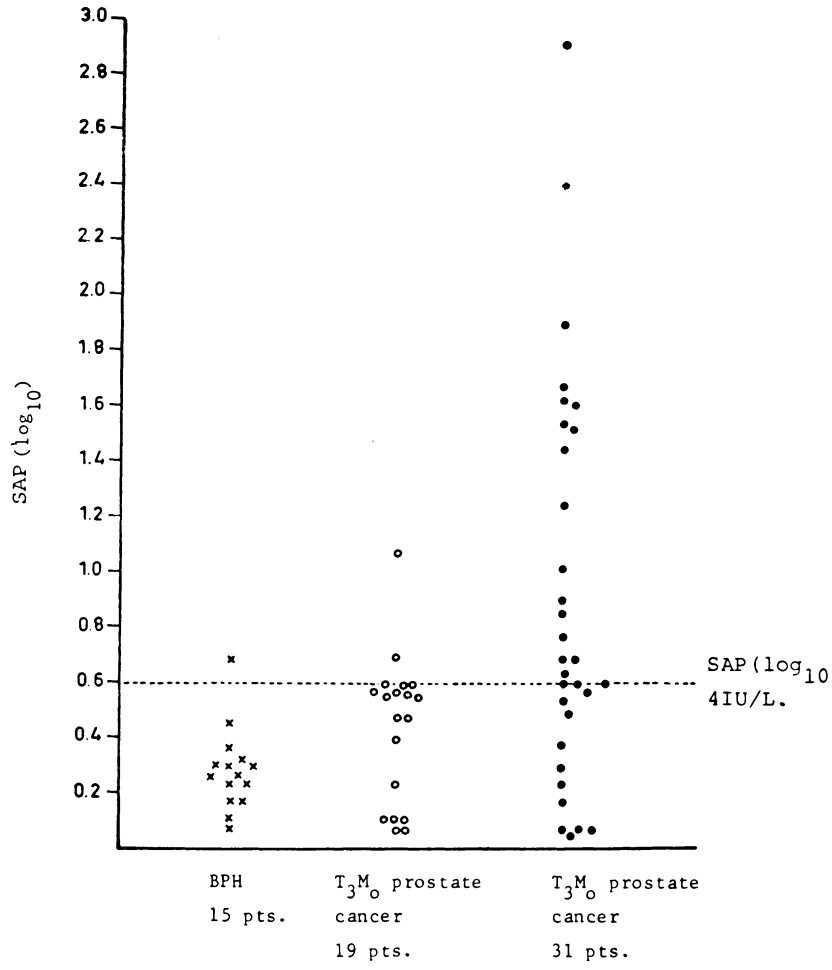

Fig 2. Pretreatment SAP levels in BPH and prostate cancer.

RESULTS

Distribution of serum CRP levels are shown in figure 1 and the SAP levels in figure 2. In order to obtain a normal distribution of this skewed data, the mean values of the results expressed as logarithms are shown in table 1. Statistical analyses employing Student's t test and Gehan's test for censored data (8) show that there is a significant difference between the CRP levels in patients with BPH and both T3 M0 and T3 M1 disease ($P < 0.05$) (Table 2).

Table 1. Pretreatment SAP and Serum CRP Levels in Patients with Cancer of the Prostate and B.P.H. (Mean ± S.D.)

	SAP (log_{10})	CRP (log_{10})
B.P.H. (n = 15)	0.2850 ± 0.1534	0.7285 ± 0.3645
T3 MO Prostate Cancer (n = 19)	0.4438 ± 0.2710	0.8634 ± 0.5000
T3 M1 Prostate Cancer (n = 31)	0.9119 ± 0.7113	1.1225 ± 0.4859
T3 MO and M1 Prostate Cancer (n = 50)	0.7332 ± 0.6248	1.0240 ± 0.5025

Table 2. Statistical Analysis of Pretreatment SAP and CRP in Patients with B.P.H. and Prostate Cancer.

Comparison Between	SAP t	SAP p	CRP t	CRP p
B.P.H. and T3 Prostate Cancer	2.7399	< 0.05	2.1121	< 0.05
B.P.H. and T3M0 Prostate Cancer	2.0245	< 0.05	0.8761	N.S.
B.P.H. and T3M1 Prostate Cancer	3.3577	< 0.05	2.7786	< 0.05
T3M0 and T3M1 Prostate Cancer	2.7403	< 0.05	1.8103	N.S.

The frequency of elevated levels of serum CRP (> 11 mg/l) and SAP (> 4 IU/l) for benign hyperplasia and the different cancer categories are shown in Table 3.

Table 4 shows the relationship between elevated and non-elevated SAP and CRP levels in patients with T3, M1 carcinoma of the prostate. In 20 of them (64.5%) the SAP and/or CRP levels were elevated and

Table 3. Frequency of Elevated Levels of CRP (>11 mg/l) and SAP (>4 IU/l) in Patients with Cancer of the Prostate and B.P.H.

Disease	CRP No. (%)	SAP No. (%)	CRP and SAP No. (%)
B.P.H.	4/15 (26.7)	1/15 (6.7)	0/15 (0)
T3M0 Prostate Cancer	5/19 (26.3)	2/19 (10.5)	0/19 (0)
T3M1 Prostate Cancer	4/31 (12.9)	3/31 (25.8)	9/31 (29)

Table 4. The Relation of Pretreatment SAP and CRP Levels in T3M1 Prostate Cancer.

	Serum CRP Levels (mg/l)		
SAP Levels (IU/L)	CRP ≤ 11	CRP > 11	Total
SAP ≤ 4	10	4	14
SAP > 4	8	9	17
Total	18	13	31

Table 5. Pretreatment SAP and CRP Levels in Patients Who Had Progression as Clinical Response After Their Hormonal Treatment.

	SAP ≤ 4IU/l and CRP ≤ 11 mg/l	SAP > 4IU/l CRP > 11mg/l	SAP > 4IU/l and CRP > 11 mg/l	Total
T3M0	1	-	2	-	3
T3M1	3	6	3	9	21
Total	4	6	5	9	24

of these 18 had progressive malignant disease within five months of commencing endocrine therapy (Table 5).

DISCUSSION

The APRP's reflect tumour load in many cancers and are also influenced by trauma and infection. Therefore they have no place as diagnostic tumour markers (4,9,10). CRP levels are not influenced by oestrogens and urinary tract infections without fever but they are powerfully stimulated by acute retention of urine (11).

Although SAP is a specific tumour marker in patients with T3, M1 disease, it is only elevated in 60% of those patients with demonstrable metastases (12). CRP levels tend to rise with tumour progression and this study is unique in demonstrating that high pre-treatment levels of SAP and/or CRP predicted clinical progression within five months in 18 of 21 patients. If these findings are confirmed in larger studies, there is a strong case for treating such patients with a combination of hormones and chemotherapy from the time of diagnosis.

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IMMUNOLOGICAL DETECTION OF PROSTATIC ACID PHOSPHATASE: FIVE YEARS' EXPERIENCE

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INTRODUCTION

In 1976, a rabbit antiserum was raised in Amsterdam against that isoenzyme of acid phosphatase (EC 3.1.3.2) which is usually designated prostatic acid phosphatase (PAP). The antigen used consisted of purified seminal plasma from vasectomized men. This antiserum has been extensively documented (1,2). The antiserum has been used principally in the field of histopathology and clinical chemistry.

PAP, a secretory glycoprotein, can be distinguished from most other acid phosphatase isoenzymes by, for example, inhibition tests (it is relatively insensitive to formaldehyde, but sensitive to L+tartrate (3,4) and by substrate specificity tests such as phosphorylcholine (5). These biochemical differences between prostatic and non-prostatic carcinomas enable the histopathologist to differentiate between them in cryostat sections using histochemical techniques (Fig. 1). But this method, which is so advantageous to the patient, cannot be applied once the tissue has been embedded in paraffin since ethanol irreversibly inhibits the PAP activity. This enzyme histochemical technique can therefore rarely be applied to small fragments of tissue such as needle biopsies or skeletal biopsies, which usually must be embedded completely for the pure morphological investigation. This is not only of importance in the differential diagnosis between bladder and prostatic carcinomas, but also in revealing the primary location of an otherwise occult prostatic carcinoma. The importance of the latter point is illustrated by the findings of Butler (6) that 18 out of 200 cases of prostatic carcinoma presented with a metastasis in the left supraclavicular lymph node. The limitations imposed by the inhibition

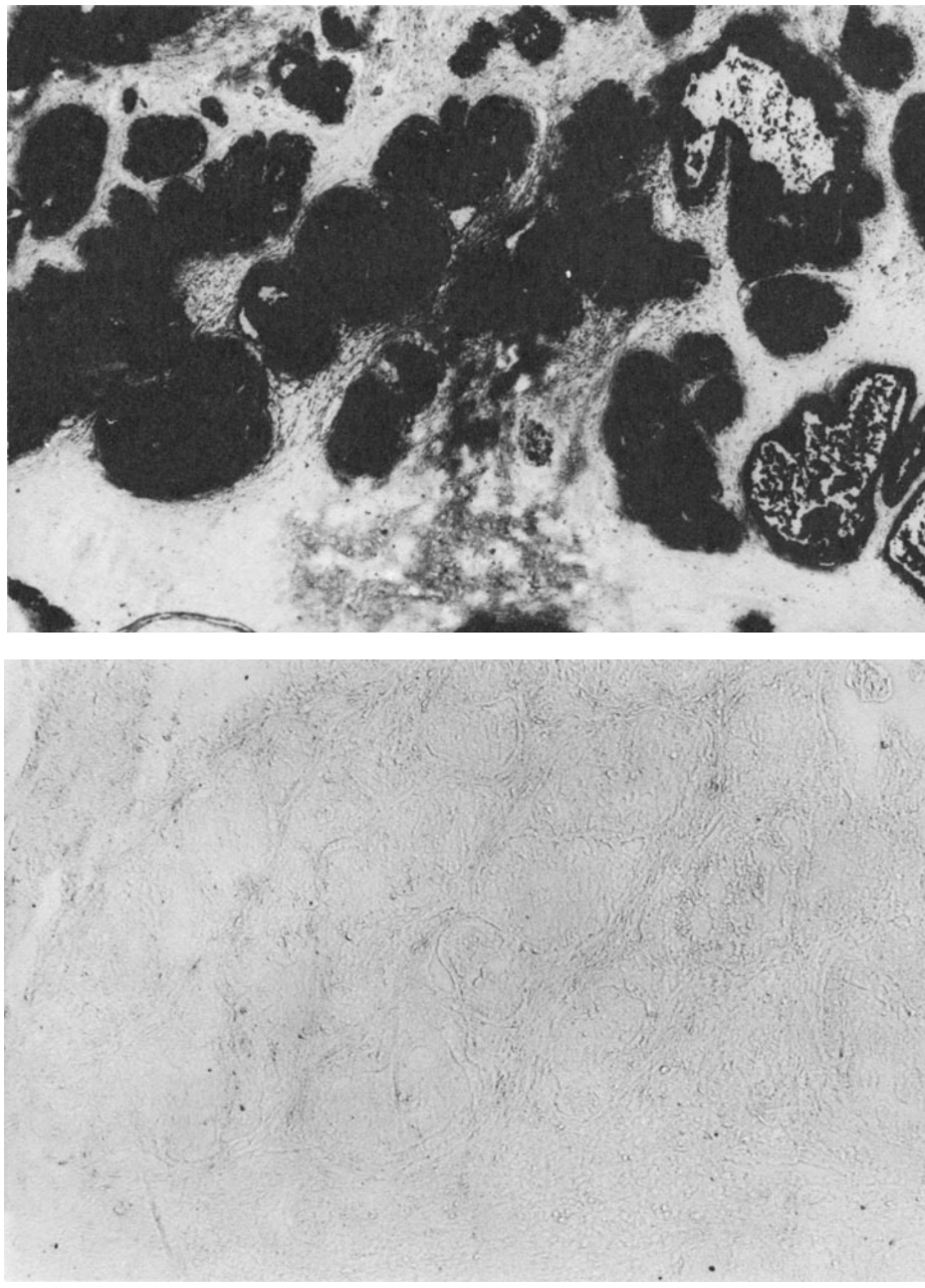

Fig. 1. Enzyme histochemical activity in two consecutive, formalin fixed cryostat sections of prostate with phosphorylcholine as substrate. The activity so strongly present in the epithelium (A) has been inhibited by L+tartrate (B).

of PAP activity by ethanol might be circumvented by the application of immunohistochemical techniques for the antigenicity of a series of secretory glycoproteins (alpha-1-antitrypsin, immunoglobulins, thyroglobulin) is not essentially altered by the procedure of paraffin embedding.

HISTOPATHOLOGY

The Amsterdam antiserum was applied as the primary antiserum in the indirect peroxidase technique (1) on paraffin sections of 300 cases of proven prostatic carcinoma (100 autopsies and 200 Surgicals) and 350 cases of non-prostatic tumour. A tumour was considered to be non-prostatic either on clinical grounds, histopathological grounds and clinical chemical grounds or on autopsy grounds. The 350 non-prostatic tumours came from 24 different organs amongst which were 70 bladder and/or urethral carcinomas, 10 kidney carcinomas, 10 testis tumours and 10 seminal vesicle carcinomas. In several cases the indirect fluorescence technique or the peroxidase-anti-peroxidase technique (7) as post-primary antiserum methods was also applied, either on paraffin sections or on cryostat sections. The results, visually assessed, did not show essential differences between these techniques except for a higher discriminative staining with the peroxidase-anti-peroxidase technique. Some cytological and haematological smears were also investigated. Control incubations were performed each time (1). The results (Fig. 2, Table 1) show that the immunological demonstration of PAP in paraffin sections circumvents the restrictions of the enzyme histochemical approach. The prostatic epithelium in smears also gives a positive reaction for PAP. The false negative percentage in the surgical material was 1.5% for the primary focus and 3% for the metastases. In the autopsy material there were 3% overall false negative results (Table 1). This is in agreement with the results in smaller series of other groups (8,9,10). Apart from the prostatic tumours none of the 100 other urological tumours gave a positive reaction for PAP. This may be of significance especially with regard to the differential diagnosis of bladder versus prostatic carcinoma or in cases of a metastasis as presenting symptom of a carcinoma (6). Only 7 positive results were obtained in the 350 patients without prostatic cancer - 6 out of the 12 insulomas (11) and one out of the 10 carcinoids, and do not detract from the practical application of this technique.

The false negatives may result from several different causes:-

1. The immunological staining of PAP in carcinoma may in general not only be less intense than in benign epithelium but may also show great variation within one section (Fig. 2). When a small tissue fragment is used, for instance a needle biopsy, the pathologist might only encounter the phosphatase negative part of the prostatic carcinoma.

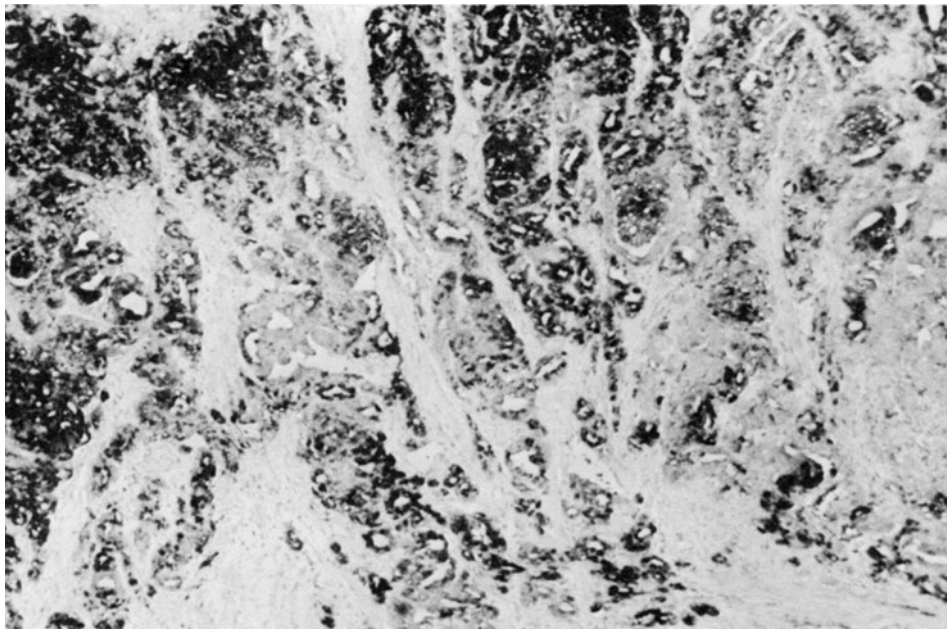

Fig. 2. Immunologically demonstrated PAP in paraffin section of prostatic carcinoma. The great variation in intensity is clearly shown. Indirect peroxidase technique with diaminobenzidine as substrate. Weak counterstain with haematoxylin.

Table 1. Intensity of Immunohistochemical Staining (300 cases, paraffin, indirect peroxidase)

	n	++	+[a]	(+)	-
Surgical material	200				
Carcinoma:					
Primary	200[b]	5	81	111	3[c]
Metastasis	69[b]		20	47	2[d]
Benign epith.	172	172			
Autopsy material	107[e]				
Carcinoma:					
Primary	90[b]		29	59	2[f]
Metastasis	46		10	32	4[g]
Benign epith.	80	71	7	2	

Notes to Table 1

a. ++ strong, + moderate, (+) focally moderate or weakly positive, - negative.
b. in 69 of the surgical cases metastases could also be investigated; in 17 of the autopsy cases only the metastases could be investigated.
c. Only tiny bits of carcinoma present in the needle biopsy.
d. Inappropriate decalcifying solution.
e. In 7 of the 200 cases in which surgical material was investigated an autopsy was subsequently performed.
f. Adenoid cystic carcinomas.
g. Only tiny bits of carcinoma present but in 3 cases the primary tumour showed focally moderate staining.

2. Autolysis diminishes the antigenicity of PAP. In general, the autopsy material and the inner parts of prostatectomy preparations stain less intensely than biopsies or prostatic "chips".

3. Some special fixatives, for instance Zenker's solution, or inappropriate decalcification (by the almost obsolete nitric acid procedure) diminish the antigenicity. Preparation of tissue for paraffin sections will, in general, reduce the antigenicity slightly. Granulocytes in smears show a weak positive reaction for PAP whereas in paraffin sections no positive results could be obtained using our technique.

4. Hormonal treatment reduces the intensity of immunostaining of PAP as could be shown in 13 cases (androgen deprivation or oestrogen therapy).

5. False negatives may also result from suboptimal circumstances during the incubation procedure (10). For instance, in 12 cases a positive reaction could only be established after application of the more sensitive post-primary antiserum procedure (peroxidase-anti-peroxidase technique). This again indicates the importance of standardising the investigation in order to obtain comparable results from different scientific groups.

Table 2 shows the results of a comparison between the visually assessed intensity of immunostaining in the dominant carcinoma field and the grade of differentiation in the same field in the preceding HE section (12). This short series clearly indicates the need for objective quantification of both the intensity of immunostaining and the parameters for morphological differentiation. In a pilot study, we measured the staining intensity photometrically (13) in paraffin sections of three positive carcinomas (grade II). The staining intensity of the carcinomas was clearly less than that of the benign epithelium in the same section. This may indicate the potential

Table 2. Comparison of Grades and Immunostaining Intensity*

Grade	n	++	+	(+)	-
0	43	43			
I	2		2		
II	14	1	10	3	
III	24	1	20	2	1
IV	10		3	7	
Totals	50	2	35	12	1

*Indirect peroxidase, immunological staining of paraffin sections (4u) of transurethral resected prostatic carcinomas (50 cases) of which 43 also contained benign hyperplastic epithelium ("grade 0"). Visual assessment. Gaeta's grading system (1981).

value of the photometric technique in the differential diagnosis of grade I adenocarcinomas and benign epithelial proliferation of the prostate.

The antiserum also has a more academic application in the field of cell biology. For instance, the more precise localization of PAP at the cell organelle level might shed some light on the background of the hormonally influenced synthesis of PAP. Whereas acid phosphatases usually are localized in lysosomes for internal cell use, PAP is a typical secretory product of the cell and might be localized in the Golgi system. Until now we have only succeeded in developing the technique of immunohistochemical demonstration of PAP at the ultrastructural level. Work is in progress to collect the conclusive data. The fact that human PAP is species specific (with the exception of the chimpanzee) presents difficulties in experimental studies. We must therefore resort to fresh surgical material or to organ or cell cultures.

CLINICAL CHEMISTRY

The immunologically determined PAP serum level values were compared with those obtained by means of the classical indirect enzymatic procedure (4). The first mentioned one - usually called enzyme immuno assay (EIA) or immuno fluorescent assay (IFA) depending on the substrate and type of measurement used - consists of the following procedure (Fig. 3): IgG is isolated from our antiserum chromatographically and used to coat polystyrene tubes. Addition of the serum sample results in a specific binding of PAP and the enzymatic activity of the bound antigen (PAP) is measured either by

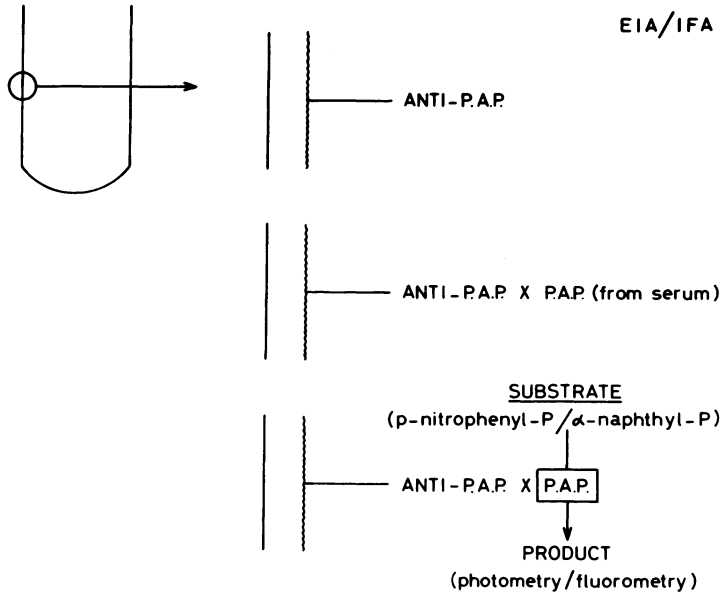

Fig. 3. Schematic representation of the immunological method applied for the determination of the PAP serum level.

spectrophotometry (paranitrophenylphosphate) or by fluorometry (alpha-naphthylphosphate). Results of the EIA and IFA correspond. These two immunoassays are of the same level of sensitivity as the radio-immuno assay (RIA) (14) and the enzyme linked immunosorbent assay (ELISA) now under development in our laboratory. The diagnostic value of our immunological techniques has been established in a number of patients (Fig. 4). The upper limit of normal is 1.0 U/l. The diagnosis in all patients was made histopathologically. A prostatic carcinoma with metastases (P.Ca + m) had a raised PAP serum level more often than a prostatic carcinoma without metastases (P.Ca). Treatment reduces the PAP values (P.Ca tr.). As can be seen in Fig. 4, these three groups consisted of 56 cases. Patients with bladder carcinoma (35 cases), bronchus carcinoma (17 cases) and other malignancies such as pancreatic (exocrine type) carcinomas (25 cases), were used as controls. Benign prostatic hyperplasia (P. hyp.) showed an elevated value in two out of 54 cases. It is especially in this group of patients where sequential determination of PAP levels is deemed necessary. Although the sensitivity of the immunological method is inadequate for the early detection of a prostatic carcinoma the specificity in the overall non-prostatic cancer patient group (99%) and the sensitivity (7 out of 26 untreated cases; 73%) are an improvement on the classical enzymatic method (specificity 85%). Our sensitivity percentage corresponds with those recently reported by other groups (15,16).

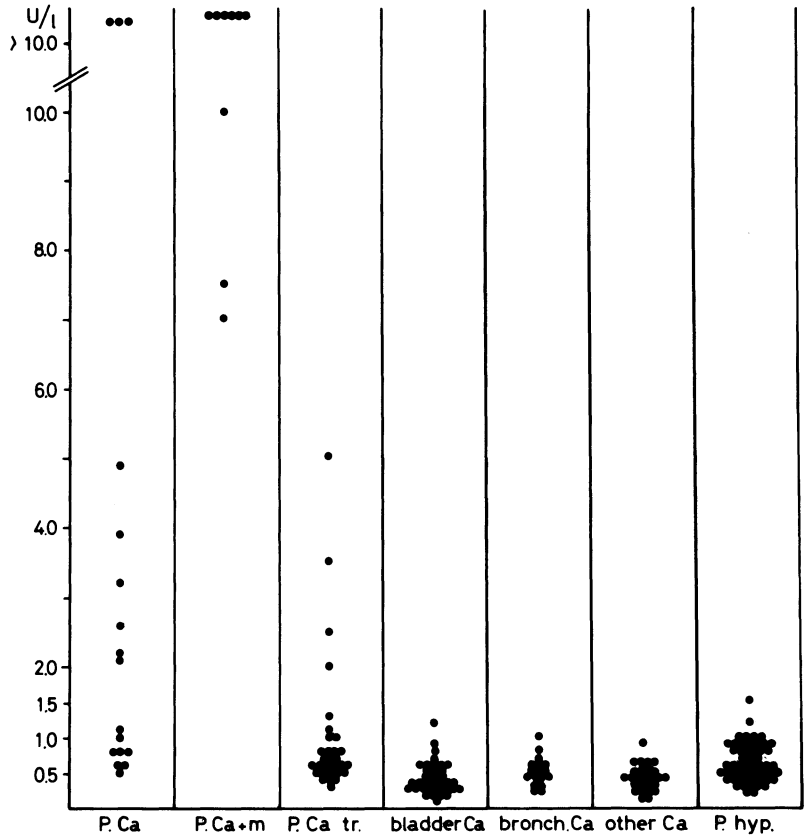

Fig. 4. Results of the determination of PAP in the serum of the various patient groups. For explanation of the abbreviations see text. The upper limit of normal is 1.0 u/l.

CONCLUSION

After five years of experience we may make the following observation: The application of the anti-PAP-serum tool has proved to be a significant advantage in the field of histopathology, but the sensitivity is less than we expected with regard to clinical chemistry. We cannot therefore speak of a screening test for prostatic carcinoma in an early stage (16,17). A new prostate specific antigen demonstrable in serum has been announced (18). The chances that this antigen will offer a screening test for prostatic carcinoma are low for this recently discovered antigen is also demonstrable in serum of normal males. The prime objective remains the demonstration of a prostate specific antigen which can always be found in the serum in the early stages of a carcinoma. The experiences with carcinoembryonal antigen and foetal alkaline phosphatase in connection with

colon carcinoma, however, indicate that the chances of success within the near future are, at best, limited.

ACKNOWLEDGEMENTS

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IMMUNOLOGICAL ASSAYS FOR PROSTATIC ACID PHOSPHATASE

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ABSTRACT

Several immunological assays for the determination of prostatic acid phosphatase (PAP) in the diagnosis of prostate carcinoma have been developed since the demonstration of a specific antibody to PAP. Some of these assays have demonstrated increased specificity and a moderate degree of increased sensitivity over standard biochemical assays. The data presented should be used as routine screening tests for the early detection of prostatic carcinoma.

Acid Phosphatase (AP) was the first "tumor marker" to be measured in the blood and over 40 years have elapsed since an elevation of the serum AP level was observed in patients with prostatic carcinoma (1). However, significant elevations in the level of this enzyme have been observed in other diseases, as well as elevations of other tissue phosphatases. Many improvements in the biochemical technique have been introduced, but none have been used successfully to detect the tissue origin of this ubiquitous enzyme. The findings that prostatic acid phosphatase (PAP) is antigenically distinct from AP of other tissues opened a new horizon in the measurement of AP in prostatic cancer. On the basis of this immunochemical specificity, various immunological methods were developed.

Counterimmunoelectrophoresis (CIEP) is a rapid semi-quantitative method for measuring PAP. Romas et al (2) have compared a standard biochemical method with CIEP on a wide spectra of prostatic and non-prostatic diseases. Non-prostatic malignancies and other disorders associated with a raised acid phosphatase by the biochemical method were found to be non-reactive for PAP by CIEP.

Patients under treatment with various stages of prostatic carcinoma showed comparable elevations by both methods (35%). In untreated patients, the CIEP was statistically most sensitive in Stage A2 lesions (39% by CIEP versus 14% by chemical). Table 1 summarizes the CIEP findings in clinically staged prostatic carcinoma in two large series.

The initial report on radioimmunoassay (RIA) by Foti and associates (2), reported significant elevations of PAP not only with disseminated adenocarcinoma of the prostate but also in those with disease limited to the prostate. Subsequently, no other investigator has reported such encouraging results. Bruce et al (4) have recently attempted to define the role of RIA for PAP in prostatic carcinoma. These investigators compared three RIA assays and demonstrated marked similarity in results for the various stages of malignant disease and an increase in the percentage of elevation of PAP with increasing tumor burden. Significantly, the mean detection rate for localized prostatic carcinoma (Stage A and B) was only 22% (from 14 to 31%). However, this study not only showed relatively low sensitivity for intracapsular adenocarcinoma of the prostate, but also a false positive rate ranging from 3 to 27% in patients with benign prostatic hypertrophy. Other investigators, with the exception of Foti et al (2), have also failed to produce encouraging results in patients with

Table 1. Results of Acid Phosphtase by CIEP (% Positive)

	Stage			
Reference	A	B	C	D
Romas et al. (2)	39	22	46	97
Murphy et al. (5)	38	35	49	69

Table 2. Comparison of Immunological Assays for PAP (% Elevations)

Stage	Biochemical	RIA	CIEP	IEA
A(27)	7	26	0	26
B(12)	33	33	25	25
C(29)	55	72	52	62
D(18)	91	100	94	94
BPH(82)	9.1	17.1	1.2	8.5

localized prostatic carcinoma. These authors concluded that findings of a low sensitivity for patients with early prostatic carcinoma and the high falsely positive rate in patients with benign disease limit severely the use of these assays in screening trials.

The present authors conducted a comparison study determining PAP by four different methodologies (biochemical, CIEP, RIA, and immunoenzyme IEA) on patients with various stages of untreated prostate carcinoma (Table 2). The study indicates that RIA and IEA were the most sensitive assays but RIA had the highest percentage of false positives in BPH (17.1%).

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IS DETERMINATION OF PROSTATIC ACID PHOSPHATASE IN BONE MARROW BY RADIOIMMUNOASSAY (BM-PAP) USEFUL IN PATIENTS WITH PROSTATIC CANCER?

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INTRODUCTION

Prostatic acid phosphatase (PAP) determined in serum and bone marrow, is claimed to be a useful tumour marker in prostatic carcinoma (1,2,3,4). The clinical significance of bone marrow PAP (BM-PAP) has, however, not been clearly defined, possibly due to methodological problems of BM-PAP analysis.

The aim of the present study is to analyse to what extent BM-PAP determinations by a specific radioimmunoassay (RIA) are of clinical significance in patients with cancer of the prostate.

MATERIAL AND METHODS

Sixty-one patients with histologically proven adenocarcinoma of the prostate were included in the study. Further details regarding the patients are given in Table 1. The stage distribution was performed according to the TNM system (5). Patients with serum PAP (Se-PAP) levels above 3.5 U/l, determined by the enzymatic method (6), were regarded as M1 patients. In general, M0 patients were treated by irradiation of the prostate and the regional lymph nodes (70 Gy). The treatment of M1 patients consisted of androgen-suppressive therapy (oestrogens, orchiectomy). Progressive disease was defined by the development of distant metastases (in M0 patients) or by death due to prostatic carcinoma (M1 patients).

Samples of bone marrow (1-3 ml) were aspirated from the posterior iliac crest as previously described (7). The aspirate was divided into two parts. One part was used for smear preparation and

Table 1. Details of 61 Patients with Prostatic Carcinoma

Age (Years) (At BM-PAP Determination)	Mean	64.9
	Range	41-83
Interval Between Initial Diagnosis and BM-PAP Determination (Months)	Median	7
	Range	0-105
No. of Patients With Androgen-Suppressive Treatment		29
No. of Patients Without Androgen-Suppressive Treatment		32
Stage Distribution (At BM-PAP Determination)	T1-2, N0/X, M0	10
	T3-4, N0/X, M0	13
	T1-4, N1-4, M0	2
	T1-4, N1-4/X, M1	36
Localisation of Metastases	Elevated Se-PAP* Only	4
	Bone $\pm$ Other Met.	27
	Lymphnode $\pm$ Soft Tissue Met.	7
No. of M0 Patients With Equivocal Findings	Se-PAP* Only	4
	X-ray/Bone Scan $\pm$ Se-PAP	8
Follow-up after BM-PAP Determination (Months)	Median	5.5
	Range	0-31

* Determined by the Enzymatic Method

for histological sections of the aspirated particles. The other part was allowed to coagulate for 30 to 60 minutes at room temperature before centrifugation. The serum was collected and stored at -20°C until final analysis.

At the same time as bone marrow was aspirated a blood sample was obtained from a peripheral arm vein for determination of Serum-PAP (Se-PAP).

The radioimmunoassay of PAP was performed by an analysis reported previously (8,9). A highly purified and immunochemically controlled enzyme preparation has been used that gave rise to a high affinity antiserum upon immunization on rabbits. The radioimmunoassay used has compared favourably with commercially available radioimmunoassays

for PAP. (Upper reference limit for Se-PAP in healthy individuals: 3.0 ug/l) (8).

The present control group consisted of 45 cancer patients with diagnoses other than prostatic cancer. BM-PAP and Se-PAP samples were obtained and analysed by the same methods as used in the prostatic carcinoma group.

RESULTS

Figure 1 shows the frequency distribution of the BM-PAP and Se-PAP values in the control group. Based on the findings obtained, the upper reference limit of BM-PAP and Se-PAP was chosen at 28 µg/l and 4 µg/l, respectively. Two of the three patients with Se-PAP values above 4.0 µg/l had a pelvic recurrence of a rectal carcinoma. The third patient had a renal carcinoma.

In patients with M0 disease the median BM-PAP level was within the normal range for all T categories. The median Se-PAP, however, was pathological for the more advanced categories (Table 2a). Raised median BM-PAP and Se-PAP levels were found in hormonally untreated M1 patients (Table 2b). Androgen-suppressive treatment had only limited influence on the median BM-PAP and Se-PAP levels in M1 patients. In general, for the whole group of prostatic carcinoma patients the median BM-PAP was less often increased than the median Se-PAP (Table 2c).

There was no clear correlation between the Se-PAP and BM-PAP levels in the control group. (Correlation coefficient R=0.07, calculated by linear regression analysis in the log-log scale (Figure 2a). In M0 patients the correlation coefficient was R=0.39 (Figure 2b). In M1 patients a good correlation (R=0.86) was found between Se-PAP and BM-PAP, similarly for hormonally treated and untreated patients (Figure 2c). No clear relationship between BM-PAP/Se-PAP and future disease progression was noted.

Histological proof of metastases at the site of BM-PAP aspiration was obtained in six patients. The Se-PAP and BM-PAP levels were distributed over a wide range and were independent of hormonal treatment (Table 3).

Twelve M0 patients had equivocal findings, either regarding enzymatically analysed Se-PAP (>2.5 U/l up to 3.5 U/l) (four patients) or with regard to radiological examination and/or bone scan (eight patients). The Se-PAP and BM-PAP values, analyzed by RIA, are shown in Table 4a and 4b. Patient number four developed distant metastases nine months after the BM-PAP determinations. In this patient BM-PAP was within the normal range, whereas Se-PAP was elevated. The other patients were without evidence of progressive disease 1-24 months after the BM-PAP anlaysis.

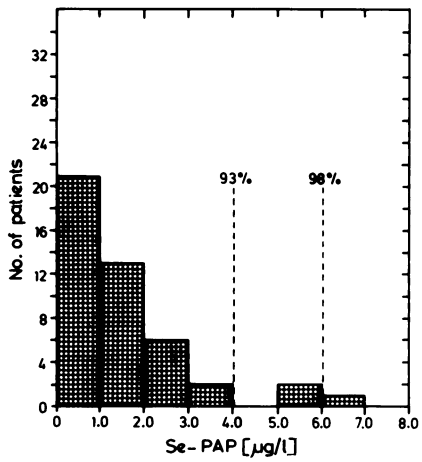


Fig. 1a. Prostatic acid phosphatase in Serum (Se-PAP), determined by RIA, in 45 patients with non-prostatic carcinoma (control group).

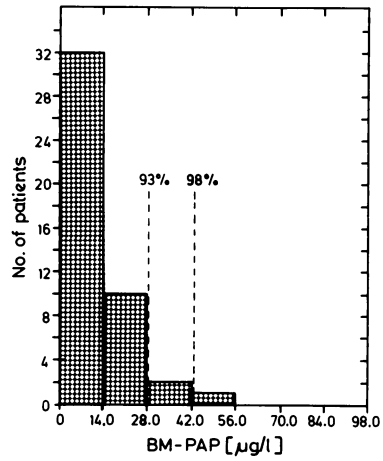


Fig. 1b. Prostatic acid phosphatase in bone marrow (BM-PAP), determined by RIA, in 45 patients with non-prostatic carcinoma (control group).

Table 2a. Prostatic Acid Phosphatase in Serum (Se-PAP) and Bone Marrow (BM-PAP) in 23 M0 Patients with Prostatic Carcinoma

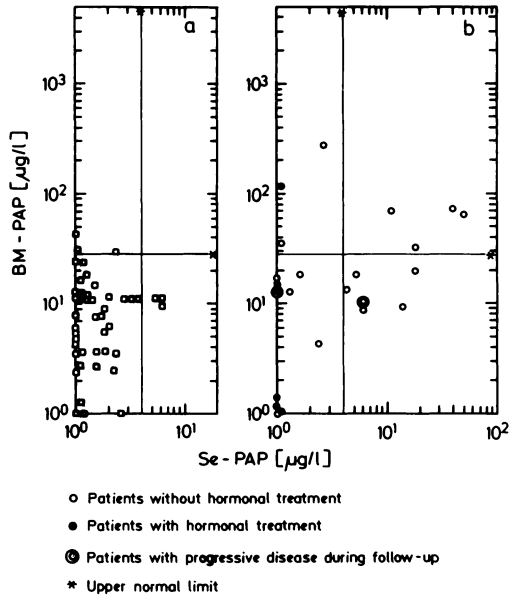
Category	Total No. of Patients	Patients with raised Se-PAP	Se-PAP (ug/l)		Patients with raised BP-PAP	BM-PAP (ug/l)	
			Median	Range (Min-Max)		Median	Range (Min-Max)
T1/2							
NX/0	10	2	1.3	0-14.2	2	13.0	0-279.0
M0							
T3/4							
NX/0	13	8	5.8	0-48.4	5	16.3	0- 73.7
M0							
T1-4							
NX/0	23	10	2.3	0-48.4	7	13.8	0-279.0
M0 (Total)							

Table 2b. Prostatic Acid Phosphatase in Serum (Se-PAP) and Bone Marrow (BM-PAP) in 38 M1 Patients with Prostatic Carcinoma

Category	Total No. of Patients	Patients with raised Se-PAP	Se-PAP (µg/l)		Patients with raised BP-PAP	BM-PAP (µg/l)	
			Median	Range (Min-Max)		Median	Range (Min-Max)
T1-4 N0/1-4/X M1 (Untreated)	13	12	20.3	0-129.8	7	30.3	3.0-313.5
T1-4 N0/1-4/X M1 (Treated)	25	16	14.7	0-310.0	11	23.5	2.2-1430.0
T1-4 N0/1-4/X M1 (Total)	38	28	17.9	0-310.0	18	25.0	3.0-1430.0

Table 2c. Prostatic Acid Phosphatase in Serum (Se-PAP) and Bone Marrow (BM-PAP) in 61 Patients with Prostatic Carcinoma

Category	Total No. of Patients	Patients with raised Se-PAP	Se-PAP (ug/l)		Patients with raised BM-PAP	BM-PAP (ug/l)	
			Median	Range (Min-Max)		Median	Range (Min-Max)
Total No. of Patients with Prostatic Cancer	61	38	9.0	0-310.0	25	19.4	0-1430.0



Figs 2a & 2b. Correlation between prostatic acid phosphatase in serum (Se-PAP) and bone marrow (BM-PAP), determined by RIA, in a control group of 45 patients with non-prostatic carcinoma and 23 patients with non-metastatic prostatic carcinoma.

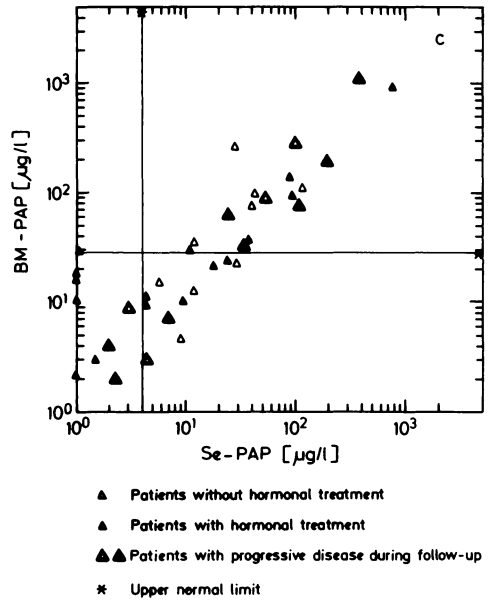


Fig. 2c. Correlation between prostatic acid phosphatase in serum (Se-PAP) bone marrow (BM-PAP), determined by RIA, in 38 patients with metastatic prostatic carcinoma.

Table 3. Prostatic Acid Phosphatase in Serum (Se-PAP) and Bone Marrow (BM-PAP) in 6 Patients with Prostatic Carcinoma and Histologically Proven Bone Marrow Infiltration at the site of Bone Marrow Aspiration

Identification		Androgen-Suppressive Treatment	Se-PAP (µg/l)	BM-PAP (µg/l)
No	26	No	3.0	7.7
	30	No	101.2	313.5
	32	No	27.1	176.0
	45	Yes	79.2	1199.0
	50	Yes	82.5	147.4
	53	Yes	42.9	95.7

Table 4a. Prostatic Acid Phosphatase in Serum (Se-PAP) and Bone Marrow (BM-PAP) Analyzed by RIA (µg/l) in M0 Patients with Prostatic Carcinoma and Slightly Elevated PAP in Serum ($\leqslant$ 3.5U/l). (Determined by the Enzymatic Method)

Identification		Androgen-Suppressive Treatment	Se-PAP (µg/l)	BM-PAP (µg/l)
No	1	No	48.4	64.9
	10	No	2.3	279.0
	16	No	14.2	9.4
	18	No	11.5	71.5

Table 4b. Prostatic Acid Phosphatase in Serum (SE-PAP) and Bone Marrow (BM-PAP) in M0 Patients with Prostatic Carcinoma and Equivocal Findings on X-Ray/Bone Scan

Identification		Androgen-Suppressive Treatment	Se-PAP (µg/l)	BM-PAP (µg/l)
No	2	Yes	0	13.8
	4*	No	6.4	11.8
	6	No	5.4	17.2
	8	No	18.2	33.0
	9	No	39.6	73.7
	11	Yes	0	12.1
	13	Yes	1.1	144.0
	23	No	2.2	4.4

* Patient who developed bone metastases during follow-up

DISCUSSION

For the control group of this study the upper reference limit of BM-PAP was found to be seven times higher than that of Se-PAP. This may indicate that the specificity of the RIA used is not as high as attempted. Acid phosphatases of non-prostatic origin, probably from bone marrow cells, seem to be partially determined by the RIA used. The amount of these phosphatases probably varies in the different samples, mainly due to inter-patient variations in the amount of peripheral blood in the bone marrow aspirate. Thus we recognise some of the same problems as observed when using the enzymatic method for BM-PAP analysis (7).

Treatment did not seem to have significant influence on BM-PAP and Se-PAP levels. However, most of our hormonally treated patients were patients whose disease had become hormone-resistant, after primary response to hormonal manipulation. The BM-PAP and Se-PAP levels may have risen again after an initial post-treatment decrease.

In symptomatic M1 patients the results of BM-PAP analysis correlated well with the serum values, but did not give any information to the clinician in addition to the information that was already obtained by the Se-PAP determination and the routine clinical/radiological examination of the patient. In particular, the BM-PAP levels, determined by RIA, did not seem to be of better prognostic value than the Se-PAP levels when survival curves were compared.

It was hoped that BM-PAP determination could indicate those M0 patients who had occult bone metastases and/or undetected lymph node metastases, especially in "borderline" cases with equivocal findings. Although the number of M0 patients examined is limited (only two M0 patients had progressive disease), the results of BM-PAP determinations so far do not seem to be correlated with the future development of bone metastases. Se-PAP analysis is probably more useful for indicating those high-risk patients (see patient no. 4, table 4b). Only by a longer follow-up period of M0 patients with known Se-PAP and BM-PAP values can the clinical significance of elevated BM-PAP and Se-PAP levels in M0 patients be determined definitely.

Bone metastases from an unknown primary tumour often present a diagnostic problem for the clinician. We had hoped that BM-PAP determinations from the metastatic area could indicate a possible prostatic origin in such patients. Indeed, five of six relevant BM-PAP analyses resulted in elevated levels. However, the Se-PAP levels were also significantly raised in these patients. Therefore the BM-PAP determination was in reality unnecessary for the diagnosis of prostatic cancer as it could have been considered on the basis of the raised Se-PAP level alone.

CONCLUSION

1. The results of BM-PAP determinations in a control group of cancer patients with non-prostatic carcinoma have indicated that the specificity of RIA for PAP in bone marrow samples is less than expected.

2. BM-PAP determinations by radioimmunoassay in M1 patients with prostatic carcinoma give no information in addition to that available from Se-PAP clinical/radiological examinations.

3. Even in M0 patients with prostatic carcinoma the clinical significance of BM-PAP analysis is most probably very limited. Further studies are, however, needed to define the role of BM-PAP determinations in M0 patients.

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STUDIES OF ACID PHOSPHATASE IN PROSTATIC CANCER

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ABSTRACT

Various analyses of data gathered in the VACURG studies demonstrate that the extent of elevation of the pre-treatment acid phosphatase correlates with the probability of having detectable metastases and with survival. Patients with increased acid phosphatase but without evidence of metastases have a worse prognosis than patients with normal acid phosphatase and no metastases. For patients in stages I and II treated with radical prostatectomy, even values in the normal range were correlated with time until progression of tumor. These findings suggest that increased acid phosphatase values probably represent undetected metastases.

Recent reports of more sensitive and specific immunologic methods for measuring acid phosphatase (1-4) have stimulated new interest in the diagnostic and prognostic importance of this serum enzyme. For this reason we decided to re-examine data collected in the large randomized clinical trials of treatment of prostatic cancer conducted by the Veterans Administration Cooperative Urological Research Group (VACURG). In all studies by that group, only newly diagnosed patients were admitted. The diagnostic work-up included digital rectal examination, a skeletal survey using ordinary x-ray techniques and a determination of the so called "prostatic fraction" of the serum acid phosphatase as measured in King-Armstrong Units (KAU). All acid phosphatase determinations were performed in a central reference laboratory under the direction of Dr U.S. Seal. Earlier studies had established 1.0 KAU as the upper limit of normal for the prostatic fraction determined by inhibition with L-tartrate, hereafter referred to simply as acid phosphatase. Radioactive scans,

lymphangiograms, and laparotomies were not used in staging the patients. Stage I refers to patients who have no abnormal findings on rectal examination, and Stage II refers to those patients in whom palpable cancer confined to the prostate gland can be detected on rectal examination. In both stages the acid phosphatase values are normal and there is no evidence of distant metastases. Stage III refers to patients with tumors extended locally beyond the confines of the prostate gland but who have normal values of acid phosphatase and no x-ray or clinical evidence of distant metastases. Stage IV refers to patients who have either elevated acid phosphatase, evidence of distant metastases to bone or soft tissue, or both. Data reported in this article are taken from all three of the major clinical trials of the VACURG (5).

We first wanted to examine the relationship between the acid phosphatase at diagnosis and the presence of detectable metastases. For this purpose we tabulated the acid phosphatase into various categories and computed the percentage of patients with metastases in each category using data from patients in all four stages of Study 1. These data (Table 1) indicate that there is a strong correlation between the acid phosphatase value at diagnosis and the probability that metastases will be found. Note for example that in the top half of the normal range (0.6-1.0 KAU) only 7.6% of patients had metastases, but when the acid phosphatase rises slightly to values between 1.1 and 2.0 KAU, the probability of metastases rises dramatically to 17.6% and continues to increase thereafter reaching a value of 76% for patients whose initial acid phosphatase was between 50 and 100 KAU. From the data in Table 1 it can be calculated that the relative odds of having detectable metastases for patients whose initial acid phosphatase is elevated ($>$ 1.0 KAU) compared to those in the normal range (0-1.0 KAU) is 11.78. This can be interpreted as meaning that patients with elevated values of acid phosphatase are almost 12 times more likely to have detectable metastases. A similar calculation using 10.0 KAU as the cut-off value produced an odds ratio of 18.3.

Using data from the same study for stage III and IV patients, we examined the effects of endocrine treatment on the acid phosphatase measurements after six months of treatment. The treatments randomly assigned in that study for stage III and IV patients were placebo, 5.0 mg of diethylstilbestrol (DES), orchiectomy plus placebo, or orchiectomy plus 5.0 mg of DES. The placebo and estrogen treatments were administered daily by mouth as a single tablet. Changes in acid phosphatase values at six months were categorized by computing the percentage change between the initial and six month values, namely the six month value minus the initial value divided by initial value. These percentage changes were then classified as <u>increased</u> if the ratio just described was 0.5 (an increase of 50% or more), <u>same</u> if the value was between $\pm$0.5, and <u>decreased</u> if the ratio was -0.5 (50% or more decrease). These results (Table 2) show that endocrine

Table 1. Relationship of Initial Acid Phosphatase to Presence of Metastases in Study 1.

Initial Acid Phosphatase (KAU)	N	Percentage of Patients With Metastases
0.0 - 0.5	948	5.0%
0.6 - 1.0	527	7.6%
1.1 - 2.0	238	17.6%
2.1 - 5.0	185	29.2%
5.1 - 10.0	112	44.6%
10.1 - 20.0	80	62.5%
20.1 - 50.0	89	68.5%
50.1 - 100.0	50	76.0%
Greater than 100.0	70	78.6%

Table 2. Effect of Treatment on Acid Phosphatase at Six Months in Stage IV of Study 1.

Treatment	N	Acid Phosphatase		
		Increase	Same	Decrease
Placebo	148	35%	37%	28%
Estrogen	157	4%	13%	83%
Orchiectomy + Placebo	152	4%	12%	84%
Orchiectomy + Estrogen	161	1%	12%	86%

treatment produces a decrease of 50% or more in the initial acid phosphatase in about 85% of the patients, whereas a decrease of this magnitude was only seen in 28% of patients assigned to placebo. These data dramatically demonstrate that endocrine therapy has a direct effect on the prostatic cancer cells; the decreases in acid phosphatase probably reflect a decreased rate of manufacture or secretion of the enzyme by the cancer cells, resulting from the lowered cellular metabolism of the hormone-sensitive tumors following endocrine therapy (6). Although the data presented in Table 2 are not broken down by the levels of the pretreatment acid phosphatase, other analyses not presented here revealed that endocrine treatment produced a 50% or greater fall in the acid phosphatase at six months for about 85% of patients no matter what the initial value was.

The staging system used in the VACURG studies is based on the assumption that elevated values of the acid phosphatase represent undetected metastatic disease, and for that reason, patients having such elevations were classified as stage IV. This viewpoint, although it has been suggested in the literature by many authors, is not universally accepted, especially since the advent of the new immunological methods of measuring acid phosphatase. Because these newer methods find substantial percentages of patients with elevations in what appear to be clinical stages I, II and III, it was hoped that these more sensitive assays could help in identifying patients who might be suitable candidates for radical surgical treatment or curative x-ray therapy (7). The data collected in the VACURG studies allow us to examine the prognostic significance of elevated acid phosphatase measurements in the absence of detectable metastases, but it must be remembered that neither bone scans, lymphangiograms, nor laparotomies were used in staging the patients. The reasons for being classified as stage IV in Study 3 are given in Table 3. Note that 62.4% of the patients in stage IV were so classified because of elevated acid phosphatase alone. By combining categories in Table 3 we can compute that 85.6% of patients with demonstrable metastases (bone, soft part, or both) had elevated acid phosphatase.

The prognostic significance of acid phosphatase elevations with or without demonstrable metastases is examined in Table 4 where death rates for various categories of patients are displayed. The death rate is defined as the number of deaths per 1000 patient-months of observation for all patients combined, whether they died or not. Rates have been computed separately for all causes of death, for deaths due to prostatic cancer, and for cardiovascular deaths. In the latter two analyses deaths from other causes are treated as withdrawals from observation at the time of death. The determination of the cause of death was made by a committee of clinicians who were unaware of the treatment assignment. The term "cardiovascular deaths" includes deaths from myocardial infarcts, congestive heart failure, pulmonary emboli, cerebrovascular accidents, and other

Table 3. Reasons For Classification As Stage IV in Study 3.

Bone Mets	Soft Mets	Acid Phosphatase 1.0 KAU	N	Per Cent
X			19	4.1
	X		4	0.9
		X	289	62.4
X	X		2	0.4
X		X	116	25.1
	X	X	16	3.5
X	X	X	17	3.7
			463	100.1

Table 4. Death Rates for Patients in Stage III and Various Categories of Stage IV in Study 3.

	Category	Deaths per 1000 patient-months			
		N	All[1]	CAP[2]	CVD[3]
Stage III	Normal acid phosphatase and no metastases	531	11.8	2.1	5.1
	↑ Acid phosphatase only	289	19.7	8.8	5.0
Stage IV	Metastases only	25	19.5	9.0	4.5
	Both metastases and ↑ acid phosphatase	149	32.7	21.5	5.6

[1] All causes of death

[2] Deaths from prostatic cancer

[3] Deaths from cardiovascular causes

cardiovascular causes. The data in Table 4 refer to the same patients in stage IV as those shown in Table 3. Examination of the death rates for cardiovascular causes reveals no appreciable differences between the four groups. However, both for all causes of death combined and for deaths due to prostatic cancer, death rates are increased when either the acid phosphatase is elevated or detectable metastases are present, and these rates are even higher when both findings are present. The increased death rates are much more striking when we examine cancer deaths only because the results for all causes of death are diluted by deaths from cardiovascular and other causes. These data clearly indicate that patients with elevated acid phosphatase alone have a worse prognosis than patients in stage III, an important factor to keep in mind when reviewing published treatment results for stage III patients from centers who do not take the acid phosphatase into account in their staging system.

Recent reports indicate that the more sensitive and specific immunological methods for measuring acid phosphatase have detected elevations in substantial numbers of stage I and II patients (1-4). These findings stimulated us to analyze our data for patients whose acid phosphatase values were in the normal range to see whether very small elevations might possibly represent undetected metastases. As mentioned earlier, the normal cut-off value in our studies was 1.0 KAU, but this figure, like any normal cut-off value, must be regarded as arbitrary to some extent. We decided to limit our attention to patients who were treated by radical prostatectomy because the operation would presumably have removed all the disease if it were confined to the prostate gland. In order to get large enough numbers of patients, we combined data from all three VACURG studies for patients in stages I and II who were treated by radical prostatectomy. We excluded patients who received estrogens as initial therapy in addition to the prostatectomy. Since there were so few deaths due to prostatic cancer in stage I and II patients, we decided to use a different end point in assessing the prognostic significance of the acid phosphatase. This was the rate of progression of the disease, expressed as the number of patients who progressed per 1000 patient-months of observation for all patients under study whether they progressed or not. Progression of disease was defined at the first appearance of definite metastases, at the first elevation of the acid phosphatase >2.0 KAU, or at death due to prostatic cancer. In fact, all patients who died of prostatic cancer had earlier had both an increase in acid phosphatase and the appearance of metastases. The value 2.0 KAU was chosen after noting that for some patients the acid phosphatase might hover in the range between 1.0 and 2.0 without a further rise later. It was thought that using a value below 2.0 KAU would result in a serious overestimation of the proportion of patients progressing. The results of this analysis (Table 5) shows that, even in the normal range, initial acid phosphatase values are correlated with the progression rate. Even

Table 5. Progression Rates by Categories of Acid Phosphatase in the Normal Range for Patients in Stages I and II of Studies 1 and 2 Treated by Radical Prostatectomy.

Initial Acid Phosphatase	No. of Patients	No. Progressing	Progression Rate[1]
0.0-0.3	58	3	0.58
0.4-0.5	68	9	1.68
0.6-1.0	62	11	2.15

[1] Number progressing per 1000 patient-months of observation.

though the numbers progressing are relatively small, a statistical test for trend for these three rates across the categories of acid phosphatase was significant at $p = 0.02$ (one-tailed test). If we use first appearance of metastases alone as evidence of progression, ignoring rises in acid phosphatase, the results are still significant at $p = 0.04$ (one-tailed test). Admitting that the fraction of the acid phosphatase inhibited by L-tartrate is not precisely the same as that detected by immunological methods, our data suggest that slight rises in the acid phosphatase indicate patients in whom the cancer has already spread, because all those patients had radical prostatectomies. If the tissue producing the acid phosphatase in the serum had been confined to the prostate gland, then no prognostic significance for the initial acid phosphatase measurements would have been observed. It appears then that rather than detecting patients who might potentially be curable by surgery or other localized modalities of treatment, even small elevations of the acid phosphatase may indicate that the disease has already spread.

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THE CLINICAL VALUE OF THE SERUM ACID PHOSPHATASE IN CARCINOMA OF THE PROSTATE

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INTRODUCTION

A phosphatase which was particularly active at an acid pH was shown to be present in red blood cells in 1924 (1). Acid phosphatases are also present in leucocytes, platelets, osteoclasts, reticuloendothelial cells, and in liver, kidney, spleen and other organs. Their clinical importance derives from the fact that the concentration of the enzyme is far greater in the prostate than in any other human tissue (2).

The relevance of acid phosphatase to the patient first became obvious in 1936 when Gutman and his colleagues showed that it was found not only in normal prostatic epithelium but also in prostatic cancer (3), being present both in primary tumours and in secondary deposits. There is less activity in carcinomatous tissue than in normal prostate, and less still in metastases (4), loss of activity being correlated with de-differentiation.

The presence of acid phosphatase in the serum of some patients with metastasising adenocarcinoma of the prostate was first demonstrated in 1938 by Gutman and Gutman (5), and since that time a great deal of attention has been paid to its use in the diagnosis and management of prostatic cancer.

One problem which presented itself at once was the separation of acid phosphatases arising from various sources. Prostatic

acid phosphatase can be distinguished from that arising from blood elements because it is inhibited by L-tartrate. Unfortunately L-tartrate also inhibits the enzymes derived from spleen, liver and kidney. Formadehyde, in contrast, inhibits the activity of red cell acid phosphatase without affecting the prostatic enzyme (6). Despite the introduction of these, and other inhibitants, enzymatic methods for determination of acid phosphatase have not proved capable of localising the prostatic enzyme precisely (7), and, in an attempt to increase the specificity of the analysis, two further techniques have been introduced.

MEASUREMENT OF ACID PHOSPHATASE IN BONE MARROW

Elevation of the bone marrow acid phosphatase has been claimed to be an early sign of metastatic spread of carcinoma to bone (8-12). Initial reports suggested a good correlation between the presence of metastases and an elevation of the bone marrow acid phosphatase level. Gursel et al, for example, noted the development of bone metastases in 5 of 16 patients in whom an elevated bone marrow acid phosphatase was the only abnormal finding (9). Unfortunately, there have been problems with false positives (13, 14), especially when enzymatic methods were employed (15), and at the present time it seems clear that bone marrow acid phosphatase determination by enzymatic techniques is of very little value (16, 17).

The use of radio-immuno assay for the measurement of bone marrow acid phosphatase may eliminate some of the false positive results (15,18). The importance of detecting bony metastases in patients whose prostatic lesions might be cured by radical therapy is obvious, and the radio-immuno assay deserves further attention (18). This subject will be reviewed by Fossa (19).

MEASUREMENT OF ACID PHOSPHATASE BY IMMUNOLOGICAL METHODS

Prostatic acid phosphatase has been demonstrated to be antigenically distinct from acid phosphatase arising elsewhere (20), and a specific antibody can be raised against it. The acid phosphatase in tumour tissue appears to be antigenically similar to that obtained from normal prostate (21) and similar also to that obtained from expressed prostatic fluid and from semen, both of which have been used as a source of antigen.

Two techniques are available for measuring prostatic acid phosphatase. In the first a radio-immuno assay (RIA) is used which measures the total antigen (22). Foti and his colleagues improved the procedure which they had originally used by changing to a solid phase technique. The assay depends upon competition of labelled and unlabelled antigen for antibody binding sites. The second depends on counter immuno electrophoresis (CIEP) and was

developed by Chu and his colleagues (23). The antigen and antibody move in opposite directions in an electrophoretic field, precipitating when they meet. The enzymatic activity of the precipitation line can be determined. There is no doubt that these techniques and their subsequent refinements (24,25) give rise to improved sensitivity - the probability that the blood test will be positive in patients with proved prostatic cancer - and specificity - the probability that the test will be negative in men already known to be free of disease - when compared with enzyme assays, and they have led to a wave of enthusiasm suggesting that measurement of prostatic acid phosphatase would detect cancer of the prostate before it had given rise to metastases - while it was still curable by local treatment. At first sight it seems very unlikely that this could be the case. The level of acid phosphatase in prostatic cancer tissue is considerably less than that in a normal prostate, and it would be surprising if a small focus of tumour gave rise to a detectable signal.

In the light of these expansive claims for the value of acid phosphatase measurement, it seems appropriate to examine critically the various uses to which it has been put. It may be employed in one or more of three ways:

1. As a diagnostic tool
   (a) in screening for the disease
   (b) in staging

2. As an indicator of prognosis

3. As an indicator of response to therapy.

SERUM ACID PHOSPHATASE (SAP) IN THE DIAGNOSIS OF PROSTATIC CANCER

Its use in diagnosis is marred by the fact that it is rarely elevated until metastases are present. Abnormalities occur when the disease is still localised to the prostate with greater frequency using radio-immuno assay than when enzymatic techniques are employed; but this must, in part, be related to the range which is accepted as normal. False positives (abnormal levels of serum acid phosphase in the absence of carcinoma of the prostate) are distinctly rare using enzymatic techniques, but were noted in between 3 and 27% of cases using radio-immuno assay (26). The incidence of abnormalities found in the various stages of the disease in a number of series is listed in Table 1.

These figures, and especially the false positives, are relevant to the enthusiastic claims for the use of serum prostatic acid phosphase as a screening test for prostatic cancer (30,31,32). It has been pointed out by Watson and Tang (33) and by Bruce and

Table 1: The percentage incidence of raised serum acid phosphatase at different stages of the disease

	Radio-immuno assay				Enzymatic ass
Stage	Foti (22)	Murphy (27)	Griffiths (28)	Bruce (26)	Woodward (29)
A	33	38	12	14	)<10
B	79	35	32	29	)
C	71	49	47	24	31
D	92	89	86	89	74

his colleagues (26) that the usefulness of a laboratory procedure for screening depends not only on the sensitivity and specificity of the test but also on the prevalence of the disease within the population tested. Given this, one can calculate the positive predictive value, the probability that a person with a positive blood test actually has the disease, and the negative predictive value, the probability that a person with a negative blood test is in fact free of disease. Combining the data of Foti and his colleagues (22) with the prevalence of prostatic cancer of 35 per 100,000 in the white male population of the United States, Watson and Tang calculated that only one in 244 subjects with a positive test would actually have a carcinoma of the prostate, one in 526 if one excluded subjects with a prostatic cancer that could be felt digitally.

Estimation of the serum levels cannot be recommended for the indiscriminate screening of men for prostatic cancer (26,33). Nevertheless, selection factors may be combined with prostatic acid phosphatase measurements to provide helpful information. Rectal examination is the basic clinical procedure used to screen patients for the possibility that they have prostatic carcinoma. Radio-immuno assay of prostatic acid phosphatase has a much better predictive value here. Watson and Tang have calculated, again using the sensitivity and specificity reported by Foti (22) that a patient with a prostatic nodule and a positive test result would have, at the time of detection, a 93% probability of carcinoma. A patient with a nodule and a negative result would have an 84% probability of having no tumour (33). Not everybody has been able to match the sensitivity and specificity rates reported by Foti and his colleagues. Even so, the technique appears to have much to offer in the evaluation of such patients.

Paradoxically, the value of acid phosphatase determinations in the diagnosis of metastatic disease has been reduced by the

increased sensitivity of the radio-immuno assay. Elevations of the serum acid phosphatase have been reported while the cancer is still confined within the prostatic capsule in 8% (15), 30% (23) and even 79% (22) of patients. These differences reflect, in part, the rigorousness of staging procedures (34), but it appears that the radio-immuno assay is positive in at least 10% of locally operable cases even when the staging technique is rigorous and includes pelvic lymphadenectomy (15). When this is considered together with the fact that the acid phosphatase is not always raised, even when the patient is known to have advanced disease (in 5% (15), 20% (23), 40% (25)), it can be seen that the test is too unreliable to be used as a definitive guide to therapy (35). It remains useful as a general guide, as most patients with a high acid phosphatase have advanced disease. Hopes that the bone marrow acid phosphatase would be more specific for the detection of early metastases to bone (8) have not stood the test of time (14-17).

SERUM ACID PHOSPHATASE AS AN INDICATOR OF PROGNOSIS IN PROSTATIC CANCER

Since the serum acid phosphatase is more frequently elevated when the disease is advanced, it would be hardly surprising if patients who had a raised SAP at the time of diagnosis tended to die sooner than those who did not, and this has been found to be the case in several studies (36-39). Curiously, in a recent EORTC study comparing the efficacy of Stilboestrol and Estramustine Phosphate as primary treatments for patients with advanced prostatic cancer, there was no prognostic advantage in having a normal acid phosphatase (40).

SERUM ACID PHOSPHATASE AS AN INDICATOR OF RESPONSE TO THERAPY

There remains the possibility that serum acid phosphatase may be used as an indicator lesion in monitoring the course of the disease and its response to therapy. It has been used in this way in the past. Huggins and Hodges used it to demonstrate that carcinoma of the prostate is hormonally responsive, regressing after orchidectomy or oestrogen therapy and growing more rapidly with testosterone (41). In their original paper, these workers made the assumption that an elevation of acid phosphatase was associated with advancing prostatic cancer, and that a fall in its level indicated a remission. Various other authors have made the same assumption, although the correlation is not always present. For example, Franks noted progression of prostatic cancer in one of seven patients having a fall of acid phosphatase. The others had stable disease or responded to therapy (42).

Assessment of the response of prostatic cancer to treatment is notoriously difficult because measurement of the local disease is

Table 2: Criteria of Evaluation of Response in EORTC Protocol 30762

Evaluation of Local Response

1. Complete Response. The disappearance of any local prostatic tumour.

2. Partial Remission. 50% or more decrease in tumour size (length x width).

3. No Change. Less than a 50% decrease or increase in the size of the prostatic tumour.

4. Progression. A 50% or greater increase in tumour size (length x width).

Evaluation of Response in Bony Metastases

1. Complete Response. Complete disappearance of all lesions on X-ray and bone scan.

2. Partial Response. Disappearance of one or more lesions seen on bone scan or X-ray with no increase in any of the others and no new lesions.

3. Progression. The appearance of new lesions on or X-ray at a site remote from known lesions.

4. No change. No new lesions and no disappearance of known lesions.

inexact and because of uncertainty about evaluating changes in bony metastases. All phase II trials require that the patient has an indicator lesion. Acceptable indicator lesions are few in prostatic cancer and it is very important to know whether the acid phosphatase is one or not.

The EORTC has just completed a study (30762) in which Diethyl Stilboestrol and Estramustine Phosphate are compared in previously untreated patients with advanced prostatic cancer. Acid phosphatase was measured each time the patient was examined but has not been used in evaluating response, the criteria of which are listed in Table 2. The acid phosphatase was measured in the local laboratory of the centre submitting the case usually by enzymatic methods. The upper limit of normal was that quoted by the local laboratory. Repeated acid phosphatase results can be correlated with local response in 172 cases, and with the response in metastatic disease (as determined by an independent review of scans and X-rays) in 50.

Two groups are apparent, those with an elevated acid phosphatase at entry to the study, and those in whom it is normal. Of cases suitable for evaluation of the local response, 44 had a SAP more than twice the upper limit of normal at entry to the study. Table 3 compares the maximum changes in SAP with the response in these patients. It will be noted that progression of the local carcinoma was noted in four of 37 cases in which the serum acid phosphatase fell. Only 22 cases have been analysed from the point of view of the response of distant metastases. Once again, progression of the cancer is noted despite a falling SAP in about 10% of cases.

Table 4 shows the results in patients whose acid phosphatase was normal at entry to the study. In 108 cases suitable for evaluation of the local response, four progressed without a change in the normal acid phosphatase. In 13 cases there was an elevation of acid phosphatase. Of these, only one showed progression. Similar changes are seen for the 20 patients with metastases in bone. Again, the correlation is poor.

The data have been analysed so far with reference to the changes in SAP. If they are considered from the point of view of the clinical response to treatment, once again two groups of interest can be isolated, those that show remission on treatment and those that show progression on treatment.

In 57 cases there was remission of tumour within the prostate

Table 3: Correlation between SAP at the time of its greatest change and the clinical response - cases with SAP greater than twice normal at entry to study.

	Cases evaluable for response of the primary tumour (N = 44)		Cases evaluable for response of metastases in bone (N = 22)	
	SAP stayed elevated	SAP fell	SAP stayed elevated	SAP fell
Progression	0	4	1	2
No change	5	21	2	7
Remission	2	12	0	10
Total	7	37	3	19

Table 4: Correlation between SAP at the time of its greatest change and the clinical response - cases with SAP normal at entry to the study

	Primary Tumour (N = 108)		Metastases in bone (N = 20)	
	SAP stayed normal	SAP rose	SAP stayed normal	SAP rose
Progression	4	1	1	1
No change	59	9	11	2
Remission	32	3	5	0
Total	95	13	17	3

on treatment. Here the correlation with SAP was good. The SAP was elevated in 22 (to above twice the normal figure in 15) and fell to normal in all cases. In the remaining 35 it was normal at entry to the study, stayed normal in 32 and rose to above twice normal in only one patient. In nine patients progression of the disease occured. The acid phosphatase rose in only one of these. It fell to normal in four cases, despite progression, and stayed normal in the other four.

It is clear that a fall in SAP correlated well with the remission of the disease as determined by other parameters. However, a fall in acid phsphatase does not mean that the patient is in remission. In fact the SAP fell to normal in association with progression of the local disease in all four cases in which it was initially elevated.

The treatment (Stilboestrol or Estramustine Phosphate) given in this study appears to reduce the acid phosphatase in the majority of cases. Changes in its level are largely independent of the progression of the disease and it is clear that it cannot be regarded as an indicator lesion by which the response of prostatic cancer can be judged in phase II trials.

SUMMARY

The clinical significance of normal and abnormal serum acid phosphatase levels is constantly being re-evaluated. Despite the greater sensitivity of the newer techniques, it has a very limited role as a screening test, and can give a rough guide only about the presence of metastases. It is of very little value as an indicator of tumour progression and its main value appears to be

the traditional one, that of screening patients with doubtful nodules, to assess the probability that the nodule is a primary or secondary prostatic cancer.

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PROSTATIC CANCER: ADVANCES IN DIAGNOSIS

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Prostatic cancer constitutes one of the most commonly diagnosed cancers in older males (1). The accumulated information concerning this disease is enormous but we are still unable to diagnose the disease in the early stages, to assess the extent of disease in a non invasive way or to select treatment according to the lethal potential of the tumor. This paper brings data together that have or seem to have value in establishing answers to these three questions. Any of these problems, if answered, would represent a true advance in diagnosis. Physical examination, histological confirmation by biopsy, medical imaging techniques and prostatic tumor markers have been selected for presentation.

1. Physical Examination

Early prostatic cancer offers no symptoms suggestive of the disease and there is a general consensus that a careful rectal examination is required in all men over 50 years on a yearly basis. The first problem is that non neoplastic disorders such as inflammatory processes may mimic the induration which suggests prostatic carcinoma. However, induration presenting as a nodule has the probability of being neoplastic in about 50 per cent of the patients (2). This is a rather rare event and applies to a selective subpopulation of patients with prostatic cancer. We feel that any area of induration in the prostate felt on rectal examination should be biopsied. We apply this simple rule to our patient population presenting with urological symptoms; because of this the detection of unsuspected cancer at the time of surgery is a rare event. Out of 100 consecutive patients with prostatic cancer only two escaped preoperative diagnosis. Scientific support for this observation has been provided by Guinan et al (3) and Catalona (4)

who biopsied respectively 300 and 70 consecutive patients admitted for a variety of urological conditions. In both series digital rectal examination proved to be a most efficient test in the diagnosis of prostatic cancer.

The efficiency of the rectal examination in prostatic cancer has also been evaluated in randomized prospective studies. In a subpopulation of the VACURG study (5) rectal examination was presented as 50 per cent correct in diagnosis in contrast to the EORTC studies where this examination got better marks (6). It should be noted that early cancers abound in the former series. The score for the correlation between rectal examination and prostatic cancer is even better in the famous Bowery series of Hudson et al (7). Of 39 cases of prostatic cancer detected by open perineal biopsy in a consecutive series of 300 symptomatic and asymptomatic men only four did not show induration of the prostate on physical examination. All four men had cancer limited to the prostate.

A second problem is the false negative prostatic needle biopsies (with the exclusion of microcarcinoma which should not be evaluated as a clinical cancer) (8). Repeat biopsy is advocated if additional suggestive evidence for prostatic cancer is present or no histological explanation for the induration is provided.

2. Histological Confirmation by Biopsy

Ever since Broders assigned a grading system to tumor differentiation we have been repeatedly warned by our pathologists about the difficulty of classification in such a notoriously heterogeneous tumor as prostatic cancer (9,10). A breakthrough in this respect is the clinical application of the Gleason scale (11) in the prediction of positive lymph nodes as demonstrated by Paulson et al (12). The addition of nuclear morphology as a separate grading factor seems to relate to the biological potential of the tumor (13). This correlates with our personal experience in 217 patients where tumor grade based on histological and cellular differentiation predicted mortality rates in a reliable pattern (14).

An important aspect of the pathological evaluation concerning the well differentiated cancers is the fact that perineural invasion may occur in normal prostates (15). It is apparent from available data that all biopsy material in a clinical situation should be examined jointly by the pathologist and the urologist and that pathology material in randomized prospective studies should be reviewed by a central pathology laboratory. The biopsy material is usually obtained by needle biopsy but transurethral resection may serve the purpose in selected instances. The division of the tissue according to resected areas improves the overall accuracy and assessment of the existing pathology (16). Repeated biopsies are necessary in selected instances as only positive biopsies are valid; transrectal

aspiration biopsy submitted to an experienced cytologist may improve on the diagnostic sensitivity.

Testing for detection of the blood group antigens (17) or association to human leucocyte antigens (18) proved inconsistent in predicting biologic aggressiveness. Pelvic lymphadenectomy on the contrary has been demonstrated to give significant information on the prognosis of the disease (19). It is of interest to note that limited lymphadenectomy provides similar information without considerable morbidity to the patient (20).

3. Medical Imaging

Two new non invasive techniques, ultrasonography (US) and computerized axial tomography (CT), have been applied to the diagnosis and staging of prostatic cancer (21,22,23). The different techniques and applications are reported elsewhere. Transrectal ultrasonography in experienced hands provides better efficiency in diagnosis and staging of prostatic cancer (24) and it is now well established that transrectal ultrasonography as advocated by Watanabe is the preferred technique for prostatic examination (25).

Ultrasound and CT constitute the only available cross-sectional imaging in urological radiology. A comparison of different aspects of the two techniques is given in Table 1. Basically CT scan utilizes the penetration of x-ray photons and detects differences in atomic composition. Its image resolution in a complete cross-section of the abdomen is unsurpassed. One problem is that the attenuation between tumoral and normal tissue is so small that detection is difficult because of the isodensity. The presence of fat or iodinated contrast agents helps to distinguish organ contours or to detect unusual masses. Visulisation of ultrasound waves reflects the elastic properties on a macromolecular structural level. Echo texture differences are visible between tissues of different composition and the main restriction in the abdomen is the presence of gas, bone or obesity.

Our experience based on more than 1,500 US examinations with five different improvements of technical material and on more than 200 CT examinations with three generations of CT scanners in patients with lower urinary tract pathology allows us to draw the following conclusions.

1. Both techniques cause minimal complications but radiation hazard and cost makes the CT scan less attractive for a routine work up.
2. The diagnostic criteria of prostatic tissue pathology are well established in transrectal ultrasonography (Table 2) and allow early diagnosis of prostatic cancer. This is impossible to diagnose on a CT scan. An example of a localized T2 tumor is

represented in Fig. 1. It should be clear that small well-differentiated carcinomas are easily missed and that areas of prostatic infection may mimic the echo pattern of prostatic cancer, especially in the newer real time technology. The echo pattern of prostatic cancer invites us to carry out a selective biopsy under visual control (26). The sensitivity in our hands oscillates around 80% without other information on the patient. This delays the possibility for mass screening by this technique though Watanabe reached a rate of sensitivity of 99.7% (27).

3. Tumor extent is appreciated in US by distortions of the prostatic capsule and by non-specific echo patterns in the seminal vesicles (Fig. 2). The mass of reflections is frequently continuous with a proven tumor in the prostate. This type of early infiltration or capsule distortion is difficult to visualize on CT.
4. Evaluation of prostatic mass is possible in both techniques by scanning at different levels to reach a tomographic reconstruction. These data are easy to obtain with US and were more reliable in our hands than with automated CT determination. For practical clinical purposes however where mass definition serves as a parameter in regression or progression of disease both methods are valid.

Table 1. Different Aspects of Ultrasound and Computerized Tomography in Prostatic Imaging

Transrectal U.S.	C.T. Scan
Sound (3.5 MH_Z)	X-rays
Structural Elasticity	Electron Density
Gas, Bone, Obesity	Needs Contrast
No Side Effects	Radiation
Technical Skill	Automatic
Artifacts	Software
Available	Restricted
Minimal Cost	Expensive
Anatomy Good	Excellent
Histology Good	Fair

Table 2. Diagnostic Criteria. Transrectal Ultrasonography in Prostatic Pathology

	Normal Prostate	Peri-Urethral Adenoma	Prostatic Cancer
Transverse Section	Triangular Semilunar	Half/Full Circle	Deformations at Different Levels
Symmetry	Present	Usually Present	Absent
Capsule	Thin/Even	Thick/Regular/Even	Interrupted/Uneven Distorted
Sonic Density	Minimal	Present	Increased
Pattern	Regular	Regular	Irregular

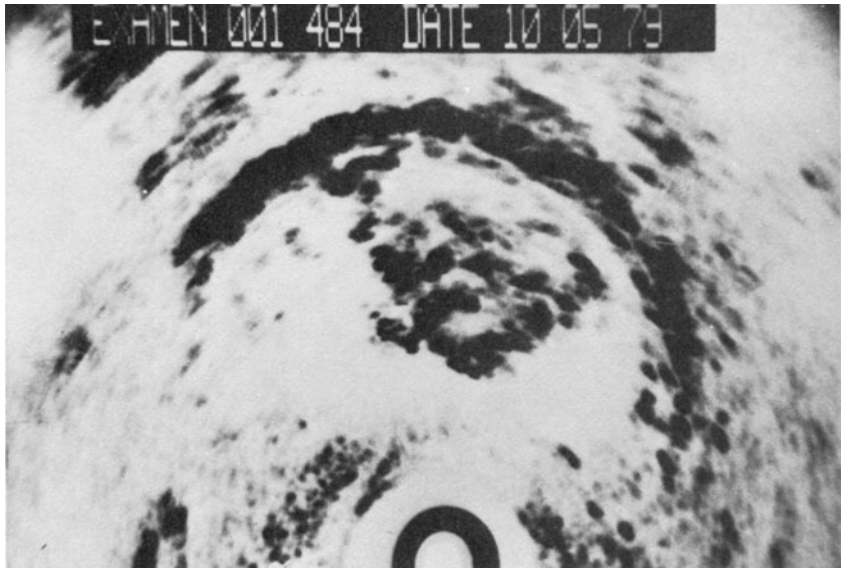

Fig. 1. Strong internal mass echoes and unevenness in capsule thickness suggest prostatic cancer in this sonogram of a T2 prostatic cancer.

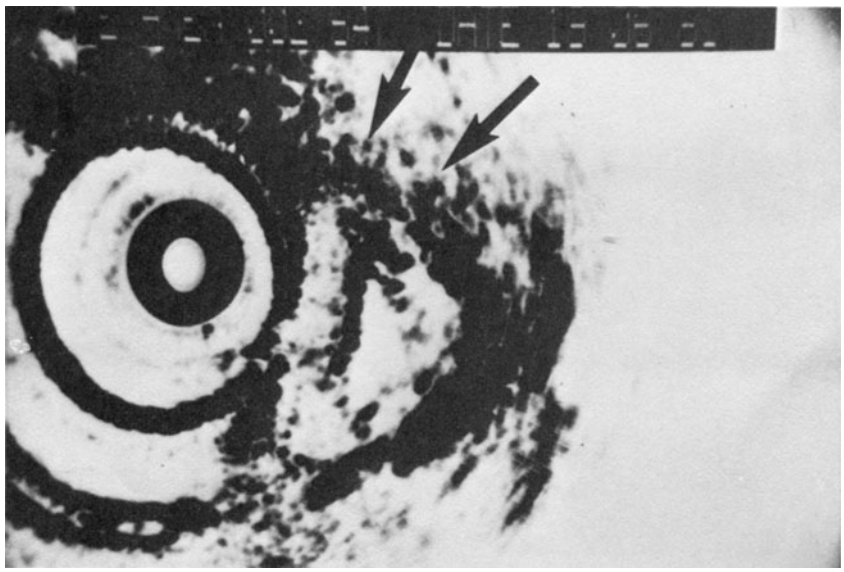

Fig. 2. Discontinuity of capsular echoes and protrusion in the seminal vesicles is diagnostic of a T3 prostatic tumor.

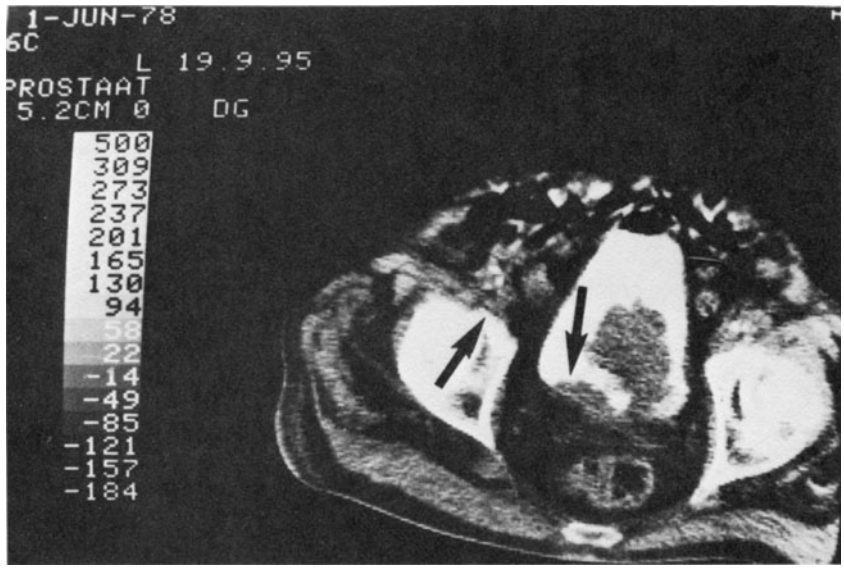

Fig. 3. CT section of pelvis in a patient with advanced prostatic cancer demonstrates nodal obturator mass. Tumor invasion diagnosed by percutaneous fine needle biopsy.

5. The diagnostic capacity of CT is remarkable in advanced tumor stages where the relationship to the surrounding organs or the pelvic bones are important with the additional benefit of the detection of nodal masses (Fig. 3) in the pelvis, in the retroperitoneum and the detection of metastatic lesions to the liver or bone in selected instances.

   Constant refinements in both techniques will adjust their proper complementary role in clinical urology.

4. Biochemical Markers

Extensive research is going on to find biochemical markers for prostatic cancer in easily accessible body fluids, especially in blood and to a lesser extent in urine and in prostatic tissue. Reliable blood markers could be useful for screening of populations at risk, as well as for helping to determine if a patient is responding to therapy and for the detection of recurrence in patients in remission. Most emphasis here will be given to some of the serum enzyme markers for prostatic cancer.

As early as 1936 Gutman et al. described an association between increased serum acid phosphatase activity and adenocarcinoma of the prostate with metastases (28). Acid phosphatases belong to an

enzyme group widely distributed in different tissues, such as platelets, leucocytes, liver, spleen and kidneys. The highest concentration is found in prostatic tissue. Acid phosphatases exist as several molecular variants or isoenzymes, and even for the prostatic acid phosphatase (PAP), several isoenzymes exist. Current enzyme activity assay methods for PAP present some problems such as specificity of the substrates used for measuring their catalytic activity (29,30). The use of inhibitors of PAP such as L-tartrate are only partly specific.

Furthermore much attention should be paid to specimen collection and storage since acid phosphatase can deteriorate rapidly. Despite these drawbacks, serum catalytic PAP assays have proven to be useful for the detection of prostatic cancer with metastases. To detect earlier stages of the disease, more sensitive and specific methods are needed. Immunological methods, using specific antisera against PAP, became available recently. To date three different immunological procedures for measuring PAP have been described:

- radioimmunoassay - RIA (32,33,34,35)
- counterimmunoelectrophoresis - CIEP (36,37)
- Solid phase immunofluorescence - SPIF (37,38,39)

What are the advantages of these immunological methods compared to conventional catalytic activity assays? In terms of stability and accuracy, RIA and related procedures seem to be better since they are much less sensitive to storage conditions. To evaluate the different methodologies, the following factors need to be compared:

- Sensitivity i.e. percentage of patients in the disease group, who have positive tests;
- specificity i.e. percentage of individuals not having the disease who have negative results (40).

According to Griffiths, RIA has more diagnostic sensitivity than the conventional colorimetric method, while the change in specificity is less striking (41). Table 3 summarizes a comparison of reported diagnostic sensitivities for different clinical stages of prostatic cancer. The very high sensitivities described by Foti et al. (42) have not been confirmed by other workers, who found lower levels of sensitivity in the first three stages where these tests should be most useful. It seems therefore premature to accept this methodology as being able to diagnose localized prostatic carcinoma in a large percentage of patients.

Total alkaline phosphatase and its bone isoenzyme have some value in patients with metastatic prostatic cancer, especially for liver and bone metastases (43). It is useful to measure them together with PAP, which can be normal in some cases presenting a high serum alkaline phosphatase activity.

Table 3. Diagnostic Sensitivity of Immunological Procedures for PAP as a Function of Disease Stage

		A (%)	B (%)	C (%)	D (%)
Foti et al.	(42)	33	79	71	92
Bruce et al.	(44)	8		35	
Wajsman et al.	(45)	38	35	49	69
Chu, T.M.	(37)	0	20	55	79
Mahan and Doctor	(46)	13	26	30	94
Griffiths, J.C.	(41)	12	32	47	86
Bruce et al.	(47)	14	29	24	89
Quinones et al.	(48)	25	8	46	52

Creatine kinase (CK) occurs as three isoenzymes, CK-MM, found in skeletal and cardiac muscle, CK-MB, predominantly present in cardiac muscle, and CK-BB, which is ubiquitous but in highest concentration in the brain, the gastro-intestinal, and the genito-urinary tract. Using conventional assay methods, CK-BB is rarely found in human serum. By more sensitive methods, including RIA (49), CK-BB has been found in abnormal amounts in the serum of patients with a variety of malignant diseases. Feld and Witte (50) found CK-BB by electrophoresis in nine out of 19 patients with stage D cancer. Similar results were obtained when CK-BB was measured by RIA (51). The possible role of BB isoenzyme as a tumor marker for prostatic cancer was suggested by Silverman et al. (52) who found elevated serum CK-BB concentrations in 15/17 stage D untreated patients. The isoenzyme was not found in appropriate control patients. When CK-BB was compared to PAP (both by RIA) as a tumor marker for prostatic cancer in 23 patients with stage D carcinoma, 6/23 had elevated CK-BB while more than 60% of these patients had increased PAP (53). The definitive value of CK-BB as a tumor marker for cancer of the prostate needs further evaluation by long term prospective study.

Besides serum enzyme markers there has been some recent work concerning hormonal markers such as steroids, sex hormone or testosterone binding globulin and prolactin. The data concerning biochemical changes in prostatic tissue such as steroid oxido-

reductase enzymes and receptor and binding proteins were recently reviewed by Kirdani et al. (54).

DISCUSSION

A number of criteria are essential for a test to be useful in the clinical diagnosis and treatment of patients, and numerous tests and technological examinations await confirmation of clinical usefulness despite early claims. It is clear that one simply cannot multiply this diagnostic avalanche for ethical and economical reasons. Since 1975 the pace of discovery has accelerated dramatically and dozens of prostatic cancer markers look for clinical recognition (54). Apart from its scientific interest in a research setting a diagnostic test could have several uses; 1. for screening populations or high risk groups, 2. to confirm a diagnosis, 3. in the evaluation of a tumor, 4. to indicate prognosis, 5. for monitoring disease during treatment to indicate regression or progression, and 6. to assist in laboratory procedures as a comparison to other tests. In any one of these functions tests must be evaluated according to the three characteristics of sensitivity, specificity and the predictive value of the test (40). Where medical imaging, especially US, needs a certain tumor mass it is obvious that these tests should be reserved for screening selected populations. Patients with lower urinary tract obstruction or found coincidentally to have prostatic induration provide a good sub-set of patients at whom we might direct a battery of tests.

A biochemical marker specific for prostatic cancer is not yet available. The available markers help us to look for cancer in patients suspected of having the disease and allow us to monitor the biological behaviour of the tumor mass. Selectivity in the use of markers in a clinical setting is indicated since the use of a battery of assays will only add confusion. Extensive clinical research and exact recording of sensitivity and specificity may prepare the way for the addition of new markers to the routine clinical tests.

CONCLUSIONS

The diagnosis of clinical prostatic cancer starts with a careful rectal examination. Transrectal ultrasound and some biochemical markers are able to confirm the diagnosis with enough specificity to insist on repeated biopsy if the first biopsy is negative.

The tissue diagnosis coupled with the same diagnostic tests is able to predict prognosis in a reliable way. The tests should play a useful role as parameters in follow-up evaluations of the disease.

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FINE-NEEDLE ASPIRATION OF SOFT-TISSUE MASSES IN PROSTATIC CANCER PATIENTS

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INTRODUCTION

Fine-needle aspiration cytology of soft-tissue masses can play a major role in both staging and monitoring of therapy for patients with prostatic cancer. The procedure is safe, inexpensive and easy to perform in outpatients. Since the lesion itself is not removed, it serves as an indicator to gauge the response of either local or systemic therapy. An interested and skilled cytopathologist and cytotechnologist are essential for this technique to be useful.

In the United States most patients with prostatic cancer have their clinical diagnosis confirmed histologically via needle biopsy or transurethral prostatic resection (1). However fine-needle aspiration cytologic diagnosis of the primary tumor has not so far received the acceptance that it has in other countries (2,3). Yet its use in the evaluation of soft-tissue masses in many malignancies has recently been received with increasing enthusiasm (4). An example is the use of this technique to follow-up an abnormal finding at lymphangiography (5).

MATERIALS AND METHODS

In the last three years fine-needle aspirations have been attempted in 18 patients with 19 soft-tissue masses. These included 10 lymph nodes (both inguinal and supraclavicular, two skin nodules and seven intraabdominal masses). The latter category has variably consisted of combinations of local extensions of a large primary tumor and deep pelvic or retroperitoneal lymphadenopathy.

The technique is simple and fairly similar to that already

reported (1). Following the measuring of the mass, the overlying skin is prepared with Povidone iodine solution. Local anesthetic infiltration using 1% Lidocaine hydrochloride is optional since the small caliber needles are usually well tolerated and too much infiltration may distort the target. The lesion is stabilized with one gloved hand while the 20 or 22 gauge needle attached to a 6-12 cc syringe is passed into the mass. It is important to create a strong suction on the needle as it is passed back and forth through the lesion. In our urology clinic, we prefer to have a cytotechnologist present for the procedure. The technologist receives the needle and syringe and immediately prepares thin smears on slides and flushes the needle and syringe into fixative. The aspirate is often repeated with a second needle and syringe. Smears and cell washings are then submitted for routine staining and examination by both the cytotechnologist and cytopathologist. A small pressure dressing or band-aid over the puncture site suffices.

RESULTS

The results of our aspiration cytologies are listed in Table 1. Aspirates are reported as either diagnostic, i.e., positive or negative for malignant cells, or non-diagnostic, i.e., insufficient or unsatisfactory for any diagnosis. The latter report definitely needs to be followed up by another attempt at aspiration. Aspirates reported as negative are repeated if clinical suspicion of malignancy is high and a sampling error is likely.

Aspirates positive for malignant cells were found in 15 specimens. In many instances the material was sufficiently diagnostic for the reading of adenocarcinoma consistent with prostatic origin. One abdominal mass proved to be a full colonic segment. No complications occurred in this instance or in any of the other patients studied by this technique.

Table 1: Results of fine-needle aspirates of 19 soft-tissue masses (18 patients with prostatic cancer)

	Diagnostic		Non-diagnostic
	Positive	Negative	
Lymph nodes	9	1	---
Abdominal masses	4	1	2
Skin nodules	2	---	---
TOTAL	15	2	2

CASE REPORT

A 69-year old man had previously been treated with external beam irradiation to the prostate and pelvis for clinically localized prostatic carcinoma. Three years later because of right hip pain he was re-evaluated and found to have an elevated serum acid phosphatase activity and a positive bone scan. In spite of bilateral orchiectomy and intravenous diethylstilbestrol diphosphate (Honvan, Stilphostrol), the disease progressed as new lesions were identified on subsequent bone scans. At this time a 2 x 2 cm firm non-tender left supraclavicular mass was palpated. Fine-needle aspiration was performed without the need of local anesthetic. The aspirate was positive for malignant cells compatible with adenocarcinoma. With the confirmatory evidence that his prostatic cancer had indeed been documented as progressing on endocrine therapy, the patient was started on cytotoxic chemotherapy. The supraclavicular lymph node remains as an additional parameter by which to gauge his response to the new treatment.

DISCUSSION

Although soft-tissue metastases are uncommon in prostatic cancer, their identification can be critical in the accurate staging of individual patients. Benign lesions such as reactive lymphadenitis or hematoma must be distinguished from metastatic involvement or extensive local infiltration by the primary tumor.

Once the soft-tissue mass had been identified as malignant, the lesions serve as a marker for any response to therapy. Many of these masses are measurable; the remainder are at least evaluable regarding response to treatment. Thus, this does not require a minor or major surgical procedure to remove the mass which, in prostatic cancer, would rarely be an isolated metastasis.

The technique can be expanded to the cytologic diagnosis of masses detected by lymphangiography, computerized tomography or ultrasonography in the evaluation of a patient with prostatic cancer (5). In these situations, the fine-needle aspirate is performed by fluoroscopic or ultrasonic guidance as has been documented in other tumor systems.

The procedure is safe, easy to perform and relatively inexpensive considering the alternatives available. The accuracy of interpretation with well-preserved specimens should be over 90 percent (4). False-positive readings should not occur; a false-negative interpretation occurs in 10% of instances and can signify a sampling error. Non-diagnostic samples, whether due to poor aspirate technique or poor cytologic technique, should be repeated at an early date.

Complications of the fine-needle aspiration procedures are infrequent and bleeding is easily controlled by local pressure. We have not experienced any tumor seeding along the needle track and no infections have occurred.

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PROSTATIC CANCER STAGING - AN OVERVIEW

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As in all malignant disease an accurate characterization of the tumour is mandatory to predict the prognosis and to guide the treatment. Most urologists and oncologists feel today that the malignancy grade is the most important parameter in the evaluation of tumour aggressiveness. Therapeutic decisions also imply assessment of the tumour extent.

In principle there are two kinds of system in use to describe tumour extent, staging systems which group the patients together according to a number of various characteristics, and a classification system which describes each parameter of the tumour disease separately.

The staging systems are variants or modifications of the system proposed by Whitmore in 1956 (1) (Fig. 1) and originally based on the pathological findings in specimens from radical surgery. In stage A the tumour is confined to the prostate and is not suspected clinically but an incidental finding on operation for obstructive disease, such as TUR or adenomectomy, or on postmortem examination.

Catalona and Scott (2), in 1978, proposed a more detailed subclassification of the Whitmore system where A1F denotes a focus of occult carcinoma, A1 a malignant lesion involving one lobe, and A2 a multifocal lesion or diffuse spread of the occult cancer. This subclassification was an improvement of the Whitmore system as widespread occult carcinoma is often of high grade and carries a much more sinister prognosis than does focal disease. Correa et al (3) reported a series of cases of carcinoma stage A, for the most part found on TUR. Forty-five patients were observed for more than a year up to a maximum of ten years. Of eight patients with diffuse

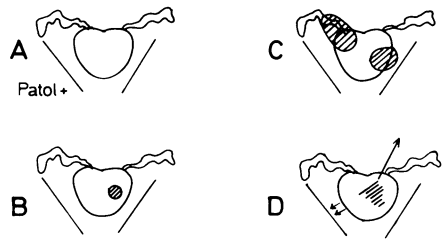

Fig. 1. Staging according to Whitmore

occult carcinoma, five had tumour recurrence. Two of these died from their tumour and three further patients died with tumour present. Among the 37 patients with focal disease, tumour recurrence was identified in three. No death from cancer occured in the latter group within the observation period. There are also other investigations on record showing that average survival in A2 patients is lower than in patients with localized stage B carcinoma.

Stage B indicates palpable tumour confined to the prostate. In the subclassification according to Catalona and Scott (2), B1N denotes a solitary nodule of estimated diameter 15 mm or less, B1 tumour involvement of an entire lobe or nearly entire lobe, and B2 diffuse involvement of the gland. The frequency of lymph node deposits increases and the prognosis worsens with the extent of tumour involvement.

In stage C there is tumour extension beyond the prostatic capsule but no evidence of distant metastases. Acid phosphatase activity, as measured by enzymatic technique, is usually normal but may be enhanced. Using radioimmunoassay, enhanced phosphatase activity is recorded in about 70% of stage C cases. Lymph node extension also occurs in a high percentage of stage C cases.

Stage D indicates cases where metastatic deposits are observed, usually in the skeleton, lymph nodes or lungs. There may be any local finding in the prostate from no palpable tumour to extensive infiltration. Serum acid phosphatase activity is elevated in the majority of stage D cases, 60% as measured by enzymatic techniques and over 90% with radioimmunoassay.

The US Veterans Administration Co-Operative Urological Research Group have used a staging system very similar to that of Whitmore, except that the symbols A-D are changed for the roman numerals I-IV (Fig. 2). The only difference of essential character is that in the VACURG system an elevated acid phosphatase activity is accepted as indication of tumour spread, and consequently stage IV, even in cases where no metastases can be observed by other methods.

Stage	Rectal Examination	Prostatic Acid Phosphatase	X-ray Evidence of Metastases
I	No Induration	< 1.0 K.A.U.	0
II	Localized Nodule	< 1.0 K.A.U.	0
III	Extra Prostatic Extension	< 1.0 K.A.U.	0
IV	Equivocal Findings	> 1.0 K.A.U.	+

Fig. 2. Staging of prostatic cancer according to the U.S. Veterans Administration co-operative Urological Research Group.

With the increasing use of the sensitive radio-immunoassay techniques with abnormal activity found in many cases of local disease the definition can hardly be accepted today, even with the reservation that our methods of identifying true local disease are still limited.

The TNM-system, originally described by Denoix in 1953 (4) and adopted by the International Union Against Cancer, is a classification system where the extent of the primary tumour, lymph node dissemination and distant metastases are indicated by separate symbols, T for the local tumour, N for regional lymph nodes, and M for distant metastases beyond the regional nodes. The TNM categorization is based on clinical evaluation and not on pathological investigation except for biopsy and thus involves the inexactitude of the clinical evaluation. The pathological description is indicated by the symbol pT which may supplement the T category but should never change it.

Fig. 3 represents the classification of the local tumour in prostate cancer according to the TNM system. The rules were specified in 1974 and it is now time to consider them for reevaluation. In T0 there is no palpable tumour but the cancer is an incidental finding in an operative or biopsy specimen, analogous with stage A. In T1 the tumour is palpable but located within the gland and surrounded by palpably normal gland tissue. In T2 the tumour is still confined to the gland but the contour is deformed by the nodule. In borderline cases it may be difficult to distinguish between T1 and T2 cases. In T3 there is extension beyond the capsule or into the seminal vesicles and T4 indicates extension to the pelvic wall or invasion into the neighbouring organs other than the seminal vesicles.

In the TNM system there is no stage grouping but lymph node dissemination is indicated by various N-symbols and distant metastases by M-symbols.

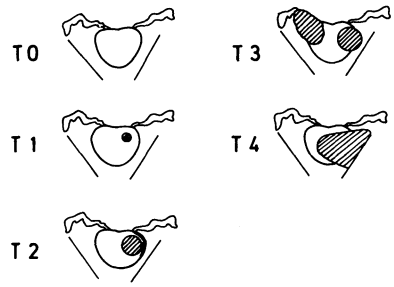

Fig. 3. TNM Classification

Even with experienced examiners the intraprostatic extent of a tumour and multifocal growth are difficult to assess. The accuracy of digital examination increases with higher T category. Extracapsular infiltration is relatively easy to identify. In a review of 103 malignant nodules, Jewett (5) noted that the extent of carcinoma had been underestimated in 72% of the cases on rectal examination. Hopefully modern imaging techniques, ultrasound scanning in particular, will improve the accuracy of estimation of the tumour extent, at least the local tumour. An exact recording of tumour spread is especially important in those cases where radical surgery or radical radiotherapy is aimed at. Assessment of the biological activity, i.e. the aggressiveness of the tumour, as reflected by the malignancy grade, DNA-pattern or some other indicator, is also an indispensable part of the tumour characterization.

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CARCINOMA OF THE PROSTATE - THE NEED FOR A REVISION OF CATEGORY T0?

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The survival of patients with carcinoma of the prostate is not always improved by commencing active treatment at the time of diagnosis. Byar (1) showed that the survival of a group of patients with unsuspected 'localised' carcinoma of the prostate was the same as that of an age-matched control population. Despite this finding it is not universal practice to defer treatment in this group of patients. Clinicians are undoubtedly influenced by the knowledge that in some cases, even with small well-differentiated tumours, metastases will develop (2). Attempts to predict those cases which will do badly have identified several prognostic factors including poor histological differentiation (3,4,5,6,7), age (8,9,10), tumour bulk (3,11) and the presence of a clear margin of resection (3).

A variety of definitions of 'local' disease have been introduced. In some the emphasis is on the extent of the tumour, in others the histological grade (12), the aim of each classification proposed being to improve prognostication and to allow the more logical selection of treatment in this group of patients.

We have reviewed a series of 34 cases of T0 carcinoma of the prostate in an attempt to correlate their clinical progress with the histological features of the tumour at presentation. The aim of this study was to clarify the spectrum of disease represented by the T0 category and to decide whether a revised category was indicated in the interests of appropriate treatment selection.

METHODS

In a series of 159 patients with carcinoma of the prostate presenting consecutively at the prostate clinic, 34 were classified as category T0. All patients had histologically proven carcinoma of the prostate and pathology of the specimens obtained has been reviewed retrospectively. Grading has been carried out by a single pathologist according to the Gleason system (13,14). This grading is carried out at relatively low magnification, five patterns of growth being assigned numbers in order of decreasing apparent histological differentiation. In order to allow for histological variation within the tumour two digits are recorded, first the predominant pattern (by area), then the lesser pattern (by area). For example, a well-differentiated tumour containing areas of poor differentiation would be represented by the figures 2-4. In addition an attempt was made, on the basis of the material available to the pathologist, to assess the amount of tissue involved with tumour. Three categories were chosen: $<10\%$ involved; 10-50% involved and $>50\%$ involved. This does not correspond exactly to the American categories of A1$_f$ focal microscopic tumour involvement, A1 microscopic involvement of one lobe and A2 multifocal or diffuse involvement (15) but it was hoped to get a reasonable measure of the extent of tumour involvement and its relation to grading and prognosis.

At the time of diagnosis all patients had levels of blood urea, creatinine, electrolytes, liver function tests and acid phosphatase determined, together with isotopic bone scan. Out patient follow-up was carried out at three-monthly intervals with clinical examination, full blood count, and acid and alkaline phosphatase on each occasion. In addition isotopic bone scans were repeated at six-monthly intervals or sooner if indicated by bone pain.

RESULTS

Of the 34 cases reviewed 26 were classified as category T0 M0 and four as T0 M1. Four patients who did not have an isotopic bone scan were categorised as MX. The patients' ages ranged from 58-88 with a mean of 72.5 years. Follow-up varied from 1-138 months (mean 22.2 months). During the course of this study eight patients have died but only three of these deaths resulted from malignant disease of the prostate. In six patients the diagnosis was made following open prostatectomy and in one case histology was obtained from a bone biopsy of a secondary deposit. In the remaining 27 patients the diagnosis was made following transurethral resection of the prostate.

Treatment was deferred in 25 cases, primary radical radiotherapy was given in three and six patients received hormonal treatment initially. Of the six patients treated initially with hormonal

therapy, three had metastases at the time of presentation and one of the other three has developed bony metastases during follow up. Of the deferred treatment cases, four have developed metastases during the course of follow-up and three have developed local progression of their disease.

Table 1 shows the relationship between histological differentiation expressed as the Gleason worst and summed score means with a theoretical maximum of five and ten respectively, and the presence or absence of metastases at presentation and during the period of follow-up. One patient is excluded from this set of results since he died before effective staging or follow-up could be carried out. It will be clear that those presenting with metastases have worse mean scores than either of the other groups, and that those who developed metastases during follow-up had higher mean Gleason scores than those patients without metastases. Sixteen of the patients selected for deferred treatment have been followed for longer than six months and these cases can be divided into two groups (Table 2) - nine who have shown no disease progression, and seven in whom local progression or metastatic disease has become evident. Once again those with disease progression had worse mean scores than those who have required no treatment intervention.

Comparison of histological scores with our assessment of tumour extent at presentation (Table 3) show a definite tendency to poorer histological differentiation in those patients with more extensive infiltration of the gland. Despite this tendency it is

Table 1. Relationship between Gleason Score and Presence or Absence of Metastases at Presentation and during Follow-up.

	No metastases	Metastases developed	Metastases at presentation
Gleason mean worst score	2.25	2.6	4.5
Gleason mean summed score	3.83	4.4	8.25
No. of Patients	24	5	4

Table 2. Relationship between Gleason Score and Subsequent Disease Progression.

	Deferred treatment No progression	Deferred treatment Disease progression
Gleason mean worst score	2.11	2.43
Gleason mean summed score	3.56	4.14
No. of Patients	9	7

Table 3. Relationship between Gleason Score and Estimate of Extent of Infiltration of Prostate.

	Extent of Prostatic Infiltration		
	<10%	10 - 50%	>50%
Gleason mean worst score	2.25	2.62	3.2
Gleason mean summed score	4.0	4.32	5.5
No. of Patients	16	8	10

important to note that of the four patients presenting with metastases, two had less than 10% of the gland involved and for these cases the mean worst score was 4.5 and the mean summed score 8.5.

In this study no correlation was found between age and histological differentiation or between age and extent of gland involvement by tumour.

DISCUSSION

This study has emphasised again the heterogeneous nature of patients falling into the TO category both in terms of tumour histology and in the extent of the involvement of the prostate by tumour.

Although patients with metastatic disease represent a distinct group both in terms of treatment and prognosis, their inclusion in this study is justified by the need to obtain an overall view of the natural history of TO disease. The impression gained from this series confirms the findings of other workers in that the poorer prognosis of more extensive tumours is really a reflection of their poorer histological grade. This suggests that greater weight should be put on the degree of tumour differentiation than upon the extent of tumour within the prostate. Although we have found no relationship between age and prognosis in this group of patients, we have relatively few aged 60 or below and it is quite possible that this younger age group form a special category. The information collected in this study does not allow us to draw any conclusions regarding the importance of a clear margin of resection nor does it indicate the potential value of assessing capsular involvement by a second resection or needle biopsy of the posterior gland.

It is concluded that since our study has demonstrated a relationship between clinical progress and histology of the tumour at presentation, irrespective of the size of the primary lesion, a decision for deferred treatment in category TO patients should be related to histological grade rather than to any measure of extent of local tumour and that the TO category should always be combined with an indication of the histological grade of the tumour.

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LYMPH NODE METASTASES IN PROSTATIC CANCER

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One of the first descriptions of the lymphatics of the prostate was that of Paolo Mascagni in his magnificent Vasorum Lymphaticorum Corporis Humani Historia et Iconographia, published in 1787 (1). A more modern work was that of Cuneo and Marcille, who completed the definitive dissections of the pelvic lymphatics at the turn of this century, and which later became the foundation for the definitive description of the pelvic lymphatics by Rouvière (2,3). According to Rouvière, the lymphatic drainage of the prostate consists of four main trunks. The first and most numerous lymphatic trunks emerge from the posterior surface of the prostatic capsule and go to the external iliac pedicle. The second group of lymphatics travels with branches of the hemorrhoidal artery to the hypogastric nodes. The third pedicle arises from the posterior surface, courses posteriorly to the pre-sacral region and encounters the first lymph node usually at the level of second sacral foramina. Other lymphatics in this group proceed to the region of the sacral promontory. The fourth pedicle descends from the anterior surface of the floor of the perineum going with the internal pudendal artery, and around the ischial spine into the pelvis, terminating in a hypogastric node.

The external iliac nodes are divided into three chains, the lateral, the middle and the medial. One of the nodes of the medial chain is placed opposite the pelvic aspect of the obturator canal and has been called "the node of the obturator foramen", and according to Rouviere, it is not be be confused with the "node of the obturator nerve."

Herman and co-authors who elegantly attempted to retrace the steps of Rouviere by modern lymphangiographic techniques appear to

believe that the "node of the obturator foramen" is the same as the obturator node described by contemporary urologic surgeons (4). This issue, however, remains in doubt because it seems that most urologists describe this node in relationship to the obturator nerve which is separately described by Rouvière as "the node of the obturator nerve." Whether or not this is important is an academic issue since whichever node the urologist selects as the obturator appears to be the one most frequently involved with prostatic cancer. It can be identified by lymphangiography and contains lymphangiographic contrast media in 100% of the obturator nodes resected by Merrin and in 94% of the obturator nodes resected in the Stanford series (Table 1) (5,6).

The significance of the pelvic lymphatics appears to have escaped serious surgical attention for many years, presumably because radical perineal prostatectomy became the principal operative procedure for the definitive surgical treatment of prostatic cancer. Of course, in this operation the pelvic lymphatics were not exposed. The fact that pelvic adenopathy was a serious problem, however, was clearly appreciated, and radiotherapists in the mid 'twenties and 'thirties were known to use orthovoltage radiation in an effort to palliate urethral, ureteral, and lymphatic obstruction secondary to massive adenopathy. Surgeons who employed the retropubic approach to the prostate were well aware of the significance of pelvic adenopathy. Flocks, and also Arduino and Glucksman were among the first to call attention to the importance of the lymphatic spread of prostatic cancer and detailed the distribution of the adenopathy by regional groupings (7,8). Recently the lymphatics of the prostate have been the subject of more intense investigation, especially for the purpose of mapping the distribution of lymphatic involvement prior to external beam radiation therapy or, in some cases, the employment of definitive node dissection in an attempt to combine the surgical removal of the lymph nodes with either radical resection of the prostate or irradiation of the prostate by interstitial implant (9,10).

In the Stanford series of staged patients, 20% of those with stage B (T1 and T2) and 59% with stage C (T3) had positive adenopathy. This incidence is consistent with that tabulated in Table 2. In addition to the sites recorded in Table 1 Golimbu has sampled the pre-sacral and pre-sciatic regions, finding involvement in about half of the cases (11).

Lymphangiography has been considered by some to have merit in the detection of lymph node metastases, especially when clearcut positivity, either alone or proven by skinny needle biopsy, can save the patient a useless extensive lymph node dissection (12). At Stanford, after a feasibility study demonstrated that lymph node metastases in prostatic cancer could be identified by lymphography (Fig. 1) (13), a prospective study of consecutively surgically staged cases demonstrated a sensitivity of 16/28 or 57% (true positive), a specificity of 36/39 or 92% (true negative), and an

Table 1. Incidence of Lymph Node Involvement by Tumor (6)

(93 Patients)

Lymph Node Group	Number of Patients Biopsied	Number (%) With Tumor	Percent Opacified*
Para-Aortic	74	13 (18%)	93
Common Iliac	76	13 (17%)	95
External Iliac	74	16 (22%)	94
Internal Iliac	63	15 (24%)	87
Obturator	51	16 (31%)	94

*Percent opacified refers to histologic evidence of retained contrast material within the lymph node specimen.

Table 2. Histologically Proven Lymph Node Metastasis in Prostatic Cancer

Series	Clinical Stage "B"	"C"	Total
Flocks et al. (1959)	2/29 (7%)	144/382 (38%)	146/411 (35%)
Arduino & Glucksman (1962)	19/71 (27%)	14/17 (82%)	19/71 (27%)
McCullough et al. (1974)	1/4 (25%)	27/46 (59%)	28/50 (56%)
Dahl et al. (1974)	3/25 (12%)	9/13 (69%)	12/38 (32%)
Hilaris et al. (1974)	6/29 (21%)	20/31 (65%)	26/60 (43%)
McLaughlin et al. (1976)	9/36 (25%)	12/24 (50%)	21/60 (35%)
Total	40/194 (21%)	226/513 (43%)	252/690 (37%)

Summary of surgical series in which pelvic lymphadenectomy was performed in patients with carcinoma of the prostate. In the Arduino and Glucksman series 17 of the 71 patients were found histologically to have tumor extending into peri-prostatic tissues and these are pathologically, not clinically, stage C tumors.

(Courtesy Spellman et al, 14)

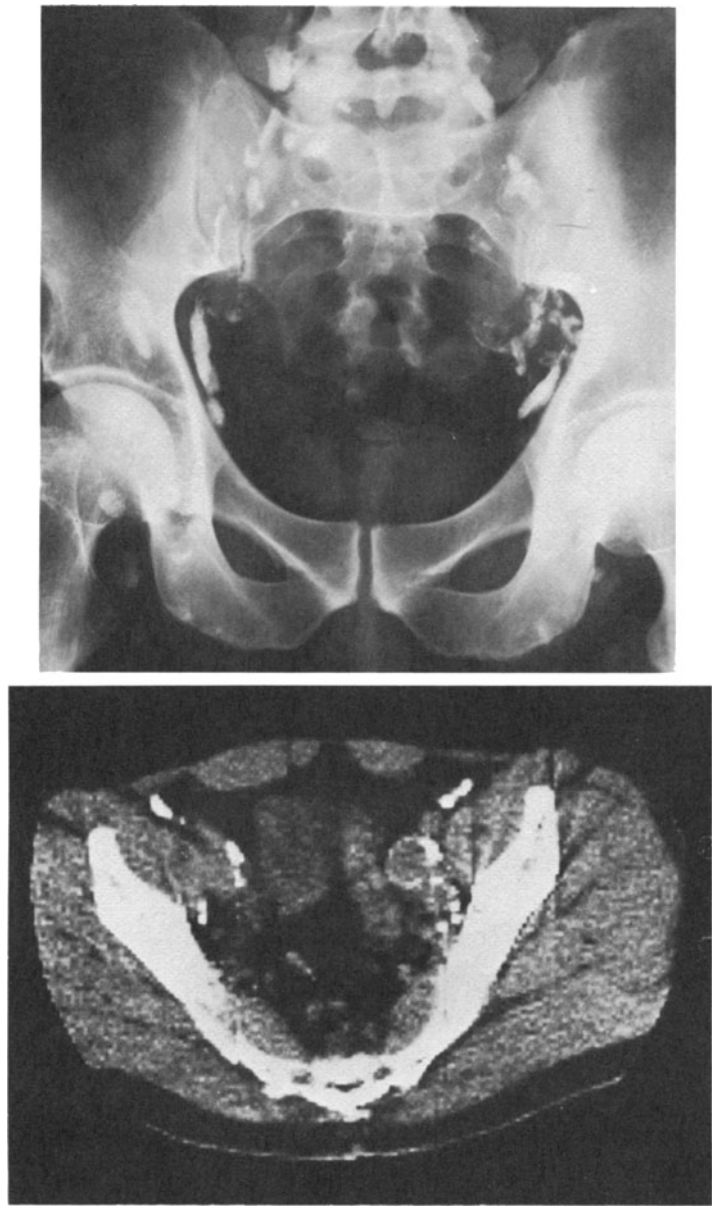

Fig 1. Lymphogram showing an enlarged left external iliac node with an extensive filling defect and classical "egg shell" sign produced by contrast in the subcapsular sinus. The node is also shown on the CT scan. There is probably a right iliac node demonstrated by CT also.

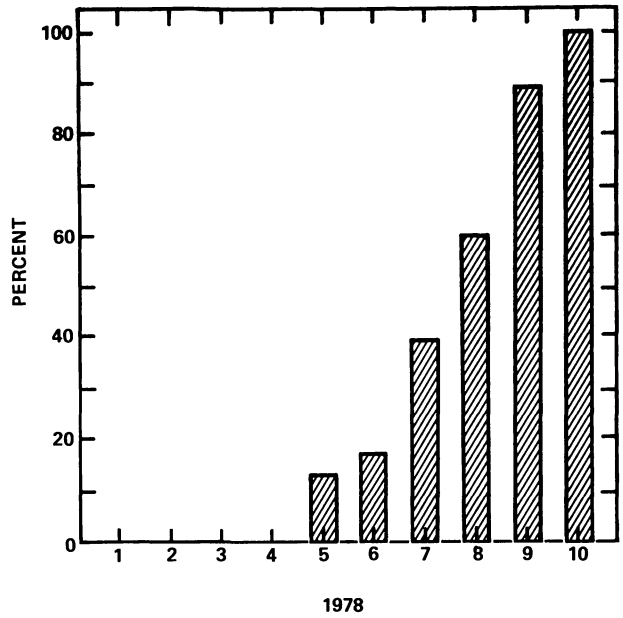

Fig. 2. Correlation of 33 patients with biopsy-proven lymph node metastases with the Gleason Pattern Scores among 93 consecutive protocol patients who were surgically staged.

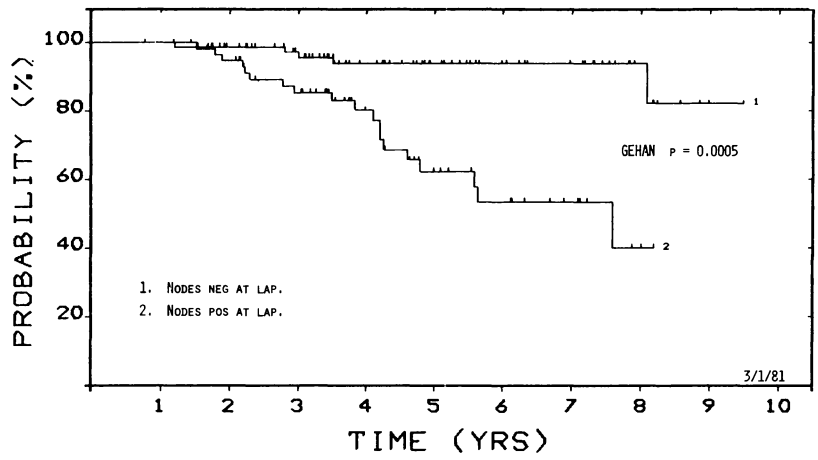

Fig. 3. Probability of surviving prostatic cancer. 151 patients staged by pelvic laparotomy. 86 were histo-pathologically negative, 65 were positive. These are disease specific survival curves, i.e., patients dying of causes other than prostatic cancer are withdrawn.

overall accuracy of 52/67 or 78% (14). These results were almost exactly substantiated by a Veterans Administration cooperative study by Liebner et al., who demonstrated a sensitivity of 56%, a specificity of 97%, and an overall accuracy of 80% (15).

In the surgically staged patients at Stanford we found positive para-aortic nodes in 20%. This appears to be a unique observation because we used a transperitoneal approach to sample the para-aortic nodes as high as the renal hila. This approach was later abandoned in favor of the more prudent retroperitoneal approach to the pelvic lymph nodes because of the unacceptably high incidence (28%) of subsequent radiation enteritis (16). The radiation enteritis was subsequently completely eliminated with a return to retroperitoneal dissection. In so doing, however, it was necessary to give up the additional information that was provided by the para-aortic biopsies. Isolated para-aortic adenopathy was never encountered.

The presence of adenopathy in our series has been an important but unwelcome prognostic indicator. In fact, its relative incidence can be reasonably predicted by the histologic grade particularly if the Gleason Pattern Scores are used (Fig. 2). In Figure 3 it can be seen that survival following radiation therapy is markedly adversely influenced by positive lymph nodes. Even though the probability of metastatic relapse is 80% at eight years (Fig. 4), still 9 of 59 patients with proven adenopathy are surviving without evident disease for 26, 26, 41, 43, 48, 61, 62, 80, and 84 months. Thus although the chances for long term disease-free survival are greatly reduced when adenopathy is present, they are sufficiently high to warrant therapy at least until additional follow-up demonstrates evidence to the contrary.

The Stanford radiotherapeutic program when pelvic or para-aortic lymph nodes are implicated is as follows. 2600 rads total pelvic irradiation is delivered by a four field box technique at the rate of 200 rads per day to the prostate and the first echelon lymph nodes of the pelvis. The treatment volume is then reduced to the prostate only and treatment is continued with left and right 120° moving beam arc therapy to an additional 2000 rads in ten treatments to the prostate only, using fields of about 7x7 or 8x8 cm as measured at the isocenter. This delivers 7000 rads in seven weeks to the prostate and 5000 rads in seven weeks to the pelvic lymph nodes. If required 5000 rads are also delivered to the para-aortic lymph nodes. More specific details on treatment have been presented previously. (17)

SUMMARY

1. It has been recognized since at least the early '50s that patients may have prostatic carcinoma metastatic to regional lymph nodes without objective evidence of tumor in skeletal or other metastatic sites.

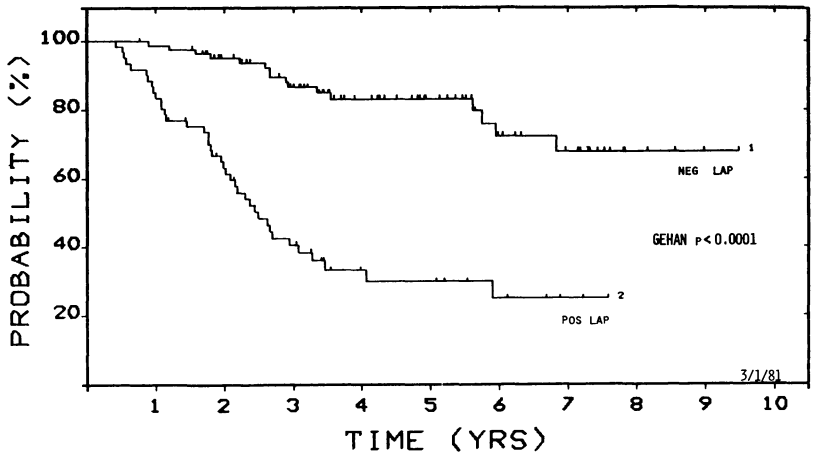

Fig. 4. Same patients as noted in Fig. 3. In this case the calculation is based upon the probability of surviving without metastases. Osseous metastases dominated by a substantial margin.

2. More recent observations have shown that when the lymph nodes are involved, however, subsequent development of osseous metastases should be expected in 75-80% of cases.

3. A more thorough understanding of the true efficacy of the irradiation of regional adenopathy must await a longer period of follow-up.

4. Certainly patients with lymph node metastases and negative bone scans are at high risk for the subsequent development of occult bone metastases and are excellent candidates for effective adjuvant therapy when such becomes available.

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LYMPH NODE DISSECTION IN THE MANAGEMENT OF PROSTATIC CARCINOMA

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Lymph node dissection (LND) may be performed either for staging or for therapy. This discussion is limited to the former. LND has added considerable data to our clinical knowledge of prostatic cancer. At present it is the most accurate method of assessing nodal status. Utilizing LND the incidence of lymph node metastases has been related to the following parameters amongst others: size of the prostate, histologic grade (1), extent of intracapsular disease, extent of contiguous extra-capsular spread, especially to the seminal vesicles, and over-all clinical stage. LND has demonstrated that a percentage of patients felt to have disease limited to the prostate by clinical staging, in fact, have cancer metastatic to the lymph nodes. This information has had a significant impact on our concept of the percentage of patients in each stage at presentation. Patients with alleged localized clinical stage A1 disease have a 2-5% chance of having positive lymph nodes. The figure for A2 disease is 3-54%, for B1 disease is 10-40%, for B2 disease 39-45%, for C disease 50-80%. A recent review of the world literature (1) clearly documents the strong influence that histologic grade exerts on the likelihood of positive nodes.

Based on these data gained from LND, attempts are being made to develop a non-invasive system for predicting the probability of lymph node metastases. Perhaps the most widely used to date is the Gleason score (2). By assessing the clinical stage and histologic appearance, a number or score between three and 15 is assigned to each patient. Patients with elevated scores have a high probability of having lymph node metastases. The validity of the Gleason score for middle and lower values is less certain (3,4).

Lymphangiography (LAG) cannot detect micrometastases. False

negative results are in the range of 15-25%. The true negative rate runs between 80-95%.

What should be the extent of the LND? A simple biopsy is inaccurate unless the biopsy is positive (5). If, on exploration, the nodes look and feel negative, a systematic LND should be performed to detect micrometastases. Serial sectioning of each node by the pathologist increases the yield. The most popular dissection currently is a bilateral extraperitoneal one which removes the common, external and internal iliac nodes and the obturator nodes. Metastasis via the lymph system is usually orderly. Combined pelvic and retroperitoneal node dissection adds little to the yield and results in a higher morbidity. Both the extension of pelvic LND to include the pre-sacral and pre-sciatic nodes (6) and the reduction of the extent of pelvic LND have been proposed (7).

The impact of LND on the immunologic status of the patient remains unsettled.

We are using the histochemical technique of Pertschuk (8) to study the relationship of androgen binding in the primary site to androgen binding in lymph node metastases.

Significant morbidity and occasional mortality is associated with pelvic LND (9). We suggest pelvic LND be limited to formal study protocols with fully informed patients. For individual patients not involved in such a study, pelvic LND should be reserved for those patients in whom the nodal status cannot be predicted with high certainty by non-invasive methods and only if knowledge of the nodal status will alter the management of the patient.

SUMMARY

1. Bilateral systematic extraperitoneal pelvic LND has provided excellent data and revised our thinking regarding the distribution of stage at diagnosis.

2. To my knowledge, no improvement in survival statistics has as yet been demonstrated secondary to the acquisition of this new data.

3. The simultaneous performance of a therapeutic procedure designed to treat localized disease, e.g. total prostatectomy or I-125 implant may be unwise. A percentage of these patients will have positive lymph nodes revealed only at permanent section and will have thus been subjected to an invasive procedure with no proven likelihood of success.

4. Routine use of surgical staging is unwarranted unless a) the patient is enrolled in a formal protocol designed to elicit new information or b) in the case of a non-protocol patient, the selection

of therapy for that patient will be significantly modified.

5. Finally, I speculate that non-invasive techniques will become increasingly reliable in predicting nodal status. In those cases where such non-invasive techniques are not reliable, minimally traumatic procedures such as percutaneous "skinny needle" node biopsy will replace open surgical staging. At that time open surgical staging will have outlived its usefulness and should be laid to an honorable and peaceful rest.

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THE VALUE OF QUANTIFIED BONE SCANNING IN THE FOLLOW-UP OF PATIENTS WITH PROSTATE CANCER AND METASTATIC BONE LESIONS

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INTRODUCTION

Bone scanning is an essential technique in the diagnosis of metastatic lesions in bone seeking cancers and is particularly important in prostate cancer, in which up to 60% of patients develop bony metastases. The bone scan aims to confirm or exclude metastatic bone lesions at diagnosis, during the follow-up and to aid in evaluating the efficiency of hormonal treatment. We have paid particular attention to this last point using a quantitative method which has allowed us to determine the variations of isotope uptake in the metastases.

THE PHYSIOLOGICAL BASIS

Bone tissue presents a double structure -

a protein matrix framed with collagenous fibers and basic substance (polysaccharide and cells such as osteoblasts, osteocytes and osteoclasts, and

mineral substance made of hydroxyapatite crystals.

This bone tissue is constantly being reshaped: loss of calcium from the bone tissue is replaced by mineral salts from the blood. A percentage of the mineral substance is in equilibrium with the blood, the exchanges leading to a steady-state so that the calcium concentration in skeleton is constant. Areas of high uptake in abnormal scans result from any process that increases the bone turnover and/or the blood flow. A metastatic lesion in bone is such a process.

The growing tumor removes calcium while the bone reaction is to try to deposit mineral substance around the lesions. The calcium balance may be either positive or negative. For bone destruction to be demonstrable roentgenologically, a lesion must be 1 - 1.5 cm. in diameter and the mineral loss must be 30 - 50%.

By using bone seeking radioisotopes, it is not necessary to have net deposition of new bone mineral since areas of osteolytic and of osteoblastic activity are both visualized.

THE RADIOISOTOPES AVAILABLE

To be used as bone-scanning agents, the radioisotopes must have precise physical properties, a short half-life in order to reduce the radiation dose to bone, exclusive γ-ray emission, an energy detectable by γ-camera, sufficiently high concentration in normal bone tissue, a higher concentration in bone lesions, a low concentration in soft tissues including bone marrow, and rapid clearance from the body of the isotope not taken up in bone in order not to hide the pelvic area when the bladder is not empty.

There are three kinds of bone seekers, those which stay in the bloodstream, those which seek the protein matrix (rarely used), and those which seek the bone tissue itself.

Calcium would have been the most natural isotope but studies with the only clinically useful γ-emitting Ca isotope (Ca^{47}) have been limited by the difficulty of production with adequately high specific activity and the difficulty of collimation due to the high γ-energy (1,31 Mev). Ca^{45} also has too long a half-life (165 days).

Strontium follows calcium accurately in its participating in the formation of new bone. Sr^{85} as well as Sr^{87m} were used but neither was entirely satisfactory because of the long half-life of the former and very short half-life of the latter, and the unduly high γ-emitting energy of both.

There has been considerable interest in certain phosphate compounds labeled with Technetium^{99m} in the presence of the reducing agent stannous chloride. The physical characteristics of Technetium are ideal and it is produced from a simple generator.

The polyphosphates whose behaviour differs according to their molecular weight are little used because of the low labeling efficiency (about 70%). The background activity and thyroid uptake level are also high. However, Methyldiphosphonate (M.D.P.) is widely used because of its high labeling efficiency and its higher bone/blood and bone/soft tissue activity. Scans can be performed as soon as two hours after intravenous injection.

METHOD

The patients are given 15 mCi of Tc^{99m} M.D.P. intravenously. The scans are recorded on films using a Picker scinticamera equipped with a whole body system and connected to a computer.

A whole body scan is performed five minutes after injection in order to measure the whole body radioactivity immediately after injection. A second scan is recorded three hours after injection. In every case whole body retention is measured after the bladder has been completely emptied. The skeleton is clearly seen. The data are computerized and recorded. Bone images of the posterior aspect of the entire axial skeleton, skull, shoulder and pelvis are obtained. Images of clinically suspect regions are also included.

From the data recorded, the whole body scan is visualized. A region of interest (R.O.I.) selection program is used to delimit areas of high uptake levels as well as an area of normal bone tissue. As a reference area we always use the humerus, because it is seldom involved in metastatic lesions. The whole body retention measured at three hours is expressed as a percentage of whole body radioactivity measured immediately after injection and is called the general or global ratio (R.G.)

$$\text{R.G. = Percentage of skeletal uptake} = \frac{\text{Radioactivity at 3 hours}}{\text{Radioactivity at 5 minutes}}$$

The ratio of isotope activity in pathological areas relative to the isotope activity in normal bone is obtained:

$$R_1 = \frac{\text{Radioactivity in pathological area}}{\text{Radioactivity in humerus}}$$

An uptake index is computed:

$$R_2 = \frac{\text{Activity in pathological areas}}{\text{Activity in "normal" area}} \times \frac{\text{Activity at 3 hours}}{\text{Activity at 5 minutes}}$$

$$R_2 = R_1 \times \text{R.G.}$$

RESULTS

In 12 subjects with normal Tc^{99m} M.D.P. bone images, the global ratio (R.G.) was about 37%

$$\text{R.G.} = \frac{\text{Activity at 3 hours}}{\text{Activity at 5 minutes}} = 0.37$$

when the patients presented metastatic bone lesions, the ratio was about 58% (R.G. = 0.58).

The average of "area under test/humerus ratio" (R_1) calculated for metastases in the spinal column was 17.8 and 9.3 for metastases in the pelvis. This ratio was respectively 7.1 and 6.6 in normal subjects. The average uptake index (R_2) was respectively 12.4 and 11.8 for the same areas where metastases were present and 2.5 and 2.4 in normal subjects.

This quantitative method allowed us to follow these patients during the treatment and to evaluate the results of therapy. We compared these different ratios and the index calculated for each patient before treatment and bimonthly thereafter. We found three groups of patients -

those whose ratio and index were lower than before treatment, i.e. returning towards normal,

those whose results did not change during the treatment, and

those with increasing ratio and index in spite of the treatment.

We then compared these quantified data with the clinical data including lessening of the pain and normalization of the roentgenograms.

Of the five patients with numerous bone metastases showing decreasing ratios, three were clinically better and two unchanged. Increased ratios were seen in three patients; of these two had areas of great pain and one showed deteriorating general health. In the group in which the ratios did not change we followed only two patients, both of whom noticed slight subjective improvement.

Although the numbers of patients are, as yet, small it seems that quantified bone scanning is likely to prove to be a good method for the assessment of changes in bone metastases and in evaluating the effect of therapy.

PULMONARY AND OSSEOUS METASTASES IN PROSTATIC CANCER:

METHODS OF QUANTITATIVE ASSESSMENT

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ABSTRACT

The extent of bone and lung metastases in each of eight sites was rated on a semi-quantitative scale using pretreatment films for 102 patients with known metastases at diagnosis. A statistical analysis revealed significant correlation between shortened patient survival and a combination of the number of sites involved and the extent of involvement in those sites. Correlations were also identified with acid phosphatase, pain and performance ratings, but not with tumor grade. Examination of several films for patients treated with endocrine therapy showed that some patients who ultimately show improvement may initially show pseudo-progression.

It is well recognised that for most cancers the prognosis is markedly different for patients having distant metastases at the time of diagnosis compared to that of patients clinically free of metastases. Generally the presence and absence of metastases define different stages of disease. However, because of difficulties of quantitation, little effort has been directed towards determing whether the extent of the metastasis, given that metastases are present, is a further prognostic variable. In order to study this question we examined the pretreatment x-ray films for 102 patients with bone metastases at diagnosis (1). The extent of metastatic involvement was recorded on a four point scale for each of eight major sites. The scale was simply based on the apparent percentage involvement in each site: no involvement, $<25\%$ involvement, 25-75% involvement, or $>75\%$ involvement. The eight sites studied (with the percentage of patients having metastases

at that site shown in parenthesis) were as follows: shoulder (39%), rib (53%), lung (24%), thoracic spine (60%), lumbosacral spine (71%), pubis-ischium (78%), ilium (83%), and femur (48%). "Femur" refers to femoral heads and necks as seen on pelvic films. The patients whose x-rays were examined were part of the first randomized clinical trial of the Veterans Administration Cooperative Urological Research Group (VACURG). They were selected simply because metastases were present at one or more sites at diagnosis and the films were available for study. In the VACURG system of staging, all these patients would be classified as stage IV. The percentages just given for each of the eight sites suggest that the most common sites of metastases are those closest to the prostate, namely the lumbo-sacral spine, the pubis-ischium, and the ileum, but an appreciable number of patients had involvement at other sites as well.

A statistical analysis using an exponential survival model revealed significant correlation between shortened patient survival and a combination of the number of sites involved and the extent of involvement within each site. Because many patients have involvement at multiple sites, the variables in the regression equation were strongly correlated among themselves and therefore not all were needed to predict survival. The variables found most useful in the prediction equation were those representing extent of involvement in the pubis-ischium, femur, and lung. On the basis of these variables, four risk groups were identified which showed marked variation in death rates due to prostatic cancer (see Table 1). Even in stage IV prostatic cancer, not all patients die of their disease because of the strong competing risk of death due to cardiovascular or other causes in aged males. In the group with the lowest risk (risk group 1) only 48% of the deaths were attributed to prostatic cancer directly, while 87% of the deaths in risk group 4 were due to prostatic cancer. Risk group membership also correlated with the average initial acid phosphatase, the average pain rating at diagnosis, and the average performance rating at diagnosis, and there was some relationship between risk group membership and the probability of having ureteral dilatation at the time of diagnosis (2). This abnormality was found in only 10% of patients in risk group 1, but it was present in 29-33% of patients in the higher risk groups. Surprisingly, the risk group membership did not correlate with the histological grade of the tumor as measured by the Gleason system (3). A possible interpretation might be that the histological grade of a tumor tells you how it might behave or what its potential is, while the number and extent of metastases at diagnosis tells you what the tumor has already done. For this reason, in the design and analysis of studies confined to patients with metastatic disease, it would be more important to stratify on radiological risk group than on the histological grade.

Table 1: Relationship of various factors to radiologic risk groups

	Risk Group			
	1	2	3	4
Number of patients[1]	21	42	24	15
Death rate (CAP)[2]	17.2	21.8	34.9	59.9
Death rate (All)[3]	36.2	33.8	46.5	69.1
% Cancer deaths	48%	64%	75%	87%
% with Ureteral dilatation	10%	29%	30%	33%
Acid phosphatase (mean)[4]	17.6	23.9	70.2	72.6
Average pain rating[5]	1.38	1.81	1.92	2.00
Average performance rating[6]	1.38	1.69	1.75	2.40
Histologic grade (Gleason)	7.000	7.024	7.304	7.143

[1] One patient studied in the 1974 paper was omitted because further study of his films suggested that he did not have metastatic disease.

[2] Death rate for prostatic cancer only, treating other deaths as withdrawals.

[3] Death rate for all causes combined.

[4] Measured in King-Armstrong units.

[5] Pain ratings were as follows: none=1, mild=2, moderate=3, and severe=4.

[6] Performance ratings were as follows: normal activity=1, in bed <50% of the time=2, in bed >50% of the time=3, and confined to bed=4.

Lung metastases have frequently been overlooked clinically in patients with prostatic carcinoma because they are usually lymphangitic rather than nodular and may easily be confused with congestive heart failure, pneumonia, or fibrosis. However, histological examination of the lungs in 566 autopsies of patients from the first VACURG study revealed that 20% of stage III and IV patients had metastases in their lungs at death. Breaking down these results by the randomly assigned treatments, the results were 29% for placebo, 13% for estrogen, 22% for orchiectomy plus placebo, and 16% for orchiectomy plus estrogen. Despite the fact that many patients in these studies originally assigned to placebo were later changed to endocrine therapy because of progression of disease, these results suggest that estrogen is more effective than orchiectomy alone or placebo in preventing the spread of the tumor to the lungs. In the present study we have noted that 24% of the patients had lung metastases at the time of diagnosis, but these were identified by careful study and might easily have been overlooked in a more routine clinical setting.

CHANGES IN LUNG AND BONE METASTASES FOLLOWING ENDOCRINE TREATMENT

From the original set of 102 patients, 52 were selected for further study because serial x-rays were available (initial, three months, and every six months thereafter up to 78 months). Of these 52 patients, seven had lung metastases at the time of diagnosis. Two of these were originally assigned to placebo, and follow-up films revealed that the lung metastases remained stable in one and progressed in the other. Of the five patients with lung metastases assigned to endocrine therapy, complete disappearance was seen in four by three to twelve months and improvement in one at three and six months. These results suggest that lung metastases, when present, may be a valuable indicator of whether or not the metastatic tumor is responding to endocrine (or other) therapy.

The evaluation of changes in bone metastases are more difficult because for prostate cancer, unlike most other cancers, metastases are usually osteoblastic rather than osteolytic. Following endocrine therapy one must distinguish between early and late changes. Early changes may include both increased number and density of blastic lesions on standard x-ray examinations, increased activity on nuclear bone scans, decreased acid phosphatase, and increased values for that portion of alkaline phosphatase arising from bone. If a true response occurs, then late changes will show either decreased number and/or density of blastic lesions on x-ray, decreased activity on bone scan, a continued decrease in acid phosphatase, and a decrease in bone alkaline phosphatase. The difficulty is that the early increased bone density on x-ray (or increased activity on nuclear bone scans) may cause one to misdiagnose the patient's condition as deteriorating rather than improving. For this reason the system of classification of changes

Table 2: System of classifying changes in appearance of bone metastases

X-ray patterns of bone metastasis change	Early	Late
Pseudo-progression	↑ Metastases	↓ Metastases
Regression	↓ Metastases	↓ Metastases
Stabilization	No change or slowed progression	No change
True progression	↑ Metastases	↑ Metastases

in the appearance of bone metastases on x-ray given in Table 2 was used in our study, where increased or decreased metastases refer to changes in number and/or density of blastic lesions. This system can only be employed when serial films are available for examination.

One of us (JH) examined serial films for all 52 patients without knowledge of the treatment to which they had been assigned and rated the response to therapy at each examination by comparing films with those from the preceding examination (4). The results of that review are given in Table 3. Progression was observed in 9 of 12 patients (75%) originally assigned to placebo versus 7 of 40 (18%) initially assigned to placebo showed pseudo-progression versus 26 of 40 (65%) of those assigned to endocrine therapy. It is very important to distinguish between true progression and pseudo-progression because failure to make this distinction could lead one

Table 3: Classification of x-ray response evaluated without knowledge of the assigned treatments

X-ray response	Placebo		Endocrine	
	N	%	N	%
Progression	9	75%	7	18%
Stable	1	8%	3	8%
Regression	0	0%	4	10%
Pseudo-progression	2	17%	26	65%

to conclude that endocrine therapy was not working when in fact it was. Pseudo-progression can only be distinguished from true progression by examining serial x-rays over considerable periods of time, often as much as one year. For this reason the evaluation of response to endocrine therapy, and presumably chemotherapy, cannot reliably be made by a simple examination of x-rays after say, three months of treatment as is common in many phase II studies of new agents. Nuclear bone scans were not generally available at the time these patients were studied. In the last decade these techniques of examination have become widely used and may provide earlier indications of response to therapy. It is suggested that researchers having data on serial changes in nuclear bone scans undertake studies similar to those we have reported in order better to define the usefulness of these methods in assessing prognostic significance and response to therapy.

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THE PRESENT STATUS OF RECEPTOR STUDIES IN RELATION TO PROSTATIC CANCER

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Research into androgen receptors in prostatic tissue started with the experimental work of Liao and Fang (1) and Bruchovsky and Wilson (2) in rats. They were able to identify the specific binding proteins for steroids, called receptors, in cytoplasm and nuclei of rat prostatic cells and they defined and confirmed their role in androgen metabolism of these cells.

For the physiological role of receptors three main models have been suggested:

1. that receptors act as storage and transport proteins protecting steroids against enzymatic attack and non-specific binding. In that philosophy steroids are the initiators of biological effects.

2. that receptors have a latent functional activity, that is activated by the steroid binding after which the steroid receptor-complex as a whole induces biosynthetic effects.

3. that receptors induce biological effects (such as transcription regulating). In this hypothesis steroids only amplify this action and thereby control the rate.

Whichever of these roles is considered to be true, there is no doubt that cytoplasmic as well as nuclear receptors play an important role in the metabolism of steroids in target cells. I should like to present here the first and the last scheme of Liao (1,3) and that of Ekman (4) for prostatic cells. The latter gives me the opportunity to show how it can be used to clarify the working mechanism of different hormonal treatments.

After the first successful attempts to determine androgen receptors in human prostate (5-7), it soon became evident that many technical (methodological) problems had to be solved. First of all, the most common circulating androgen, testosterone, was not the most potent and in fact not the one that is active within the prostatic cell. It has to be reduced to 5α-dihydrotestosterone (DHT) in the cytoplasm by 5α-reductase and this is then in turn bound to the specific receptor. But the most important difficulties encountered were those listed in Table 1.

Gradually these problems have been surmounted in the last 10 years. Though it is still not easy to get normal prostatic tissue, there are some contributions that deal with receptor content in normal prostate (8,9). For benign prostatic hypertrophy (BPH) receptor research and the whole picture of steroid metabolism has largely solved the origin of this disease. But still prostatic cancer (PCA) and BPH are usually compared to each other in consideration of receptor content.

The problem of endogenous androgens and SHBG-contamination has been solved in different ways. Wagner (10) invented cold agar-gel-electrophoresis to separate SHBG-bound and receptor-bound steroids and Raynaud and co-workers (11) contributed largely to this by the use of the artificial ligands of the Roussel-Laboratories (R-1881, R-5020). Both methods found their (more or less fervent) supporters and contributed to the solving of methodology. Others (12,13) worked on exchange incubation techniques to determine nuclear receptors and total receptors and so indirectly cytoplasmic receptors, which in men are normally occupied by endogenous androgens. Today the opinion is gaining ground that nuclear receptors are probably more important than those in the cytoplasm as far as the prognosis for hormonal therapy is concerned.

Table 1. Receptors in human prostate. Problems to be solved.

1. Normal prostatic tissue not usually available.
2. Therefore only tissue from patients with benign prostatic hypertrophy is available for comparison with neoplastic tissue.
3. Difficulties in obtaining enough suitable cancer tissue.
4. The endogenous androgens and plasma contaminants (SHBG).
5. The relation between stromal and epithelial elements in any given piece of prostatic tissue.

One must consider finally the relation between stromal and epithelial elements in the usually very heterogeneous prostatic tissue. The work of Bartsch and Röhr on stereology has to be mentioned in the first place (14), but others by way of statistical analysis (15) or by separation of stroma and epithelium (16) have solved this and established that both stroma and epithelium have their own steroid hormone metabolism. It is the interaction between the two that plays such an important role in the embryonal development of the prostate as well as in BPH and perhaps in the lack of inhibition of growth of prostatic cancer. Mc Neal's hypothesis (17) that BPH is the induction of normal epithelium by an altered stroma, while premalignant hyperplasia arises as an embryonic alteration of epithelium that becomes unresponsive to a normal stroma, is worth mentioning here. It probably also explains why oestrogen receptor, E-r, can be found in prostatic tissue as well as those for DHT (and also receptor for progesterone and corticosteroids).

Some difficulties still remain. Though there is increasing evidence that a correlation exists between the occurrence of receptors and the success of anti-androgenic therapy (4,18), we have as yet no definite proof of it in such a way that it could be used on a routine basis for the choice of treatment.

Also the problems of obtaining PCA tissue for biochemical assay are not yet solved. Surgical excision or thick needle biopsy (5) has to be used and there is increasing demand for micro-assays, especially for those that could use the cell material obtained by aspiration biopsy for cytologic diagnosis.

In my opinion the work of Nenci (19) and Pertschuk (20) deserves our attention. They use steroid ligand conjugates, labeled with fluoresceine isothiocyanate (FITC) to localize receptor sites by way of fluorescence. They still need frozen sections of tissue-biopsies to perform this histochemical receptor assay.

More promising is the use of the naturally fluorescent plant estrogen coumestrol or the small molecule of dansyl-hexestrol as fluorescent probes (21) that can be used in whole cells (for instance by incubation of thin needle aspirates or cell cultures). When these methods have been elaborated for all steroids and developed into reliable and simple techniques we will have taken a large step forward. In the same context should be mentioned the attempts to purify receptor proteins in order to allow for immunological assays, as well as for better understanding of the mechanism of action. Though much has been achieved in this respect for oestrogen and progesterone-receptors, the purification of androgen receptors has so far met with unsurmountable technical problems. However the future prospects for investigation of the mode of action of androgen receptors are favourable (22).

Finally, back to the experimental laboratory: I would like to end this paper by mentioning some very recent results of Rao and co-workers on the Dunning tumor in Copenhagen rats (23), one of the very few reliable animal models of prostatic cancer. Using a hormone-independent anaplastic (R 3327-AT) cell-line of this tumor they found neither DHT nor E_2 receptors. After continuous culturing of these cells in the presence of high concentration of estradiol an estrogen-adapted cell-line resulted. After a few passages E_2 -R were detected in these cells and when they were injected to female rats tumors grew in 12 days. Androgens and anti-estrogens were cytotoxic to these cells.

This adaptation phenomenon, which resembles in many ways the work of Noble (24) could throw some new light on the problems which remain: First, as I mentioned earlier, it might explain the presence of receptors in non-target tissues, such as renal cell cancer. Secondly, it could explain the question of hormone-resistance at least partly, and thirdly it contradicts Coffey and Isaacs' hypothesis (25) of the survival of androgen-insensitive cells.

Though much work still has to be done to confirm this in human prostatic cancer cells, future prospects for receptor research are encouraging and this work is worthwhile pursuing.

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METHODOLOGY AND PRINCIPLES OF IMMUNO-CYTOLOGICAL ASSAYS OF STEROID RECEPTORS

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Recent development in cytochemical techniques for steroid receptors may contribute to bridge the gap between molecular biology in vitro and cell biology in vivo, thus leading to significant progress in knowledge of cancer which can influence the treatment approach.

Since the first report (1) many generations of methods have rapidly followed.

To visualize steroid receptors under the microscope, the cytochemical techniques exploit the same binding properties of the receptor site as are used in biochemical assay.

The first generation belongs to immunocytochemistry; these methods utilize a specific anti-steroid antibody which is then traced by a second anti-immunoglobulin antibody labelled with a fluorescent or enzymatic tracer (2). By the antibody technique, the steroid molecules specifically retained by receptor sites in target tissues can be visualized under ultraviolet light and by electron microscopy.

The immunocytochemical techniques need accurate control tests of immunological specificity in addition to the tests of steroid binding specificity which are in common with other techniques.

The second generation methods, of labelled-ligand cytochemistry, involve steroid hormones coupled to a macromolecular protein carrier, such as bovine serum albumin, which is highly

substituted with fluorescein or horse radish peroxidase (3,4). These labelled steroid derivatives may be applied only to the static evaluation of steroid binding on frozen tissue sections since they are prevented by their size from getting through the same plasma membrane.

The third generation of techniques is the use of affinity cytochemistry (5,6) allowed by the synthesis of steroid molecules directly labelled with a fluorescent tracer. These micromolecular derivatives bind to cytosolic receptor with a relative binding affinity higher than the macromolecular ones and interact with intact cells in a way similar to the native hormone. Therefore, these fluorescent hormonal probes can enter the cells, thus yielding information on the overall intracellular kinetics of steroid receptor complexes under appropriate experimental conditions (6). The latest technique, of affinity cytochemistry, exploits the intrinsic fluorescence of estrogen molecules such as Coumestrol, a blue fluroescing phytoestrogen whose relative binding affinity is within the range of that of physiologic E_1 and E_3 estrogens (7).

The fourth and last generation is now in gestation; these newer techniques attempt to exploit a specific anti-receptor raised in animals or hybridomas to trace the receptor protein itself (8). It is now possible to use this technique for glucocorticoid receptors and for estradiol receptors in breast cancer. In this way, a major breakthrough in the cytochemical approach to steroid receptors is being achieved. The demonstration of receptor through its binding capacity may be an advantage since not only the presence but also the primary function of the receptor can be assessed. The results of the various cytochemical techniques should be interpreted with strict attention to accurate control tests since the demonstration of binding site does not necessarily provide evidence for their receptor nature.

According to current concepts, the demonstration of finite binding capacity, high affinity, steroid and tissue specificity constitutes reasonably good evidence for the receptor nature of the displaced binding sites. Non specific binding would be, on the contrary, of limited capacity, rapidly dissociating, non steroid specific and ubiquitous. In cytochemical investigations the above criteria can be appropriately checked.

Some other data support the specificity of histochemical tracing of steroid binding sites; they are the consistency of the positivity by the same (intra-assay validation) or different (inter-assay validation) techniques when applied to tissue sections of the same specimen and chiefly the physiological validation since these techniques visualize specific binding sites which fulfil the intracellular requirement for steroid receptor sites.

In fact, the cytochemical techniques can display the site of steroid synthesis, the presence of binding sites in different cell compartments of target tissue and, of especial importance, the intracellular kinetics of steroid hormones. In this way, the precise identification of the structural expression of the mechanism of steroid action can be achieved, exploiting different experimental conditions.

Breast cancer has been the testing bench of all cytochemical approaches to steroid receptors, but the results can be applied to other tumors growing from steroid responsive tissue, such as the prostate.

The heterogeneity in hormone receptivity of tumor cells and the presence of several defects of the steroid action mechanism downstream to cytoplasmic uptake, such as impaired nuclear translocation and defective or absent nuclear retention, are responsible for the variable tumor hormone responsiveness and can be easily visualized by cytochemical procedures (9).

As far as the prostate is concerned, the above cytochemical techniques visualize a differential binding of estrogen and androgen with a very interesting dualism between the stroma and the epithelium (Fig. 1.).

While the androgen binding sites are predominantly located at the glandular level, the estrogen binding sites are chiefly distributed in the stroma. This result, besides confirming some recent results concerning cytosolic binding sites or separated epithelium and stroma of human prostatic hyperplasia (10), draws attention to the already suggested differential control of stromal and epithelial growth by sex steroid hormones at the prostatic level.

SUMMARY

Cytochemical techniques visualize steroid receptors under the microscope exploiting the same properties of the receptor site as do the techniques for biochemical assay.

Many generations of methods have followed in rapid succession including immunocytochemistry, labelled-ligand cytochemistry and affinity cytochemistry. These techniques, under different experimental conditions, can display the site of steroid synthesis, the presence of specific binding sites in target tissues and the intracellular kinetics of steroid hormones.

As far as the cancer is concerned, the cytochemical procedures easily visualize the heterogeneity in hormone receptivity of tumor cells and the presence of several defects of steroid action mechanism which are responsible for the variable tumor hormone responsiveness.

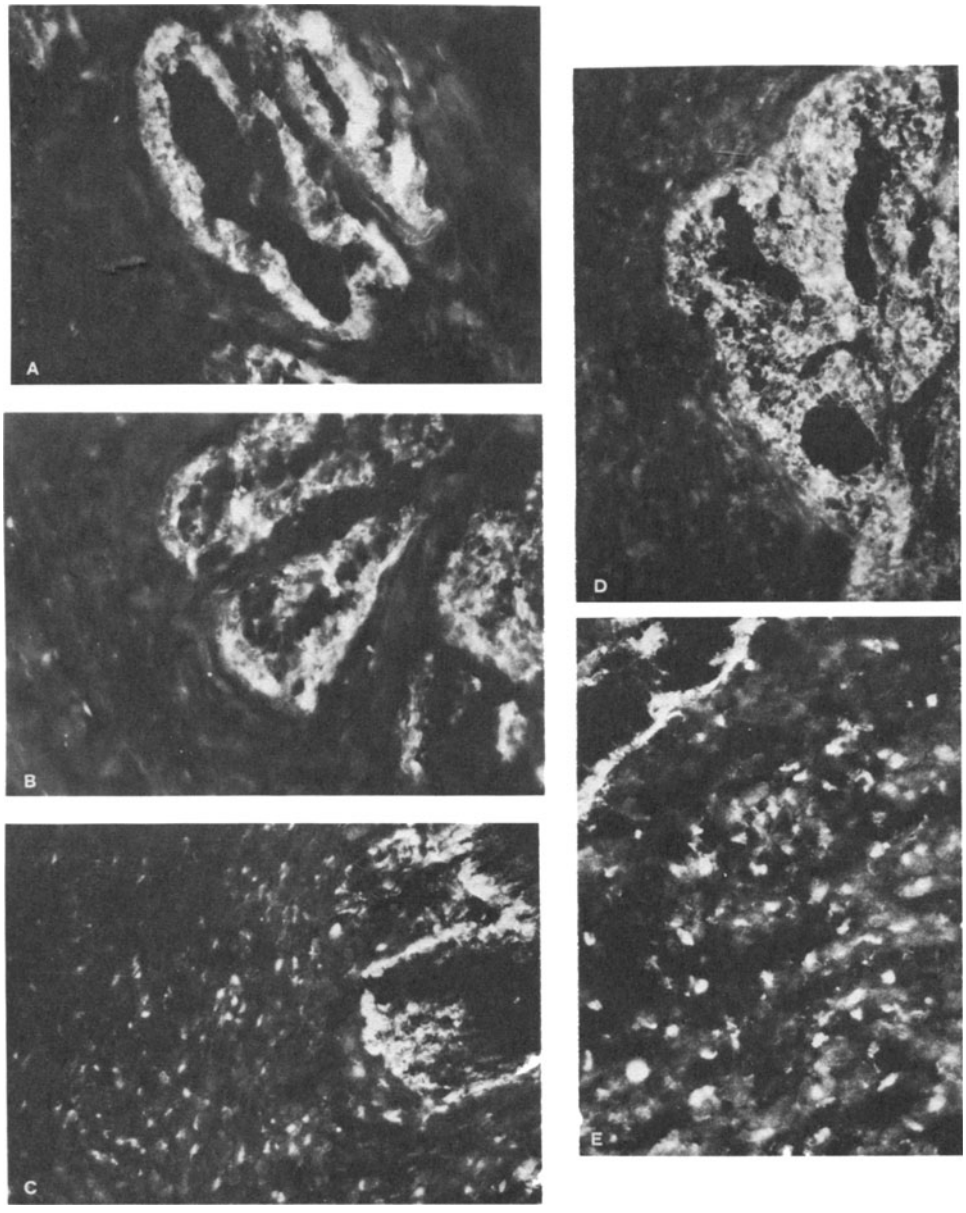
A
B
C
D
E

Fig. 1. Sex steroid binding sites detected in human prostate by cytochemical techniques.

A. Normal gland: Evident cytoplasmic uptake of labelled androgen by epithelial cells; stroma is unstained.

B. Benign hypertrophy: Labelled androgen is taken up by hyperplastic epithelial glandular cells only; the fibromuscular stromal cells are unstained.

C. Benign hypertrophy: Fluorescent estrogen shows specific estrogen binding sites in both the epithelial and the hyperplastic stromal cells.

D. Cribriform carcinoma: Androgen binding sites are clearly displayed at the cytoplasmic level of neoplastic epithelial cells.

E. Infiltrating carcinoma: Infiltrating neoplastic cells show a predominantly nuclear localization of androgen binding sites.

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HISTOCHEMICAL DETERMINATION OF ANDROGEN BINDING SITES IN PROSTATIC ADENOCARCINOMA: CLINICAL CORRELATION

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The figure of 80% is classically quoted as the percentage of patients with advanced prostatic adenocarcinoma who respond at least initially to hormonal manipulation. At the present time there is no method of predicting whether a given patient will respond to such therapy. It is not unreasonable to hypothesize that those patients with androgen dependent tumors should have high levels of androgen binding sites. A non-immunologic histochemical method has been developed by Pertschuk (1,2,3,4,5) to measure androgen binding sites. The principal purpose of this report is to update the clinical trial we are currently conducting to determine whether or not the test can be used to predict response to hormonal manipulation.

The clinical trial is being conducted as follows. Whenever prostatic tissue is removed from a patient, a portion is reserved for histochemical androgen binding site analysis and a portion for biochemical analysis. Each patient is entered into a computerized data retrieval system together with all the clinical and pathological facts pertinent to his case. The trial is double blind in that the clinician is unaware of the histochemical and biochemical assay results and the assayists are unaware of the clinical data. Response to therapy is measured by the criteria of the National Prostate Cancer Project (6).

The Pertschuk test has been completely described (1,2,3,4,5). Briefly, a solution is prepared of testosterone linked to a fluorescein moiety (7). The specimen of prostatic cancer, which is immediately frozen in liquid nitrogen after removal from the patient, is subsequently sectioned (four microns thick) and placed on a glass slide. After warming to room temperature, the section is covered

with the androgen fluorescein solution for two hours, then examined under an ultraviolet light microscope. A step section of the frozen specimen is taken and processed by the routine H & E technique. The results of competitive and non-specific binding studies have been published (1,2,3,4,5). To date 177 specimens of prostatic adenocarcinoma have been so studied.

Specimens in which, by visual estimate, 0-8% of the cells displayed fluorescence were designated as binding site poor, those in which 9-14% were stained as borderline, and those with 15% or more stained as positive. The predominant site of staining - cytoplastic, nuclear or mixed - was noted.

RESULTS

Of the first 107 cases the specimen was obtained by TURP in 45, by needle biopsy in 41, by open prostatectomy in 11, by open biopsy in 5 and by needle biopsy and TURP in 5.

Histological grade was assigned using the Gleason system (8) and clinical stage by the method of Whitmore (9). Tables 1 and 2 show that neither estrogen nor testosterone binding correlated with histologic grade or with clinical stage.

The method of biochemical assay which we utilize has been described (5). Table 3 demonstrates the agreement between biochemical testosterone (AR) assay and histochemical testosterone binding. Our method of statistical analysis for this and subsequent tables has been described (5). The agreement of qualitative histochemical androgen assay to biochemical androgen receptor assay is indicated by the Kappa (K) statistic: K = .365 and p .0001.

Table 1. Histological Grade and Steroid Binding by Histochemistry in Prostatic Carcinoma (171 cases)

Tumor Grade	% E and T+	% E and T-	% E+ /T-	% E- /T+	Total
1 - 2	87	8	4	1	97
3	82	5	10	3	40
4 - 5	79	15	3	3	34

E = Estrogen binding, T = Anrogen binding.

Table 2. Clinicopathologic Stage and Steroid Binding by Histochemistry in Prostatic Cancer (177 cases)

Stage	% E and T+	% E and T-	% E+/T-	%E-/T+	Total
A	86	14	0	0	29
B	87	7	2	4	45
C	92	8	0	0	15
D	83	7	6	4	88

E = Estrogen binding, T = Androgen binding

Table 3. Agreement Between Biochemical AR Assay and Histochemical Androgen Binding Assays in Prostate Cancer (103 cases)

	Biochemical Assay		
Histochemical Assay	Positive	Negative	Total
Positive	77	9	86
Negative	9*	8	17
Total	86	17	103

* In three of these cases, histochemistry revealed the tumor to be poor in androgen binding and adjacent benign glands to be positive.

Table 4 further analyzes the histochemical to biochemical agreement by examining the relationship with regard to subcellular localization of binding sites. For this K = .546 and p .0001.

We have described a method for semi-quantitating the results of histochemical assay (5). Table 5 related the quantitative biochemical assay to the semiquantitative histochemical assay. For this K = .317 and p .0001.

Table 4. Agreement Between Histochemical and Biochemical Detection of Subcellular Androgen Binding Sites in Prostate Carcinoma (103 cases)

	Biochemical Assay				
Histochemical Assay	Cytoplasmic	Nuclear	Mixed	None	Total
Cytoplasmic predominant	38	3	7	6	54
Nuclear predominant	5	5	-	3	13
Mixed (circa 1:1 nuclear vs cytoplasmic)	3	1	21	-	25
None	1	1	1	8	11
Total	47	10	29	17	103

Includes correlations in histochemically processed tissue sections designated as poor where <10% of cells were positive.

Table 5. Comparison of Quantitative Biochemical AR and Semi-quantitative Histochemical Androgen Binding Assay Results in Prostate Cancer (77 cases)

	Biochemical Assay			
Histochemical Assay	Zero-Trace	Low-Intermediate	High-Very High	Total
Zero-Trace	9	6	4	19
Low-Intermediate	10	15	7	32
High-Very High	5	3	18	26
Total	24	24	29	77

Biochemical designation of positive, non-quantifiable (+NQ), not included as no comparative histochemical designation available.

Histochemistry (Semiquantified)	Biochemistry (fmoles/g tissue)
Zero-trace 0-<5	0- 150
Low-Intermediate >5-18	>150- 550
High-Very High >18->30	>550->950

Table 6. Comparison of Androgen Binding by Semiquantitative Histochemistry and Biochemistry to Clinical Response to Hormonal Therapy (38 cases)

	Histochemistry			Biochemistry				
Response	Z-T	L-I	H-VH	Z-T	L-I	H-VH	+NQ	NA*
Complete	0	0	3	0	2	1	0	0
Partial	0	8	8	1	5	5	2	3
Stable	2	5	0	0	0	0	2	5
Progressed	7	5	0	3	1	4	1	3

Histochemistry (Semiquantified)	Biochemistry (fmoles/g tissue)
Zero-trace (Z-T) 0-<5	0- 150
Low-Intermediate (L-I) >5-18	>150- 550
High-very high (H-VH) >18->30	>550->950

*NA - Not Suitable for Assay

Table 6 examines the ability of histochemical and biochemical analysis to predict response to hormonal manipulation. The clinical records of 38 men with stage C and D cancer where more than one year had elapsed since specimen acquisition were available. Both methods exhibit trends whereby patients with zero or trace androgen binding progress clinically and those with high binding have a response other than progression.

Statistical analysis indicates that treatment response was significantly associated (p .0001) with the histochemical result but not with the biochemical result (p .60).

COMMENTS

The histochemical technique has many advantages and few disadvantages when compared to biochemical techniques. It is simple, rapid and inexpensive. It can be performed in a simple community hospital laboratory by regular laboratory personnel. The technique allows for subcellular binding site localization (cytoplasmic vs nuclear). The test can be performed on minute pieces of tissue, even aspiration biopsy specimens. Binding capacity heterogeneity means that within a given specimen not all cells exhibit the same binding capacity. Variation of fluorescent intensity of a given specimen, we believe, represents this heterogeneity and is easily appreciated by

the histochemical method. Most specimens demonstrated significant heterogeneity.

Of the first 108 patients known to have prostatic cancer, six failed to have tumor on the particular specimen submitted for histochemical assay. Verification of the presence of tumor is an important advantage of the histochemical technique.

In three specimens analyzed histochemically, the tumor was seen to be poor in androgen binding while adjacent, non-malignant prostatic glands were rich in binding sites. In all three cases, biochemical assay was positive.

The histochemical technique allows for visual identification of heat artifact in TUR specimens. Heat can destroy or alter androgen binding sites and thus cause a falsely negative assay.

Do the biochemical and histochemical techniques measure the same thing? At this point an answer cannot be given with certainty. The ultimate test is not whether they agree but whether either can accurately predict response to hormonal manipulation.

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PROSTATIC CANCER: CLINICAL SIGNIFICANCE OF RECEPTOR STUDIES

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In recent years, there has been a substantial increase in our knowledge about the mechanism of action of sex steroids in hormone responsive target organs. It is now believed that the high concentration of hormones measured in cells of organs such as breast, uterus and prostate is due to a binding (receptor) protein in the cytoplasm; this protein binds the appropriate hormones specifically and with a high affinity. Following the binding, the receptor protein undergoes a process known as activation - permitting or causing its translocation to the nucleus. Activation and translocation occur within minutes of the hormone entering the cell and the whole process has been shown to be energy dependent. Once within the nucleus, the hormone receptor forms a complex with the chromatin. This final step induces several responses characteristic of the steroid sensitive tissue and it should be noted that these processes are not manifested before the hormonal stimulation. This is, however, an over-simplification - since in some target tissues hormones entering the cell may also be subjected to metabolism (1). Nonetheless, current theories attach major emphasis to the translocation of the steroid receptor complexes into the nucleus and with a few minor exceptions, hormonal responses are, on the whole, believed to be centred on the selective activation of nuclear processes in target cells (2).

Although receptor studies are now widely used for the identification of hormone responsive tissues in gynaecological and breast tumours (3), there is still little information on the relevance of these studies in the treatment and management of prostatic cancer (4). These attempts have been frustrated in part by the failure to characterise a specific receptor in the prostate and partly by the lack of a reliable assay for the measurement of these receptors.

Furthermore, the interpretation of biochemical data is complicated by the heterogeneous nature of the prostate gland. Prostatic tissue consists of a mixture of stromal and epithelial cells and the relative proportion of these components in specimens removed at surgery reveals considerable variation from patient to patient (5).

The most recent developments in prostate/steroid receptor investigations and some of the more common pitfalls associated with the measurement of these molecules are reviewed. The results of studies on the distribution of hormonal binding proteins within the separated prostatic tissue components are reported.

CYTOPLASMIC RECEPTORS

Most of the earlier receptor studies concentrated on the cytosol fraction. Although androgens remain an important aspect of this type of study, recent investigations have also established the presence of oestrogen and progesterone receptors in the prostate.

Androgen Receptors

Androgen receptors have been demonstrated in normal prostate, benign prostatic hyperplasia and carcinoma of the prostate (6,7,8,9, 10,11,12,13,14). The studies were characterised by a broad range of receptor concentrations. Furthermore, preliminary correlation with response to treatment has been mixed and inconclusive. Although Mobbs et al, (12) and Ekman et al (7) observed partial responses to hormonal manipulation, when the androgen binding capacity of cytosol protein was between 0.3 and 2.0 fmol/per mg tissue, other workers (6) were totally unsuccessful in demonstrating a consistent pattern between a receptor concentration and clinical response.

There are many factors which may account for these reported differences. Firstly, the contamination of tissue with sex hormone binding globulin; this blood protein possesses similar properties to the cytoplasmic receptor and it is more difficult to distinguish between the two components. The second problem concerns the high endogenous concentrations of dihydrotestosterone present in prostatic tissue; although these steroids frequently mask the potential hormone binding sites, reports on patients with low endogenous androgens suggest that hormonal sensitivity of prostatic carcinoma is not directly related to the concentration of androgen receptors (12). Thirdly, the presence in the receptor of a protein with similar characteristics to a progestin receptor which also binds to androgens (15) may erroneously lead to higher estimates of androgen receptor than is actually present in the tissue. Perhaps even more significant are our own recent observations revealing that the cytosolic androgen receptor concentration per gm of tissue was higher in the

Table 1. The Relative Receptor Concentrations in the Cytosol of Stroma and Epithelial Components Obtained from Seven Patients with Benign Prostatic Hypertrophy.

Patients	Epithelium (fmol/gm wet tissue)	Stroma (fmol/gm wet tissue)
1	55*	23
2	56	62
3	12	9
4	56	31
5	47	58
6	86	58
7	146	120

* Mean of three separate readings

epithelial components than in the stroma (Table1). This suggests that the measurement of receptor concentrations in whole tissues is probably related to the epithelial cell content in the analysed specimens. Thus the stroma rich periurethral regions of the prostate could maintain lower binding capacities than the predominantly glandular peripheral sections of the gland. A recent study on various lobes obtained from the same prostate have revealed that different parts of the same tumour possess different degrees of hormone dependence (16). This further confirms the above hypothesis and underlines the inherent danger of single sampling analysis in receptor studies.

Oestrogen Receptors

The extensive use of oestrogen therapy for the treatment of prostatic cancer has led many investigators to examine the role and function of the steroid in the prostate gland. Extensive studies on the human prostate have revealed that oestrogens inhibit the binding of dihydrotestosterone to the cytosol fraction; this may account for some of the beneficial effects detected in carcinoma patients treated with oestrogen based drugs. More important, however, are the reports of a specific 17β oestradiol receptor in the cytosol of the human benign hypertrophied prostate (17). Although the presence of receptors has not been universally accepted, more recent investigations by Sidh et al. (18) have suggested that patients with

tumours deficient in oestrogen receptors but containing a full measure of androgen receptors are less likely to respond to endocrine manipulation.

Progesterone Receptors

There is increasing evidence that progesterone is important in the development of benign prostatic hypertrophy. A number of studies on the normal and pathological prostate demonstrated the presence of progesterone receptors in most cases of B.P.H. (19) but they are less common in normal prostatic tissue. It is possible that progesterone receptors in B.P.H. are a phenomenon secondary to changes in androgen/oestrogen blood levels and it may also be that progestational anti-androgens are more logical drugs to consider in the control and even reduction of benign prostatic growth. Progesterone receptors were also detected in prostatic carcinoma (7) and in view of the favourable reports following progestin therapy, carcinoma patients with progesterone receptor rich tumours should possibly be considered for this type of treatment.

NUCLEAR RECEPTORS

In view of the technical problems associated with the accurate measurements of androgen receptors in the cytoplasm of the human prostate, a number of attempts were made to quantify receptors localised in the purified nuclear fraction (14,15,20). A high affinity binding for androgens was demonstrated in all cases and it is now believed that approximately 75% of the total measurable androgen receptors in benign prostatic hypertrophy is located in the nucleus. These results were further confirmed by our own studies which also revealed that the bulk of these androgen binding proteins was located in the stromal components of the nuclear extractions (Table 2). The importance of these receptors as discriminant for hormone responsiveness can not yet be determined but assuming that nuclear receptor studies in malignant tissue will also yield a correspondingly high localisation of binding sites in the nuclei, this may prove a useful measure in assessing treatment of carcinoma of the prostate. There are reports of patients with metastatic carcinoma of the prostate who have a good response to hormone therapy despite poor or negative levels of cytoplasmic receptors (21). It is possible that nuclear receptor measurements might have identified these patients as responders.

Preliminary studies on the nuclear components have also identified the presence of oestrogen and progesterone binding proteins, but again as in the case of the androgen binding components the significance of these molecules has not yet been established.

Table 2. Variation in Nuclear and Cytosol Androgen Receptor Concentration in Tissue Components of Eight Patients with Benign Prostatic Hypertrophy.

	Cytosol fraction (fmol/gm tissue)	Nuclear fraction (fmol/mg tissue)
	Mean (range)	Mean (range)
Whole tissue	108 (71-209)	108 (62-385)
Epithelium	65 (12-146)	102 (43-326)
Stroma	51 (9-120)	128 (43-280)

Conclusions

The measurement of hormone receptors in human prostatic tissue has been greatly limited by many difficulties and until these have been resolved the success of the hormone receptor test in predicting the hormonal status of prostatic cancer will be of limited use in the management of this tumour. Several attempts have already been made to provide new methods for the measurement and the specific localisation of receptors in individual samples of human cancer. Perhaps the most promising of these new techniques are the immunocytochemical tests which will enable the direct identification of the specific binding proteins on the histology slides, thus allowing for a histological analysis and a biochemical test to be carried out on the same section of tissue. Another important approach will be the production of monoclonal antibodies which it is hoped will dramatically improve the sensitivity and specificity of the immunological procedures. At the same time, the possibility of alternative approaches for predicting clinical response, for example, the measurement of whole tissue concentrations of dihydrotestosterone, the assessment of androgen metabolism in subcellular fractions or further fundamental studies on receptor proteins, may still be necessary and should not be ignored.

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RADICAL PROSTATECTOMY

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INTRODUCTION

Treatment of prostatic cancer is an interesting subject. One can expect cure from a radical prostatectomy providing there is no spread beyond the prostate gland. Radical perineal prostatectomy offers this cure without the advantage of sampling and testing the lymphatics which drain the prostate, staging only. Nonetheless, it is a time honored treatment which has excellent results and which is much less damaging to the patient than radical retropubic prostatectomy with node dissection. It is also less discomforting to the patient in the sense that the complications are much less and, in my opinion, the opportunity to do a direct anastomosis to the urethra under vision, allows for a better control and a decrease in the chance of urinary incontinence. There are no available figures that I know of to support my view, but I feel quite certain from watching the results of retropubic radical prostatectomy versus radical perineal prostatectomy, that the statements I have made are true.

When radical retropubic was a new operation, I tried to do it and subsequently tried to do a combination of radical retropubic and perineal, but I gave this up because it seemed to me that the patients fared much better with a radical perineal and I believe that when the figures are finally in, it will be seen that the idea of doing a radical node dissection, plus a radical retropubic prostatectomy does not justify the trauma to the patient.

PART I: RADICAL PERINEAL PROSTATECTOMY

My view is that a radical perineal prostatectomy is the surgery of choice in A-2 and B-1 and B-2 lesions. There may also be a particular place for it in stage C lesions, simply to relieve the patient of any further difficulties with obstructive symptoms. I am completely committed to this operation as the operation of choice for early prostatic cancer. I would not do it in the presence of known bony metastases or metastases elsewhere, but I believe that some stage C lesions with lymphatic metastases are best handled in this manner.

Radical perineal prostatectomy is one of the most intriguing and exacting operations in the field of urological surgery. It requires a perfect understanding of the anatomy and meticulous care whilst trying to "sail 'twixt wind and water".*

There are many variants of technique, but the basic procedure was described by Doctor Hugh Hampton Young. He describes the history and genesis of this procedure in his classic article, "The cure of cancer of the prostate by radical perineal prostatectomy (prostato-seminovesiculectomy): History, literature, and statistics of Young's operation" (1). In that article, he records his first operation note in 1904 and the technique of his operation is beautifully illustrated by William P. Didusch. It my view, it is impossible to improve on Doctor Young's description. However, I am going to try to summarize it.

In this discussion I wish to refer to figures 11 - 21 by Didusch illustrating Hugh Young's operation (1) and to the illustrations in Elmer Belt's article (2). I will not attempt to reproduce them because they are so numerous and because they are easily available to almost everyone with access to a library.

The first and most important step is to position the patient in the exaggerated lithotomy position in such a way that the perineum is parallel to the floor of the operating theatre. This allows the rectum to drop backward and gives the surgeon the best access to the prostate (3).

After meticulous cleansing of the penis, scrotum and perineum, including the anal region, the anus is draped out of the field in such a way as to give the operator access for a rectal examination if he needs to do it during the operation. The important steps in the operation are described on the following pages.

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* "Young in heart; young in hand,
Young is known throughout the land.
Lord preserve us from the slaughter
That he creates
'Twixt wind and water." (Keyes)

1. The incision is an inverted "U" made, according to Young's plan, three scalpel handles anterior to the anal margin. A curved sound is inserted into the urethra to allow the prostate to be levered upward into the wound.

2. After the skin has been opened, the ischiorectal fossa is developed on each side of the central tendon by blunt dissection with the fingers and the handle of the scalpel.

3. Young's bifid posterior tractor is used to pull the rectum backward which accentuates the central tendon which is then cut. This allows the rectum to drop backward and the transverse perineal muscles to go forward with the bulb and the urethral sphincter.

4. Dissection is then carried downward bluntly until the fibers of the levator ani are found. Doctor Young identified the decussation of these fibers in the midline as the "rectourethralis muscle". The surgeon stays well posterior to the external urinary sphincter, which really should never enter into the field. The muscles of the levators, or the rectourethralis muscle as identified by Young, are either transected near the apex of the prostate, or pushed laterally until the fascia of Denonvilliers is encountered. It is a glistening white fascia which looks very much like peritoneum which, indeed, it was embryologically. There are two layers to it. Doctor Young used to describe this as "the pearly gates".

5. The lateral fascia, together with the levator muscles, is pushed laterally to expose the lateral sides and the apex of the prostate. The prostate is pulled downward on traction to elongate the intrasphincteric part of the urethra, and a very careful transverse incision is made just at the apex of the prostate without involving the urethral sphincter. The urethra is then completely divided and dissection is carried upward along the anterior surface of the prostate. Young's prostatic tractor is placed through the apex of the prostate into the bladder and used for traction to pull the prostate downward into the wound and identify the anterior portion. There is often excessive bleeding from the area of Santorini's plexus; and it is important, if possible, to secure these vessels with a good ligature before cutting the pubo-prostatic ligaments in that area. The endo-pelvic fascia is entered bluntly and dissected to free the prostate laterally on each side.

6. The prostate is pulled downward into the wound with Young's prostatic tractor and the anterior fascia is pushed away from the bladder until one can see the junction between the prostate and the bladder. A knife is plunged through the bladder in the median line at its junction with the prostate to open the bladder anteriorly.

7. The anterior bladder incision is then enlarged by cutting downward on each side exactly at the place where the prostate lies next

to the bladder neck. Young describes removing a small cuff of bladder with the prostate. The trigone and ureteral orifices are visualized and the incision is made distal to that point.

8. The incision is carried all the way across to free the prostate completely from the back of the bladder and this opens the retrovesical space to expose the anterior surface of the seminal vesicles. The bladder is lifted off the seminal vesicles until the ampullae of the vasa can be seen and the vasa are identified and transsected. They may either be clipped or tied. The most difficult part of this dissection now comes when the blood vessels leading into the tips of the seminal vesicles have to be transsected between clamps, or, as is now more usual, metal clips. The vascular pedicle of the prostate and seminal vesicles is cross-clamped, cut and then ligated, usually with a heavy chromic suture.

This allows removal of the total prostate together with a cuff of the bladder and the seminal vesicles with the anterior and posterior layers of Denonvilliers' fascia covering them, intact. Didusch has made a perfectly beautiful drawing of one of these specimens which is demonstrated in Young's article.

9. The closure is now performed after narrowing the neck of the bladder. This can be done in several ways. I usually prefer to do it by taking sutures at all four quadrants and simply bringing them in closer and closer until the neck of the bladder is approximately No. 20 French. It is then necessary to sew the neck of the bladder to the severed urethra, usually with interrupted 3-0 chromic sutures. The anterior sutures are placed first and then, working around the circumference of the open bladder neck, other interrupted sutures are placed. A No. 22 Foley catheter is inserted after the anterior sutures are placed. Young's article illustrates the use of Vest's suture which comes out through the urethra to pull the bladder neck down. This is useful but not necessary when it is possible to obtain an excellent direct anastomosis.

10. After the careful anastomosis performed over a Foley catheter, a search is made to be sure there are no bleeding areas deep in the wound. Occasionally the artery that comes in near the seminal vesicle can be a troublesome bleeding spot and difficult to find.

11. Many perineal surgeons will, at this point, put on a new glove over their already gloved hand and do a rectal examination to be sure there has not been any rectal injury.

Following this, a Penrose drain is placed and the levator muscles are brought together with interrupted chromic sutures to give further support. This also seems to help in the control of urinary continence. Then the subcutaneous structures are brought together with interrupted chromic sutures and the skin is closed

with interrupted nonabsorbable sutures, allowing the Penrose drain to come out one side of the wound.

The drain is usually left in for 24 - 48 hours and then removed. The urethral catheter is usually left in for approximately ten days.

A significant modification of the approach with a new anatomical observation was described by Elmer Belt in 1942 (2). His contribution was to show that it was anatomically feasible, and in his hands, desirable, to approach the prostate by dissecting underneath the anal sphincter and using the anterior wall of the rectum as a guide, to expose the prostatic area. His perineal incision is a flatter inverted "U" shaped curve, a very short distance, perhaps half a centimeter, in front of the mucocutaneous junction of the anus.

As soon as the skin has been opened, the posterior flap is turned downward to exclude the anus from the field to prevent contamination. One immediately encounters the anal sphincter, either external or internal, and by blunt dissection with the scalpel handle, lifts the anal sphincter off the rectum.

After identifying the levator ani muscles which Doctor Young had called the rectourethralis they are separated from each other in the midline and pushed laterally far enough to reveal the whole posterior aspect of the prostate, which is apparent as the gleaming fascia of Denonvilliers. The vertical fibers of the rectum are pressed below the lower margin of the prostate.

After separating the lateral aspects of the prostate from the surrounding fascia by blunt dissection the prostate is pulled upward from behind to identify the vessels entering at the lateral inferior border. These are isolated and either clamped and ligated or they may be handled with metal clips.

At this point, his procedure differs from Doctor Young's in that Denonvilliers' fascia which covers the back of the seminal vesicles is opened by a transverse incision across the base of the prostate and the vesicles are identified from behind while pulling the rectum backward.

The seminal vesicles and their ampullae are then bluntly dissected from the fascia surrounding them and the large artery which enters at the apex on each side is carefully ligated. The vas is dissected free on each side and transsected and ligated, or it may be clamped with a metal clip.

At this point, the operator turns his attention to the apex of the prostate and Belt cuts across the gland "leaving a collar of prostatic tissue to attach to the membranous urethra" to improve urinary control. Hugh Young criticized this, saying that it was not

a true radical operation if some of the prostate was left behind. The fact is that most patients would rather have residual cancer than be incontinent of urine.

Traction is obtained on the apex of the prostate with two Lahey type thyroid clamps, and the anterior surface is exposed by blunt dissection. Santorini's plexus is pushed away from it in an attempt to stay out of those veins.

This finally leaves the prostate attached to the bladder at the bladder neck. This is cut across with scissors at a right angle so that the prostate with its capsule and both seminal vesicles and ampullae are removed as a single specimen.

The anastomosis is carried out under direct vision; usually 3-0 chromic is used. Two or three anterior sutures are placed under direct vision and than a No. 22 Foley urethral catheter is introduced and the rest of the anastomosis is carried out posteriorly with interrupted chromic sutures.

The posterior layer of Denonvilliers' fascia which has been dissected in order to bring the seminal vesicles into the wound is now seized and brought forward to cover the anastomosis and give added support in that area.

The levator ani muscles, which instead of being transsected, as in Young's procedure, have been separated laterally, are now brought together with interrupted sutures in the midline to give further support.

The edges of the rectal sphincter are picked up with a circular suture which tends to pull the sphincter down and close the area that has been opened between the sphincter and the rectum in the early dissection. A Penrose drain is placed. This is brought out through an angle of the wound and the skin is closed with a continuous subcuticular stitch. The drain usually remains in place for 24 - 48 hours and is then removed. The urethral catheter usually remains in place for approximately 8 - 10 days and is then removed, after which the patient resumes his normal voiding.

If at any time during the procedure there is an injury to the rectum, it is, of course, important to recognize and close it (3). If such an injury should occur before the urinary tract is opened, it has been my practice simply to get a biopsy to confirm the diagnosis and then retreat after draining the wound and closing the opening in the rectum. It is then possible to re-operate at a later date and perform a radical procedure (4). If the injury to the rectum occurs after the urinary tract is opened, one may as well go ahead with the procedure, finish it, and then close the rectal injury in two layers with a continuous chromic suture, inverting for the mucosal layer.

Then interrupted, nonabsorbable or Dexon sutures are used for the muscular layer of the rectum. Obviously the wound is contaminated and must be particularly well drained. It is probably a good idea under those circumstances to put in a suprapubic catheter as well, in order to ensure perfect urinary drainage, because if a fistula is going to develop, it usually occurs in relation to leakage of urine and abscess formation. In my opinion, it is not necessary to do a diverting colostomy (5).

Radical perineal prostatectomy is a difficult operation to learn and probably that is why it is not used more. In the hands of an expert, it is an exquisite experience and the patient has much less difficulty in the post-operative period. As far as I am concerned, it is the operation that should be one of choice in the treatment of early prostatic cancer.

PART II: RADICAL RETROPUBIC PROSTATECTOMY*

When I first accepted the assignment to speak about radical prostatectomy for cancer of the prostate, I had the illusion that I was to speak about my favorite method which is perineal prostatectomy; however, I learned later that I should cover all aspects of the field and regret that I had not done it in a more methodical fashion.

There is a great deal to be said about radical retropubic prostatectomy and I should like to include it here.

One of the most important things is to talk about the advantages and disadvantages concerning radical retropubic and total perineal prostatectomy, and this has been admirably summarized in the excellent article by Joseph D. Schmidt, "Indications and Surgical Approaches for Prostatic Cancer" (6).

On page 6, Schmidt has given us Table III, "Advantages and disadvantages of radical retropubic prostatectomy and total perineal prostatectomy". I am going to reproduce it here -

Prostatectomy	Advantages	Disadvantages
Radical retropubic	Direct assessment of regional nodes	Exposure poor for anastomosis
	Simultaneous prostato-seminal-vesiculectomy	Greater pelvic bleeding, lymphoceles
	Low urinary fistula rate	Increased cardiopulmonary complications
	Easier bladder reconstruction	Problem of frozen sections

*With the cooperation of J.B. deKernion.

Prostatectomy	Advantages	Disadvantages
Total perineal	Better patient selection	Unknown state of regional nodes
	Excellent exposure for anastomosis	Higher urinary fistula rate
	Easier control of vascular pedicle	Higher risk of rectal injury
	Decreased operating time	Need for special training in perineal surgical anatomy
	Decreased cardiopulmonary complications	

As I have already said in writing about radical perineal prostatectomy, I think it is the operation of choice, but there certainly is a place for radical retropubic prostatectomy. This is particularly true as concerns the staging that can be gained from lymph node biopsy. In my opinion, lymph node removal is not curative, and not therapeutic, but it does have a valuable prognostic function.

In our previous publication, we described 329 patients who had radical prostatectomy at UCLA, the majority of this work being undertaken by Dr. Boxer, then a resident. Unfortunately we missed the great opportunity to compare the results and the patients' reaction to the two different operations (7).

We stated "the perineal approach of Young, later modified by Belt, was chosen by the attending and resident staff in 265 cases (80.5%) and the retropubic approach with pelvic lymphadenectomy was performed in 64 cases (19.5%). The radical prostatectomy was not performed in two cases after frozen section analysis of lymph nodes proved metastases. This fact has been given as a reason for using the retropubic approach. Furthermore, because 80.5% of the cases had the perineal approach, accurate staging of the lymph nodes was not possible in those patients". It is a sorrow to me that we did not go on to compare the radical perineal in 80% versus the radical retropubic in 20%, but we did not seize that opportunity and I suppose it is lost unless some other more vigorous soul comes along to make the comparison. In my view, the radical perineal is kinder to the patient, and the radical retropubic, although it has its place, has only the advantage of the opportunity for staging with lymph node biopsy.

Having said that, I should like to go on to say something about technique. Probably the original article on this subject was written by Chute (8). The illustrations and the description of the operation are extremely clear. Although there have been many modifications since by numerous surgeons, the basic procedure is well described. If one would like to look further for a beautiful anatomical illustration of the procedure by Didusch, it can be found in the article by Memmelaar (9).

The operation is further described in some detail, without so many frills, in the excellent article by Kopecky et al (10).

When radical retropubic prostatectomy was a new procedure, I tried it in a number of cases and felt that it lacked the surgical finesse of allowing a careful and visible complete anatomic anastomosis of the bladder neck to the urethra. For this reason, I abandoned it because I thought that most patients would rather not know whether they had cancer in their lymph nodes, but would certainly rather be continent. Thus the radical perineal operation is my preference.

My colleague, Dr. Jean deKernion, also expresses support for perineal prostatectomy -

"Perineal prostatectomy, in my hands, is associated with much less blood loss and less post-operative morbidity than that accompanying retropubic prostatectomy. I, therefore, think it is still the treatment of choice in the poor risk patient with obesity or severe pulmonary disease, and in patients who are unlikely to have lymph node metastases (Stage B-1).

As we learn more about the implications of lymph node metastases, and develop new, more accurate methods of identifying tumor in lymph nodes, these indications may be further expanded. It would seem, therefore, that perineal prostatectomy is still an art worth learning and teaching."

Because of little recent personal experience with the radical retropubic operation, I asked Dr. deKernion to give some ammunition, and he did that with an operation report from June 1981, plus a special message, and I am going to include that here.

"Stage A-II cancer of the prostate. Bilateral pelvic lymphadenectomy, radical retropubic prostatectomy - 63 year old male, two months status post transurethral resection of the prostate. His metastatic workup was negative.

After adequate general anesthesia was obtained, the patient was placed in the supine position with some hyperextension at the sacrum. A catheter was placed in the bladder. An incision was made in the midline just below the umbilicus and carried through the abdominal fat and Scarpa's fascia down to the rectus fascia which was then incised in the midline. The rectus muscle bellies were dissected and retracted laterally, and the prevesical space was entered. The rectus muscles were taken down just very slightly at the symphysis pubis to gain adequate exposure. Blunt dissection was employed very carefully to dissect the perivesical fat and the peritoneum away from the pelvic sidewall on either side. The spermatic cords were identified on each side, and the iliac vessels were then gradually exposed. Dissection was begun by placing Harrington

retractors deep into the area surrounding the bladder to expose an extremely tortuous external iliac artery. Pelvic lymphadenectomy was performed from the ureter to the recurrent iliac circumflex vein on the right."

I will not continue with that surgical note but will go on with the special description which he gave me at my request. He said, "I thought it might be helpful if I emphasized a few points".

"We perform the operation through a low midline incision. The lymphadenectomy is done extraperitoneally and extends from the bifurcation of the common iliac vessels, distally to the inguinal ligament. We no longer dissect the tissue on the lateral aspect of the external iliac arteries since preservation of this lymphatic drainage seems to decrease the incidence of lymphedema. Distally, we make a big point of securing the large bundle of lymphatics that enter the pelvis near the femoral canal, just medially and posteriorly to the external iliac vein. Gene Carlton brought this to my attention six years ago, and I now assiduously clip these lymphatics and have not had a single lymphatic leak or lymphocele since that time. We then continue down into the obturator fossa. I do not dissect any deeper than the obturator nerve which is, of course, well dissected and visualized. The obturator vessels are only removed if necessary, but they are often not in the way. I am also very cautious about accessory obturator veins in a surprisingly large number of patients. We then dissect the obturator fat pad upward as far as the area of bifurcation of the iliac artery. Frozen sections are obtained as each lymph node is removed. If the patient has more than one or two microfoci of tumor, we abandon the plan for prostatectomy.

After lymphadenectomy, we begin the prostatectomy. A large catheter is inserted into the urethra. The prostatectomy is begun by incising the bladder neck fibers at the junction of the prostate in the bladder. I like to put large figure of 8 hemostatic sutures in each corner, both on the prostate and the bladder neck. These serve both as retractors and hemostatic sutures. I identify the ureteral orifices and then proceed directly across the posterior bladder neck to expose and tie the vasa. We then isolate the seminal vesicles and dissect them completely free. The vascular pedicle then is obvious on each side, adjacent to the rectum. This is ligated with large chromic sutures. (Note A).

I then lift up on the sutures to elevate the prostate gland and develop the plane behind the prostate. This develops a nice pedicle on each side of the prostate which extends between the lateral aspect of the prostate and the rectum and contains much of the prostatic blood supply. I then serially clip these pedicles deep into the pelvis down toward the end of the pelvic fascia. At this point, I narrow the bladder neck with a continuous running 2-0 chromic suture beginning in the midline posteriorly. This is

done with ureteral catheters in place to avoid ureteral obstruction. A vesical opening approximately one centimeter in diameter is left. (Note B).

I then like to remove a trapezoid of the pubis to facilitate the rest of the dissection and the incision of the urethra. We never take a full thickness of the pubis.

Dr. Charles Robson put me on to this idea, and I think it is a very good one. We do this very simply with an osteotome and a mallet and then apply bone wax to the raw area. I have never had a problem from this part of the procedure. Following the removal of this segment of bone, we are right down on the urethra. We then secure the dorsal vein with a 2-0 chromic tie; we never use clips in this area because they could occasionally erode into the anastomosis. I then gently incise the endopelvic fascia and the puboprostatic ligaments. There are large veins under them which I then secure by passing a curved clamp around and tie these vessels with chromic ties. (Note C).

At this point we have the prostate attached only by the urethra. Even if there is some bleeding from the pelvic veins, the operation is almost completed and one can quickly finish the final steps. I begin incising the urethra under direct vision, using a scalpel. I never leave a button of prostate and I never cut into the urogenital diaphragm. (Note D).

I like to divide the urethra part of the way and then insert the first 2-0 chromic anastomatic suture. Then, by rotating the prostate on the catheter, I can gradually incise the urethra and insert other sutures until finally the entire specimen is free on the catheter and I have inserted at least four or six sutures through the urethra only. We then quickly pass these through the bladder neck and the operation is completed. This is an excellent anastomosis which is done under more or less direct vision. Of course, a fresh catheter is inserted before tying the sutures.

I like to leave the catheter in for about 10 days and sometimes send the patient home with the catheter indwelling. I always use a drain, but almost never use a suprapubic tube."

Notes - W.E. Goodwin

A. This is one of the advantages of the retropubic over the perineal approach. It is sometimes difficult to identify and secure these arteries which can be sources of considerable bleeding in the perineal approach.

B. This is similar to the radical perineal approach where we narrow the neck of the bladder, usually from four quadrants, to make a vesical orifice of about 24 French.

C. In the perineal procedure, this is done, of course, from below and the same maneuver is carried out more or less blindly by passing a large curved clamp around the large veins and the puboprostatic ligaments and making a tie just as he describes from above.

D. This should be compared with Belt's perineal approach which leaves a small piece of the apex of the prostate in order to facilitate the anastomosis and prevent urinary incontinence.

I believe that Dr. de'Kernion's description is as graphic as any I could imagine, and putting it together with the excellent illustrations of Chute and the recent contributions of Wettlaufer, I believe this represents an adequate explanation of radical retropubic prostatectomy which certainly has its place in the urologist's armamentarium for dealing with prostatic cancer by radical surgery.

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CRYOSURGERY OF PROSTATIC CARCINOMA

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INTRODUCTION

Since the advent of cryosurgical equipment, first developed by Cooper and Lee in 1961 (1), much attention has been centered on the field of cryobiology and the destructive effect of cold on human tissues. In 1964 Gonder et al (2) first reported on experimental cryosurgery of the dog prostate. Two years later they introduced transurethral cryosurgery in the treatment of benign prostatic hyperplasia (3) and subsequently published their experience utilising the transurethral cryoprobe in 50 patients with prostatic carcinoma to produce local prostatic destruction (4).

The original purpose of this operation was to destroy obstructing prostatic tissue by freezing to provide an adequate urinary passage. Experience, after initial enthusiasm, has led to the conclusion that the procedure has a very limited field of application and is certainly not the panacea for prostatic obstruction as was originally hoped.

We decided to abandon this technique after an experience of over two years with more than 20 patients. We found that transurethral cryosurgery, as a blind procedure, was not without danger and had a large range of possible complications apart from the usual protracted course (Tables 1 and 2). In our opinion this technique has no advantage over transurethral resection or open prostatectomy, even in high risk patients, as the improvement of preoperative diagnostic procedures, anesthetic techniques and pre- and post-operative care makes it possible to treat older and poor risk patients by transurethral resection or, if necessary, by open prostatectomy.

Table 1. Transurethral Cryosurgery of the Prostate. Immediate Postoperative Complications

- Haemorrhage
- Oligo-anuria (especially in lesion of the trigone and those involving the ureteric orifices)
- Acute pyelonephritis
- Epidydimitis and orchitis
- Periurethral abscesses
- Oedema of the penis
- Cardiopulmonary or cerebrovascular accidents

Table 2. Transurethral Cryosurgery of the Prostate. Late postoperative Complications

- Urinary incontinence
- Secondary haemorrhage
- Retropubic infection
- Bladder calculi
- Protracted (severe) urinary tract infection
- Urethral (meatal) stenosis

In the last five years we have performed 679 prostatic operations, a substantial proportion of them in patients with cardiovascular or pulmonary disease. In this period hardly any patient was excluded from transurethral resection. Despite this our perioperative mortality is less than one percent (Table 3).

We are therefore surprised by recent pulbications (5,6,7), suggesting an indication for cryosurgery in 15 to 25% of patients suffering from prostatic obstruction. A comparison of the worst results of cryosurgery with those of transurethral resection (8) suggests that this figure should be less than 5% as recently

Table 3. Prostatic Surgery

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1975 - 1980

Transurethral Resection	482	(71%)
Open Prostatectomy	191	(29%)
Perioperative Mortality	3	(o.4%)

postulated by Groenteman and van Haga (9) in our country.

Refinements of transurethral cryosurgery by application of "cryoresection" (transurethral resection after freezing of the prostate) or "cryocautery" (the combination of freezing and subsequent heating) do not seem to us to offer any improvement of the initial technique.

CRYOSURGERY IN PROSTATIC CANCER

Control of the Primary Tumor

When Soanes and Gonder (3) introduced transurethral cryosurgery it was a blind procedure and exact placement of the cryoprobe was difficult unless it was used in combination with a suprapubic trocar cystoscopy. Also, since at least 65% of the prostatic cancers develop in the dorsolateral part of the gland, the lesion is difficult to reach by transurethral freezing. Furthermore the bulk of prostatic cancer in large lesions cannot be destroyed transurethrally. Therefore Flocks in 1972 (10) introduced cryosurgery via the perineal route. With this approach, direct application of the cryoprobe to the prostatic lesion is possible. The purpose of this method is local destruction of the tumor while maintaining the integrity of the adjacent bladder and urethra.

Crysurgery produces tissue death by intracellular dehydration and toxic electrolyte concentration, crystallization with secondary membrane rupture, denaturation of proteins, thermal shock and vascular stasis. This degenerative phase, characterized by tissue edema and necrosis, lasts one to 10 weeks and usually progresses to a reparative phase between two and three months after treatment (11). The depth of tissue destruction by freezing is difficult to assess.

This depends on many variables such as tissue sensitivity to freezing, velocity of temperature reduction and subsequent thawing, and the duration of the freezing period. Nowadays liquid nitrogen is used as the cooling agent, reducing the temperature in the heat exchanger tip of the probe to -180 to -190°C.

Perineal exposure provides excellent visualization of the prostatic cancer and presents the least risk of cold injury to the rectum. It also allows visualization of the seminal vesicles which can be frozen to control extracapsular extension of the disease. The freezing process can be repeated to ensure adequate tissue destruction. However one is never sure if all prostatic tissue in the center of the gland is destroyed. Therefore Klosterhalfen et al (12) combined the perineal technique with transurethral freezing to control centrally located tumors. Perineal cryosurgery and the combined method are well tolerated by the patients. It seems clear that prostatic cryosurgery can be effective in destroying the local lesion. Rectal examination after three months shows striking changes in the local prostatic lesion. Loening et al (13) found an empty prostatic fossa with no clinical evidence of residual tumor in 161 of 215 patients (74.8%). In 32 patients (14.9%) no three months follow-up or rectal examination was recorded and the remaining 22 patients (10.2%) had incomplete destruction of the local prostatic lesion. Klosterhalfen et al (12) and O'Donoghue et al (14) had identical observations concerning local control of the prostatic lesion three and six months after cryosurgery.

Loening et al (13) also noted that the cryosurgery patients had a probability of survival equal to that seen in the total prostatectomy patients of each stage. The actuarial survival curves related to clinical stage from their study gave a five year survival after cryosurgery of about 75% for stage B lesions. This is almost identical with the five year survival rates reported by Boxer et al (15) following radical prostatectomy for surgical stages A and B prostatic cancer. For patients with stage C disease the results of radical surgery as reported by Boxer et al (15) was 67% whilst Bagshaw et al (16) found that external radiation therapy resulted in 73% five year survival with stage B disease and 46% survival in patients with stage C prostatic cancer. Again the results of cryosurgery as reported by Loening et al (13) are comparable with these figures. Therefore to those authors and to others, cryosurgery appears to be another modality of treatment for local destruction of prostatic carcinoma. Further they found that ablation of the primary site in locally advanced disease avoided late urinary incontinence, decreased the incidence of repeated transurethral resections and avoided late complications such as ureteral obstruction or bladder neck infiltration by the neoplasm. In brief they favour perineal cryosurgery for local tumour control.

Possible Immunological effect of Cryosurgery

The second reason to advocate cryosurgical therapy of the prostate is the possibility that cryosurgical destruction may elicit an immune response in the patient and, by immunologic means, may favourably influence distant metastases. Soanes et al (17) first postulated a cryo-immune response because two patients with metastatic disease had regression following this type of operation and Gursel et al (18) noted relief of pain from metastases following cryotherapy in eight of 11 patients. In only one patient was an objective response of bone metastases evident. Since then occasional remissions have been reported. Recently Gittes, in an editorial comment on a study of this subject by Milleman et al, in which the authors could not identify indirect evidence of an (humoral) immune response to cryosurgery, stated that cryosurgery in patients with prostatic carcinoma has not been shown to yield any proved immunological benefit.. However, the findings of Ablin and Fontana (20) contrast with the results of Milleman et al (19) as their data support the hypothesis of a systemic (humoral) immune response.

With respect to host cell-mediated immunologic activity there is now ample evidence that cellular immunity is depressed in many prostatic cancer patients. The aim of the clinical studies conducted by Ablin and the Cryoimmunotherapeutic Study Group was to prove an augmentation or inducement of host resistance as a result of in situ cryosurgical destruction. This would imply that cryosurgery may be effective as a means of immunotherapy. These studies are very difficult to conduct in man, since the antigenic properties of an individual prostatic carcinoma are not known and the humoral and cellular immune responses after cryosurgical treatment are difficult to assess and may be non-specific.

Therefore studies with an experimental animal model with known antigenic capacities are mandatory in the first instance. A suitable animal model seems now to be available in the Dunning R 3327 prostatic tumor system, induced in the Copenhagen rat, which is an appropriate model to use for studies of the immunobiology of prostatic carcinoma. Lubaroff and co-workers (21) developed a research program on the immunological responses after cryosurgery of this experimental animal tumor. The results to date demonstrate the production of humoral and cell-mediated immune responses. On the other hand other investigators (22,23) demonstrated the development of contradictory effects following cryostimulation including tumor enhancement. However all these data are still preliminary.

Many immunological problems still exist in the animal model and even greater questions remain in man. As long as these questions remain unanswered, we believe that certain favourable immunological effects on metastatic lesions are not sufficient indication for the general use of cryosurgery for prostatic carcinoma in man.

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CRYOSURGICAL AND CRYORESECTION TREATMENT OF PROSTATIC CANCER

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SUMMARY

This paper reviews 160 cases of advanced prostatic cancer (category T3 and T4) treated by cryosurgery and cryoresection. In 52 patients cryosurgery only was undertaken, in 20 cases at first cryosurgery and then transurethral resection (TUR) was performed, and in 88 patients TUR was followed by cryosurgery. The last method yielded the best results.

PATIENTS AND METHODS

In the Urological Clinic of Ljubljana cryosurgery and cryoresection has been used since 1975 for the treatment of advanced prostatic cancer (category T3 and T4). From 1975 to 1980, 160 patients have been treated by this method (Table 1).

All the patients were older than 60 years, 53 (33%) aged 61 - 70, 90 (56%) aged 71 - 80, and 17 (11%) over 81 years of age. Before treatment all the patients were classified according to the TNM system. Of our patients, 66 were category T3 and 84 were category T4. The presenting symptoms before the cryosurgical treatment are listed in Table 2.

Before treatment we searched for metastases by biochemical examination, skeletal survey, chest X-ray and radioisotopic examination. The results are shown in Table III.

Histological or cytological evidence of tumour was available before cyrosurgical treatment in 142 patients (88%); in 18 patients

(12%) we were content to proceed, for various reasons, on the findings of rectal palpation. The results of histology and cytology in 160 patients are shown in Table 4.

Table 1. Number of patients treated by cryosurgery (1976 - 1980)

1975	1
1976	30
1977	23
1978	31
1979	35
1980	40
Total	160

Table 2. Patients' symptoms prior to cryosurgical treatment

Symptom	No. of patients	
Difficulty in micturition	142	(88%)
Retention of urine	66	(41%)
Haematuria	19	(12%)
Bone pain	42	(26%)
Other symptoms	51	(31%)

Table 3. Incidence of metastases in patients with prostatic cancer

Site of metastases	No. of patients	
Skeleton	62	(38%)
Lungs	13	(8.6%)
Other organs (liver, palpable lymphatic glands)	6	(3.6%)
No metastasis demonstrable	79	(45.4%)

Table 4. Histological and cytological findings in 160 patients with prostatic cancer

Well differentiated	89	(55%)
Moderately differentiated	48	(31%)
Poorly differentiated	23	(14%)

In these five years we have used three methods of cryoprostatectomy. In the beginning cryosurgery only was practised, but soon TUR was also carried out before or after cryosurgery. The duration of freezing of the prostatic cancer was between three and eight minutes, depending on the size of the prostate. After freezing the phase of warming followed, the duration of which was equal to that of freezing or about one minute longer. All these methods were combined with bilateral subcapsular orchidectomy and a high dose hormonal therapy - 1000 mg of Honvan daily for 10 days.

Cryosurgery alone was undertaken in 52 patients (32%). The greatest problem of this method however was the elimination of necrotic tissue, which led to numerous complications including haematuria and retention of urine. Post-operative catheterization was necessary for up to six weeks.

In 1976 and 1977 TUR of frozen tissue was performed after cryosurgical treatment in 20 patients (12.4%). Because of changes in the frozen tissue transurethral resection presented a greater risk (especially towards the periphery) and control of haemostasis was difficult.

RESULTS

The best results were obtained when TUR was performed first and cryosurgery afterwards. In this way only the peripheral tissue is frozen; 88 patients were treated by this method.

The advantages of this last method mentioned include -

1. lesser quantity of necrotic tissue than after cryosurgery alone,
2. easy elimination of necrotic tissue through the urethra (after the TUR),
3. obstructions after such treatment are less frequent,
4. the number of infections is minimal, and
5. the duration of use of the catheter and hospitalization is shorter.

Follow up examinations were made every three months. Among the postoperative complications (Table 5) incontinence was the most frequent (17 patients - 10.5%) because the malignant tissue spread

to the external sphincter. After cryosurgery alone, haematuria occurred in nine patients (5.5%) and recurrent retention of urine in eight (4.9%), associated with the elimination of necrotic tissue. In 115 patients (72.2%) no complications were observed.

In the postoperative period after cryosurgical treatment two patients died (1.2%). In the first case perforation of the bladder occurred and in the second case mortality was due to urosepsis.

At follow up examination 98 patients (61%) reported an improvement in general health. In 110 patients (69%) obstructive symptoms were relieved and 28 patients (13%) mentioned a reduction of skeletal pain. Twelve patients (7.4%) had no changes after cryosurgery and 25 patients (15.5%) were totally free of symptoms after treatment.

In 95 patients (59%) a large reduction of tumor mass was observed by repeated rectal examinations and only in 20 patients (12.4%) was enlargement of the prostate found.

The amount of residual urine after cryosurgery is also of interest, a great improvement being observed (Table 6). In four patients skeletal metastases regressed and pulmonary metastases resolved in one. The survival of our patients as recorded by the central cancer register in the Institute of Oncology of Ljubljana is shown in Table 7.

Table 5. Complications after cryosurgery

Symptom	No. of patients	
Incontinence	17	(10.5%)
Haematuria	9	(5.4%)
Recurrent retention of urine	8	(4.9%)
Stricture of urethra	5	(3.1%)
Epididymitis	3	(2%)
Perforation of bladder	1	(0.6%)
Urosepsis	2	(1.2%)
Without complications	115	(72.2%)

Table 6. Residual urine before and after treatment

Residual	No. of patients	
	Before	After
None	5	70
Up to 100 ml	14	84
From 100 - 200 ml	34	5
Above 200 ml	107	1

Table 7. Survival following treatment

Year of treatment	Patients surviving
1976	12 (40%)
1977	16 (71%)
1978	24 (78%)
1979	30 (84%)
1980	36 (90%)

Editorial Note (P.H.S.)

Treatment in all patients included cryosurgery, bilateral subcapsular orchiectomy and hormone therapy of ten days' duration. In addition, 108 of the 160 patients also had a transurethral resection. The procedure of cryosurgery is not without complications and the results of the combined therapy demonstrate no obvious benefit in terms of control of local symptoms, remission of distant metastases or survival which is directly attributable to the cryosurgery.

RADIOTHERAPY OF PROSTATIC CANCER

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In the 1950's, new sources of deeply penetrating radiations, such as Cobalt-60 units, linear accelerators, and betatrons, became available and have become the standard for modern radiation therapy.

Clinical research has demonstrated that doses of 7,000 to 7,600 rads in 7 to 7-1/2 weeks can be delivered to the prostate and immediately adjacent tissues, and doses of 5,000 rads in 5 weeks can be absorbed in regional lymph nodes without undue morbidity. As the treatment techniques evolved, attention to tumor localization, individualized treatment planning, multi-portal treatment, daily fractionation, optimal radiation dose, and other therapeutic details have become relatively standardized.

In a recent review of over 1200 cases of prostatic cancer, five-year actuarial survivals ranged from 60% to 75%, while the 10-year actuarial survival was 40% (1). Updated reports of these series, along with new reports, further extend survival rates to nearly 80% at five years for patients with disease limited to the prostate (DLP), and to approximately 60% at five years for patients with extra-capsular extension (ECE) (2,3,4).

In spite of this evidence for the efficacy of the radiotherapy of prostatic cancer, one must interpret these statistics with caution, especially when comparing results between different institutions or between treatment by different modalities. For example, we will discuss in another presentation that among patients with no initial demonstrable hematogenous spread, the most important prognostic determinant for later metastases is the presence or absence of lymph node metastases at presentation and that lymph node metastases may be predicted with considerable accuracy by careful

clinical staging and histologic grading of the primary neoplasm (5). Therefore, unless these prognostic indicators have been carefully determined and analyzed, rigorous inter-comparison of survival statistics is impossible. In recognition of these limitations, the Stanford experience as reported here, which now extends over 20 years, may be taken as a reasonable reflection of what might be achieved with external beam radiation therapy in carcinoma of the prostate.

PRE-THERAPEUTIC PATIENT EVALUATION

1. Biopsy and Histopathologic Grading: Patients are usually referred for radiotherapy after the biopsy has been obtained. This is usually accomplished by trans-rectal or trans-perineal biopsy, although transurethral resection is nearly equally as common.

In our material, the close correlation between histologic grade especially by the Gleason grading system and the degree of lymph node involvement has been of inestimable value in predicting the probability of lymphadenopathy (6).

2. History and Physical Examination: A simple diagram of the prostate as perceived by digital examination, complete with metric dimensions, should be mandatory. The physiologic sexual status of the patient is often overlooked in the history. There is no way to evaluate the influence of treatment on sexual potency without this key information.

3. Acid Phosphatase Determination: Conventional acid phosphatase determinations, using citrate or tartrate as a buffer, should be carried out until the newer techniques such as radioimmune assay have been more thoroughly evaluated.

4. Chest X-Ray: Although early pulmonary metastases are rare in prostatic cancer, they should be ruled out before proceeding with definitive therapy.

5. Intravenous Pyelogram: Ureteral obstruction occurs in about 15% of patients with relatively early cancer and can be identified by an IVP. Hydronephrosis, while a grave prognostic sign, is not necessarily lethal.

6. Bone Scans: Currently Technetium-99m labeled methylene disphosphonate is widely used for detection of osseous metastases. Positive or equivocal findings should be confirmed by roentgenograms.

7. Special Examinations: Techniques for visualization of the prostate include computerized tomography and ultrasound (7,8). These methods are useful in establishing whether extracapsular extension has occurred and should be used as an adjunct to the more

conventional localization procedures for the preparation of the patient for radiation therapy.

8. Lymphangiogram (LAG): The lymphangiogram, although controversial in prostate cancer, can be extremely useful. One careful efficacy study correctly detected 30 of 54 patients with nodal metastases for a sensitivity of 56%; and an independent study correctly detected 16 of 28 with nodal metastases for a sensitivity of 57% (9,10). Thus, in many cases, the LAG combined with fine needle aspiration biopsy offers an opportunity to prove the presence of lymph node metastases without formal surgical intervention (11).

9. Surgical Staging of Lymph Nodes: Surgical mapping of lymph node involvment in prostatic cancer has had a profound impact on understanding its natural history, especially as a predictor for disseminated disease (12,13).

RESULTS OF RADIOTHERAPY

This treatment for prostatic cancer was started at Stanford in 1956. Since then 1293 patients have been referred. 382 were eliminated from this series because they were referred for consultation only or because metastases were discovered. 226 patients have been referred since 1 January 1977 and are not included because of short follow-up. 24 patients were rejected from the analysis for miscellaneous reasons, leaving 661 for this analysis.

This series has been updated recently. All patients at risk for 15 years or more and for 10 years or more were reviewed. The clinical stage at the time of treatment was reevaluated and a TNM designation was assigned. The original designations of disease limited to the prostate (DLP) - 351 patients, and extracapsular extension (ECE) - 310 patients, were not modified. All patients had a histologic diagnosis (Table 1).

The current status of the patients was updated as of 1 March 1981. The cutoff date for patients entering this study was 31 December 1976, and therefore the minimum follow-up through 1 March 1981 is four years and three months.

Clinical Stage Versus Survival

Figure 1 presents the overall survival of the 661 patients divided between patients with disease limited to the prostate and those with extracapsular extension. The same data at 5, 10, and 15 year intervals are presented in Table 1.

Figure 2 displays the survival by T stage. The T1, T2, and T3

Table 1. Summary of Stanford Results in External Beam Radiotherapy of Carcinoma of the Prostate.

Date of Report	Total No. of Cases	Actuarial Survival at Intervals of 5 Years DLP	5 Years ECE	10 Years DLP	10 Years ECE	15 Years DLP	15 Years ECE	Follow-up
1965[14]	73	All cases combined 54%						1-8 years
1973[15]	160 DLP	72%		48%				2-15 years
	150 ECE		48%		30%			
1981 (March)	351 DLP	78% ±4.8*		57% ±6.4*		39% ±8.4*		4-20 years
	310 ECE		59% ±6*		39.5% ±6.8*		30% ±7.4	

DLP = Disease Limited to the Prostate, Nominal Stage B, or T1

ECE = Extracapsular Extension, Nominal Stage C, or T2 and T3

* 2 Standard errors

categories are essentially the same as those advocated by the UICC. The lesser subdivisions within the T categories are unique to the Stanford TNM system and have been described elsewhere (5). Without going into the statistical differences between these curves, one can see that there is clearly a difference between the T0 patients and the rest. The T1 and T2 patients are not much different from each other; however, they differ significantly from the T3 and the T4 patients.

The differences in survival between T0 focal and T0 diffuse were not significant. The large group of T1 patients were segregated according to their respective subgroups, and substantial differences in survival patterns between T1a, T1b, and T1c were observed (Fig. 3). We then added the T1a and T1b patients together, because these patients appear to be, by stage, the closest to those who are regarded by Jewett, Culp, Walsh, and others as candidates for radical

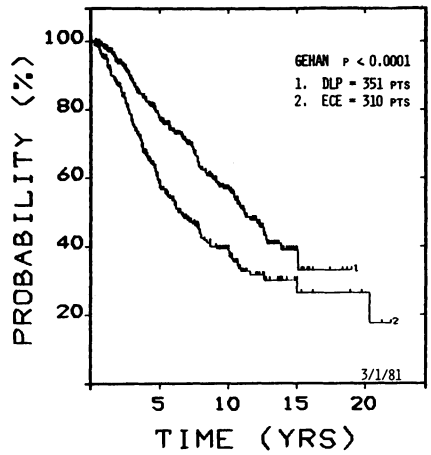

Fig. 1. Prostatic cancer survival - death due to all causes. Time 0 is the first day of radiation therapy. The survival curves are after the method of Kaplan and Meier (16). In this instance we are plotting the probability of survival as a function of time from the first day of treatment. When a patient dies, the curve drops one unit, and each time a patient is withdrawn from the population - such as the end of the period of observation for that patient - there is a vertical tick. You can see with such large numbers as these that the curves are quite smooth and obviously quite different. Their difference can be quantified by the Gehan test, which shows that the probability of these two curves representing the same population is less than one chance in 10,000.

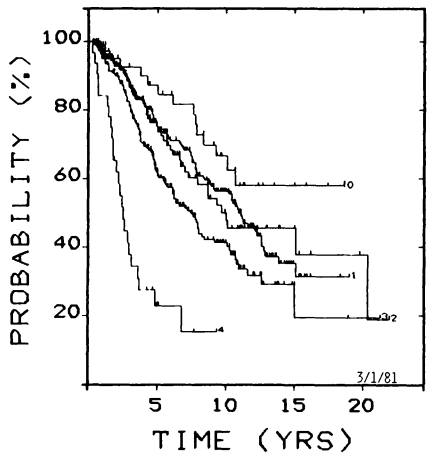

Fig. 2. Prostatic cancer - survival by T stage. Time 0 is the first day of radiation therapy. Curve 0 is limited to T0 patients, N=43. Curve 1 is limited to T1 patients, N=225. Curve 2 is limited to T2 patients, n=119. Curve 3 is limited to T3 patients, N=242. Curve 4 is limited to T4 patients, N=32.

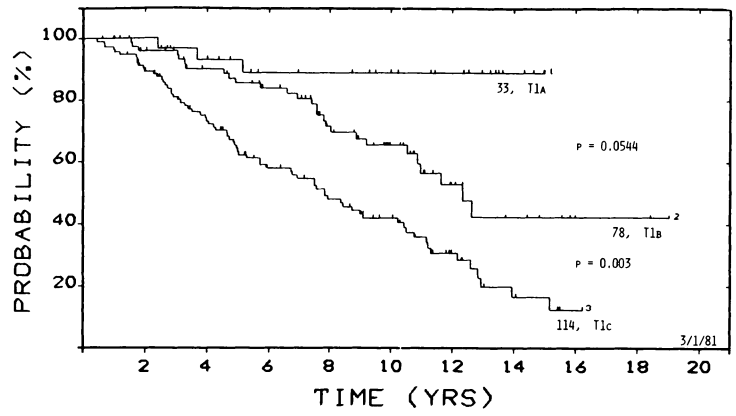

Fig. 3. Prostatic cancer - survival by T stage. Time 0 is the first day of radiation therapy.

T1 Palpable tumor limited by the prostatic capsule without distortion of the superior or lateral anatomic boundaries.

T1a Solitary nodule equal to or less than 1 cm in diameter with normal compressible prostatic tissue on 3 sides (the Jewett nodule, amenable to radical prostatectomy).

T1b Palpable tumor greater than 1 cm occupying less than 50% of a lobe.

T1c Palpable tumor occupying greater than 50% of one lobe, multiple nodules limited to one lobe, or involvement of both lobes.

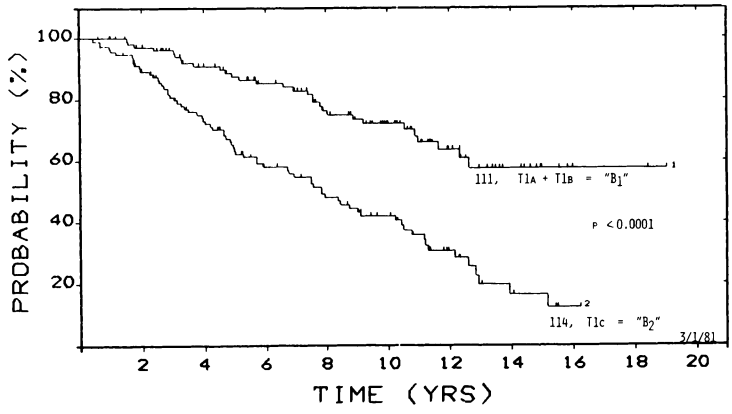

Fig. 4. Prostatic cancer - survival by "B" stage. Time 0 is the first day of radiation therapy. "B" stage refers to the American Urological Staging system. B1, or surgically resectable stage, corresponds to the Stanford T1a or T1b, whereas B2, considered by most authors to be too advanced for surgical resection, corresponds to the Stanford T1c group.

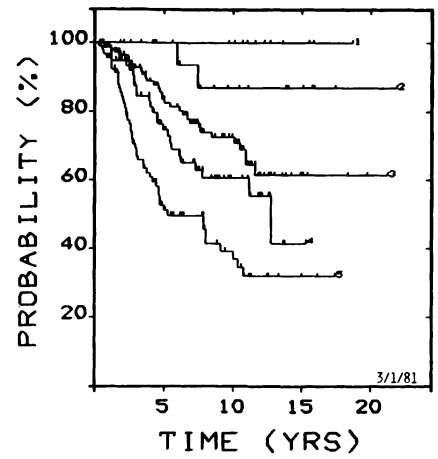

Fig. 5. Survival as a function of Gleason histopathologic pattern scores. Survival is calculated as a function of death due to prostatic cancer only, i.e., death due to intercurrent disease is withdrawn. The same patterns are seen when death due to all causes is calculated but the relationship is crisper in this presentation. Curve 1, Gleason 2,3,4; Curve 2, Gleason 5; Curve 3, Gleason 6; Curve 4, Gleason 7; Curve 5, Gleason 8,9,10.

prostatectomy (17,18,19). In other words, the Stanford T1a and T1b appear equivalent to the American Urological Stage B1. A highly significant difference in survival between these B1 patients and B2 patients was observed (Fig. 4). It is important to recall that the B2 patients have been lumped togther with the B1 patients in the DLP, (Disease limited to the Prostate) category, and therefore this group as a whole is obviously more advanced than the patients subjected to radical prostatectomy in the reports of Jewett, Culp, Walsh, and others. Here we have tried to segregate a group of patients treated by irradiation who are comparable by stage to those treated by radical prostatectomy. We have been unable to stratify them by histologic grade.

Histopathologic Grade Versus Survival

Both Dr. Richard Kempson of Stanford Department of Pathology and James Gleason have graded approximately one-half of the neoplams in this series. The Gleason grading system is based upon microscopic pattern recognition of the glandular elements at a relatively low power. Gleason assigns an integer of 1 through to 5 to the major and also the minor patterns recognized upon scanning the specimen. These integers are added, giving a 9-step grading system, 2 through 9, which is called the Gleason Pattern Score.

Figure 5 demonstrates the correlation of disease-specific survival with the Gleason Pattern Score in 324 of these patients. It is clearly evident that death due to prostatic cancer for Gleason Pattern Scores of 2,3, and 4 is negligible, slightly greater for a Gleason Pattern Score of 5, and progressively greater as the Pattern Score approaches the maximum of 10. We have previously reported a close correlation between the incidence of lymph node involvement and an increase in the Gleason Pattern Score. (5) This relationship will be covered in more detail in the session on lymph node metastases.

Palliative Irradiation

Disseminated osseous metastases present by far the most common indication for palliative irradiation. Curiously, although a patient may have many metastases and be responding generally well to hormonal management, only one or two metastatic sites become painful. Often these are near major joints, e.g., the acetabulum or within the spine. They usually respond promptly with pain relief. The dose should be relatively high and well fractionated, e.g., 4000 rads in 4 weeks, because the overall survival could be quite long and adverse sequelae should be avoided. Occasionally the dose is increased to 5000-5500 rads in 5-5 1/2 weeks when one is trying to prevent fracture of a weight bearing bone or prevent or relieve spinal cord compression.

SUMMARY

1. A substantial world experience in external beam irradiation of cancer of the prostate has been accumulated over the past 25 years.

2. Survival rates approaching 80% at 5 years, 60% at 10 years, and 40% at 15 years are being achieved for disease confined within the capsule. Comparable figures for extracapsular disease approach 60% at 5 years, 40% at 10 years, and 30% at 15 years, respectively.

3. The degree of aggressiveness of prostatic carcinoma is often telegraphed by the interrelationships between clinical stage, histopathologic grade, and lymphadenopathy. Unless these parameters are clearly described in any given series of patients, it is quite useless to try to compare treatment results.

4. Painful bone metastases are often alleviated by radiation therapy.

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Editorial Note - Iridium 192 Wire Implantation in Prostatic Carcinoma (C.C. Abbou).

Since irradiation has been considered as the treatment of choice for localised prostatic carcinoma, many attempts have been made to replace tele-irradiation with interstitial irradiation. The latter delivers a specific high radiation dose to the involved area only.

Radium needles, colloidal radio-gold, radio-gold grains and iridium 192 needles have been tried with variable success and complications. Encapsulated iodine 125 has been used the most often.

Iridium 192 wire has a half life of seventy four days, a low energy gamma irradiation of 0.3 mev and a half attenuation constant (HAC) of only 0.2 mm of lead. Because of these physical properties and its low cost iridium 192 is used in our unit as the radio active element of choice for interstitial irradiation. The active zone is 1 cm broad (5 mm each side of the wire). The technique of insertion is completely harmless to the surgeon's hand.

Two techniques have been described:

In Miller's Technique (1) a preoperative dose of 20 Gy in 10 fractions is given before operation after which a Pfannenstiel incision is made and the retro pubic space opened. The lateral surfaces of the prostate are exposed. Steel needles are inserted into the prostate by perineal puncture and guided by rectal and supra pubic palpation of the prostate. The needles are inserted at one centimeter intervals. When in place they are replaced with blind nylon tubes.

Postoperatively active iridium wires are loaded after dosimetry. The entire prostate is irradiated with a dose of 45 to 50 Gy for four to five days.

In the technique of Court and Chassagne (2) the patient is placed in a modified lithotomy position with the thighs flexed to approximately 30° - 45° on the trunk and abducted approximately to 30° - 45 from the central axis. A double abdominal and perineal operating field is prepared. A subumbilical transperitoneal approach permits one to proceed to a bilateral ilio-obturator node dissection for a frozen histologic section. Patients with metastatic nodes are excluded from the protocol.

The radiotherapist inserts the plastic tubes by means of stainless steel needles 15 cm long from the perineum towards the prostate. The needle is pushed by the left hand and guided by the right hand which also serves to palpate the prostatic nodule between the thumb and index finger. A loop is formed by means of an intermediate nylon obturator. The target volume includes the whole prostate. Two or three loops with a separation factor of 10 - 20 mm are implanted. These wires can be placed either in a frontal or a sagittal plane. During the operation non active lead wires are placed in the plastic tubes for radiologic control and to carry out dosimetric calculations.

Two or three days later, after obtaining precise dosimetry the lead wires are replaced with active iridium wires of 0.3 mm diameter. The technique allows a dose of 60 to 70 Gy to be given to a small volume at a low dose rate. Continuous irradiation is given for about six days.

This technique is suitable only for category T_1 and T_2 carcinoma of the prostate of the international UICC classification which are also N0 and M0. N0, M0 indicates that the patient must have had previously a normal bone scan and normal lymphangiogram. The absence of node metastases is confirmed by the ilio-obturator lymphadenectomy with frozen sections.

Miller used his protocol on 16 patients with major complications in three (one pulmonary embolism, one proctitis, one anal ulceration). His series was too small and the follow up time too short to give a meaningful statement of survival experience. His minimum follow up was less than two years.

Court and Chassagne used their protocol on 13 patients. There was one post-operative death due to pulmonary embolism and one surgical infection with renal failure. The follow up was six months to six years with satisfactory control of the tumour.

As yet the place of this technique is not yet well defined

and the results of these small series are not significantly better than those obtained by external irradiation.

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Editorial Note (M.P.M.)

Interstitial irradiation of cancer localized to the prostate has gained wide acceptance in the U.S.A. following the experience of various workers who employed I^{125} needles by open retropubic or by perineal percutaneous implantation. Whitmore's experience is particularly encouraging, but his results may be biased by case selection. They are however very stimulating, especially as the rate of secondary sexual impotence is negligible, if compared to distant irradiation and, in particular, to radical prostatectomy. Since I^{125} needles are not readily available in Europe, iridium may represent an alternative choice, as pointed out in Dr Abbou's note. I wish to add that, in general, the addition of distant irradiation to interstitial implantation of radioactive sources is not advisable. The results are not improved, but the rate of untoward effects can be greatly increased, according to Shipley (personal communication).

EXTERNAL IRRADIATION OF POORLY DIFFERENTIATED PROSTATIC CARCINOMA

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INTRODUCTION

Esposti (1) reported on 469 cases of prostatic carcinoma of all grades who were given hormone treatment at the Karolinska Sjukhuset in Stockholm. The survival rate up to five years in this series is given in Fig. 1. However in the patients with grade 3 carcinoma and without evidence of dissemination the five-year survival was only 14%.

Because of these depressing results in grade 3 tumors the therapy schedule was changed at the beginning of 1965 from hormone therapy to external irradiation.

MATERIAL AND METHOD

From the beginning of 1965 over 350 patients with poorly differentiated carcinoma of the prostate have received local irradiation of the primary tumor in our hospital. The majority had progressive disease after previous hormone therapy. Many were also given estrogens after the radiotherapy.

The evaluation comprises 107 patients treated between 1965 and 1973, initially free of clinical metastases and who were observed for at least five years or to death. The age varied between 47 and 81 years, average 66 years.

In all cases the diagnosis was verified by transrectal fine needle aspiration biopsy. Some patients had already had a histopathological diagnosis of poorly differentiated carcinoma but were

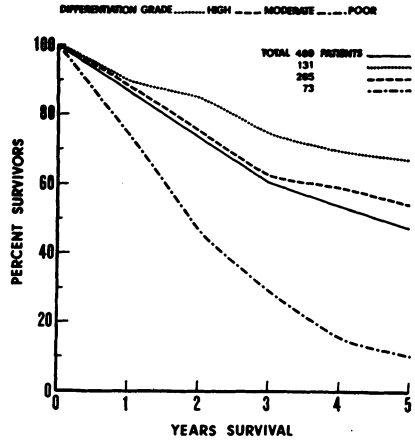

Fig 1. Histological grade and survival in 469 patients with prostatic cancer treated with hormonal agents (1)

re-diagnosed with cytology. Skeletal x-ray and/or bone scans and determination of the acid and alkaline phosphatase activity were carried out. The tumors were categorized according to the TNM system. The distribution by T category was T2-10 patients, T3-62 and T4-35 patients. Initially the treatment was given with a cobalt-60 unit and thereafter by a 6 MV linear accelerator. The irradiation was given with two oblique beams anteriorly and one open beam posteriorly. All treatment was individually planned. The tumor dose including the volume of the true pelvis volume was a mean calculated tumor dose of 5400 rad (54 Gy) given over six weeks.

RESULTS

The five-year survival rate was 33% after correction for non-cancer deaths. This figure is significantly higher than that for the patients treated only with estrogens (Table 1). There was a slight decrease in survival rate associated with a higher T category (Table 2).

The five-year survival was approximately the same in those patients who were given estrogens after irradiation as in the group receiving no hormones (Table 3).

In the majority of patients there was a slow regression of the palpable tumor mass over the first three to six month period following irradiation. In 67% of cases there was no longer any tumor palpable at the five-year review or at death (Table 4).

In 33% of the patients no cancer cells were detectable on

Table 1. Survival in 107 Patients with no Detectable Dissemination Before Irradiation After Exclusion of Non-cancer Deaths

Survival (y)	No.	%
1	91/106	86
2	77/103	75
3	52/100	52
4	35/98	36
5	32/96	33

Table 2. Five Year Survival Related to T Category

	T2	T3	T4
Number of patients	10	62	35
Dead intercurrent disease	3	5	3
5-year survival	3	20	9
Corrected survival	43%	35%	28%

Table 3. Influence of Subsequent Estrogen Therapy on Five Year Survival After Irradiation

	No Hormone	Hormone
Number of patients	42	65
Dead intercurrent disease	4	7
5-year survival	13	19
Corrected Survival	34%	33%

Table 4. Local Tumor Response

	No.	%
Complete response	72	67
Partial response	18	17
Regression - progression	9	8
Unchanged	2	2
Progression	2	2
Not evaluated	4	4
Total	107	100

Table 5. Cytologic Findings After Radiotherapy

	No.	%
Persisting cancer cells	44	41
No cancer cells found	35	33
Not evaluated	28	26
Total	107	100

repeated aspiration biopsies up to five years or more. In 41% the smears contained malignant cells (Table 5). A number of these patients had no palpable tumor. 26% of the patients were not evaluated because of early death within six months of irradiation, the earliest time at which cytological evaluation can be carried out.

Side Effects

Proctitis, usually slight to moderate, occurred in many patients, starting in mid-treatment. Bladder irritability was less frequent and, as a rule, mild. Two patients had long-term intestinal haemorrhage and persistent obstruction, necessitating surgery.

DISCUSSION

In the hormone treated patient with grade 3 carcinoma and without evidence of dissemination Esposti reported a 14% five-year survival rate. The 33% five-year survival rate in a comparable group in the present study is significantly higher.

The finding of tumor regression, as judged by palpation, in 67% of the patients was in good agreement with the experiences of Cantril et al (2) and Mantyla (3) who reported local control of poorly differentiated prostatic carcinoma in 79 and 64% respectively following radiotherapy. On the other hand, aspiration biopsy, when performed with an adequate technique, is no doubt a more sensitive indicator of tumor existence than the digital examination.

The incidence of serious complications following radiotherapy with this modern high voltage technique is low. A few years ago we introduced an extended field technique, including the para-aortic lymph nodes in the irradiated volume. The pelvic and para-aortic nodes receive 5000 rad in seven weeks and the local tumour 7000 rad.

SUMMARY

At least in cases of poorly differentiated carcinoma of the prostate, with no identified dissemination, it appears that radiotherapy may offer a chance of tumor control superior to hormone treatment and with an acceptable incidence of side effects.

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Editorial Note (M.P.M.)

According to the Stockholm workers, both estramustine phosphate and irradiation are valid methods for treating high grade prostatic cancer. In the paper by Andersson et al (this volume) of 90 patients with grade 3 cancer treated with estracyt, 42 had bony metastases and 15 had soft tissue lesions. This leaves 33 patients in whom the disease was apparently limited to the primary tumour.

The results are not given in detail and no survival figures are presented, but it is stated that the local tumour regressed objectively in 11 of 14 patients not previously treated with oestrogens, which correpsonds to a 78.6% response rate. In this article 62 patients had extensive local spread (62 T3 and 35 T4) with an expected rate of lymph node metastases of 60% (see Bagshaw's article on lymph node metastases, in this book), 80% of which will be followed by bone metastases. One wonders, therefore, if an effective systemic treatment such as estramustine, given alone or in combination with irradiation, might not prove to be superior to radiotherapy, at least in stage C patients.

IODINE-125 IMPLANTATION FOR PROSTATIC CARCINOMA

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The technique and rationale for iodine 125 implantation for prostatic adenocarcinoma has been fully described by its originators (1). This article will be devoted to recent modifications and continuing controversies.

Clinical Stage

Whitmore and his colleagues had originally suggested that clinical stage B and the majority of clinical stage C patients were potential candidates for this procedure. Stage C patients are no longer considered amenable to implantation. Ill defined borders increase the probability that some tumor tissue will escape adequate irradiation. Because of their spongy nature the seminal vesicles cannot be implanted properly. Finally, the incidence of undetected metastases or metastases to the lymph nodes is high in stage C disease.

Tumor Grade

This is still not a factor in patient selection in most institutions. However, the incidence of early metastases, undetectable with current clinical staging techniques, is extraordinarily high. This is proven by the dismal survival statistics for very high grade tumors regardless of what modality of therapy is utilized. It is my practice to exclude very high grade tumors from iodine 125 implantation.

Lymph Node Metastases

The reader is referred to the article by this author elsewhere in the book entitled "Lymph Node Dissection in the Management of Prostatic Carcinoma". There has never been any solid evidence that lymph node dissection for prostatic adenocarcinoma has any value as a therapeutic maneuver. If a patient is known to have metastases, it is difficult to imagine how any treatment confined to the primary site can be of benefit. The pre-operative detection of lymph node (or any other) metastases should exclude a patient from 1-125 implantation.

Let us imagine that a patient is thought from clinical and pathological evidence to have disease limited to the prostate. Suppose at exploration a positive lymph node is demonstrated. What should be done? It has been the usual custom in some centers to proceed to implant these patients. It is my practice, however, not to implant. In my opinion iodine 125 implantation is of no proven value in this setting. Some would argue that since the exploration has exposed the prostate, one should proceed with the implant. My retort is that even simple implantation without a full node dissection is not without complications (2).

Patient Selection

Our current practice is to divide patients into three groups based on the Gleason score (3). Patients with low Gleason scores have a very low likelihood of having lymph node metastases. We suggest that these patients have an exploration. If no suspicious lymph nodes are palpated, iodine 125 implant is performed without a lymph node dissection. Patients with high Gleason scores are very likely to have metastases. We offer these patients radiation therapy with or without hormonal manipulation. Middle level Gleason scores cannot predict lymph node status. We suggest an exploration. If palpation at exploration does not reveal any suspicious nodes, we proceed to modified systematic extra-peritoneal bilateral lymph node dissection.

Seed Placement

The accurate placement of the iodine seeds is essential to ensure adequate radiation of all cancerous tissue. Because of the retropubic location of the prostate and the limited range of the iodine 125 radiation, this has been a problem and the transcoccygeal and percutaneous perineal approaches either alone or with retropubic placement have been described.

Obstructing Glands

It has been common practice to perform a "conservative" TURP if a gland has produced retention or symptoms of severe obstruction to prevent a post-implant acute retention secondary to enlargement of the gland due to mechanical or radiation induced edema. Such a maneuver, while sometimes necessary, renders adequate implantation more difficult. Perhaps no more that 5% of the patients really need a pre-implant TURP. It is better to accept temporary post-operative retention, treating it with an indwelling catheter until the edema resolves.

Dosimetry

It is mandatory for any physician performing this procedure to have access to sophisticated computerized dosimetry techniques and to monitor that placement is accurate (4,5). If implant distribution has led to inadequate radiation, supplemental external radiation is essential.

Local Treatment Failure

As with other radiation techniques post-implant biopsies have documented the persistence of apparently viable tumor. The significance of this is not clear and the incidence decreases as the time between implantation and biopsy increases (6).

Sexual Function

An overall post-implant incidence of impotency of approximately 10% has been documented (7).

Complications

The significant complication rate has been discussed in the literature (2). It is my opinion that most of the morbidity is due to the lymph node dissection and not to the implant and it will be interesting to note the complication rate in patients treated with iodine 125 implantation without concomitant lymph node dissection.

Supplemental External Radiation Therapy (XRT)

Pelvic XRT following implantation and node dissection is associated with a high complication rate and pre-operative XRT has been proposed. However most patients with cancer-bearing lymph

nodes already have bony metastases (even if undetectable). One must therefore question the use of XRT in this setting even if it is able to sterilize lymph nodes as some claim.

Over all Cure of Cancer and Survival Statistics

Sufficiently long follow-up of patients is not currently available. We are still debating the relative indications and merits of total prostatectomy versus external radiation therapy despite the fact that they have been with us for a long time. I fear the same fate will befall iodine 125 implantation.

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CONTROVERSIAL ASPECTS OF HORMONE MANIPULATION IN PROSTATIC CARCINOMA

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INTRODUCTION

The biochemical similarity between the patient's normal and his cancerous prostatic tissue is one essential prerequisite for successful hormone manipulation basically directed at the deprivation of androgenic influences upon the prostate. In achieving this goal every therapeutic modality still relies on classical androgen control principles; however, new developments in the field of chemotherapeutic drugs and antihormones are being tested in multicenter phase II and III trials, e.g. under the auspices of the National Prostatic Cancer Project and the EORTC.

The aim of hormone manipulation may be achieved by the following basic mechanism described by Walsh (1),

1. Ablation of androgen sources,
2. Suppression of pituitary gonadotropin release,
3. Inhibition of androgen synthesis, and
4. Interference with androgen action at target tissues (= end organ antagonism).

INDICATIONS FOR HORMONE TREATMENT

Whether or not hormone treatment may be regarded as curative is far from clear, particularly when comparing the results of hormone therapy with those of radical surgery or irradiation. Thus at the present time those stages of carcinoma of the prostate (PCA) where a cure may be achieved should be excluded. A borderline situation is encountered in stage C tumors with few positive pelvic lymph nodes, i.e. T1-4 N1,2 M0. In the operable patient a thorough pelvic

Table 1. Metastatic PCA: Variety of Treatment Modalities

(67 pts. seen in one year: Murphy 1977) (47)

Hormones	35.8%
Hormones + Surgery	19.4%
None	18.2%
Surgery	14.9%
Radiation + Hormones	2.9%
Radiation + Hormones + Surgery	2.9%
Hormones + Surgery + Chemotherapy	2.9%
Radiation	1.5%
Hormones + Chemotherapy	1.5%

lymphadenectomy with irradiation should precede attempts at hormone manipulation (2). Progression despite local therapy with curative intent would leave the prostatic carcinoma still amenable to systemic treatment. In patients with proof of metastases hormone therapy is usually indicated.

A special situation is created by ureteral obstruction due to seminal vesicle invasion or direct extension of the tumor to the bladder. Michigan and Catalona (3) studied the charts of 1065 patients and found 10% with uni- or bilateral ureteral obstruction. Orchiectomy improved upper tract dilatation in 22 of 25 patients, whereas estrogen and antiandrogen were successful in only one of six patients. Grade and stage did not correlate with response rate. This favourable effect of orchiectomy upon ureteral obstruction may even be encountered after long-term medical treatment. Irradiation was far inferior in treating these patients.

Principal Methods of Hormone Manipulation

The variety of treatment modalities used in patients with metastatic prostatic carcinoma is remarkable and reflects the urologists dilemma in trying to cope with advanced disease (Table 1). Four controversial treatment categories evolve:

1. Orchiectomy plus estrogens,
2. Orchiectomy only. Other hormonal therapy is withheld until symptoms arise,
3. Hormones only. Orchiectomy is postponed,
4. Hormones plus chemotherapy.

Table 2. Androgen Control - Regimes Accepted as "Standard Treatment"

Subcapsular orchiectomy

Oral estrogens:
3 mg DES or equivalent

Parenteral estrogens:
80 mg polyestradiol phosphate

or

100 mg estradiol undecylate

Pain:
Honvan-infusion 1.2 g q.d. over 10 days

Orchiectomy plus estrogens. This is widely practiced in M1 prostatic carcinoma and may be considered a standard treatment (4,5), (Table 2). Once staging is completed, subcapsular orchiectomy is carried out followed by a diethylstilbestrol diphosphate-infusion. Maintenance therapy consists of four weekly injections of 80 mg. polyestradiol phosphate or 100 mg. estradiol undecylate (6). Some advise additional intake of an oral estrogen, e.g. Tace™ or diethylstilbestrol (DES) (4). However, the drawback of all oral medication in chronically ill patients is their poor compliance (7).

This treatment provides an excellent but transient palliation in advanced disease. There are also some long term survivors even with prostatic carcinoma of this stage (8). A recent retrospective study revealed 40% (17/43 pts.) five-year and 12% (5/42 pts.) 10-year survival following orchiectomy and estrogens (9). The results of the best known phase III trial (first VACURG study) showed that 5 mg. DES was superior to placebo, orchiectomy plus placebo or orchiectomy plus DES at the five-year follow up. At nine years, orchiectomy and placebo appeared to be the worst therapy.

In analyzing the causes of death (Table 3) in study I for stage D prostatic cancer it is obvious that estrogens increase the non-cancer mortality, but it is tempting to speculate that if these patients had lived long enough they would have succumbed to their cancer (10). This appears to be the case when taking into account the results of VACURG II (11) comparing: placebo, 0.2, 1 and 5 mg. DES. The two highest doses of estrogens slowed down the progression of stage C and D prostatic cancer.

The widely discussed data of the VACURG I have been reconsidered by Jordan et al (12) who arrived at the conclusion that estrogen is

Table 3. Causes of Death in First VACURG Study.

	Cancer		Cardiovascular	
	Stage C	Stage D	Stage C	Stage D
Placebo	18%	47%	33%	24%
Placebo + Orchiectomy	13%	48%	35%	27%
Estrogen	6%	38%	42%	36%
Estrogen + Orchiectomy	9%	38%	42%	27%

more effective than orchiectomy in preventing deaths from prostatic carcinoma and that the addition of orchiectomy to estrogen does not offer any clear-cut advantage over estrogen therapy alone. If cancer symptoms necessitate treatment these authors prefer estrogens. Orchiectomy should, according to these authors, be reserved for those circumstances in which a patient is not reliable, cannot tolerate estrogens or has severe cardiovascular disease.

Orchiectomy only. Other hormonal treatment is deferred. This concept is favored by Paulson (13) in the asymptomatic patient. Likewise, Menon and Walsh (10) prefer orchiectomy alone with relapses treated by chemotherapy. However, two large-scale retrospective studies have demonstrated the inferiority of orchiectomy vs estrogens (14,15). At the nine-year follow up even a phase III trial, VACURG I, revealed a slight, but insignificant inferiority of castration vs estrogens. This trial clearly demonstrated that estrogens are more effective than orchiectomy in preventing deaths from prostatic carcinoma (12).

The main advantage of orchiectomy alone for distant prostatic carcinoma - its excellent toleration by the patient - is more than offset by Brendler and Prout's observation that patients resistant to castration did not respond to later estrogen application (16).

Hormones only. Orchiectomy deferred. Estrogens remain the basis of all hormone preparations used for the palliation of prostatic carcinoma. A multitude of reports dealing with a variety of estrogenic preparations have appeared in the literature. Besides, two large-scale retrospective studies (14,15) two phase III trials (VACURG I and II) have exemplified the effectiveness of DES in comparison to orchiectomy plus placebo, placebo, and orchiectomy plus estrogen. The use of estrogens has been discredited, however, for the following reasons: cardiovascular toxicity, hyperprolactinemia, impairment of immune response, hepatic toxicity (17) and salt and water retention (18).

Gestagens. The availability of well-tolerated 17α-hydroxy-

Table 4. Gestagens Used For The Treatment of Prostatic Carcinoma: 17α - hydroxyprogesterone Derivatives.

Compound	Dose	Remarks
Hydroxyprogesterone caproate	1.5 g i.m./x 2 per week	---
Gestonorone caproate	400 mg/week i.m.	ineffective
Chlormadinone acetate	0.1 - 2.0 g q.d.	weak
Megestrol acetate	160 mg q.d.	active
Medroxyprogesterone acetate (MAP)	30 - 5000 mg/day	active

progesterone derivatives (Table 4) led Geller et al (19) to use these compounds in patients with prostatic carcinoma. The rationale for their use was the virtual lack of serious side-effects (20), even in a phase II-study employing daily injections of 1500 mg medroxyprogesterone acetate (MAP) over 30 days (21) and despite the dopaminagonistic action induced by gestagens with subsequent prolactin-suppression (22). However, in a survey of the various phase II-trials conducted by Bouffioux (23) none of the 17 -hydroxyprogesterone derivatives appeared to be clearly effective. Another drug, gestonorone caproate, was tested in a phase III-trial vs orchiectomy alone with negative results (24).

In the third VACURG study including 424 patients with stage IV or D carcinoma of the prostate 30 mg medroxyprogesterone acetate was not worse than 1 mg DES (25) (Table 5). The EORTC compared cyproterone acetate, medroxyprogesterone acetate, and 3 mg DES in their protocol no. 30761. At a preliminary evaluation the progression rates were 33%, 41%, and 19% respectively. However, these differences did not reach the level of significance (26).

Table 5. Third VACURG Study for Stage D PCA (1969-1974)

No.	Treatment	
105	Premarin	2.5 mg
104	MAP	30.0 mg
105	MAP (30 mg) + DES	1.0 mg
110	DES	1.0 mg

Evaluation (1981): no significant difference in survival (36).

Table 6. Medroxyprogesterone Acetate (MAP) Versus Diethylstilbestrol (DES) (27)

	Number of Pts	Survival 1 year	2 years	3 years
DES (3 - 5 mg /day p.o.)	20	16/20	14/20	11/20 (55%)
MAP (1.0 - 2.0 g/week: then 0.1 g q.d.)	20	19/20	15/20	6/17 (35%)

The most recent randomized prospective trial was conducted by Bouffioux (27) employing 500 mg medroxyprogesterone acetate 2-4 times a week for one month, followed by an oral dose of 100 mg q.d. (Table 6). It turned out that the gestagen seemed less efficient than DES in the treatment of advanced prostatic carcinoma with shorter periods of remission; but the drug was well tolerated. The 60% impotence rate was reversible after discontinuation of the medication. Gynecomastia or mastodynia did not occur.

Among the antiestrogens only tamoxifen has been tested, in a few estrogen-resistent patients, resulting in some short term remissions (28,29). A phase II-trial is expected.

Among the antiandrogens 3 compounds have been tested in patients with carcinoma of the prostate: megestrol acetate, flutamide, and cyproterone acetate.

1. Megestrol acetate was employed in a phase II-trial; four of nine patients with prostatic tumor experienced a 12 month partial remission (30). The "escape" of plasma testosterone-suppression beginning at two to six months after use of the drug was embarrassing and was possibly responsible for the development of resistance.

2. In a phase II-trial involving 13 men with previously untreated stage D carcinoma of the prostate 750 mg flutamide was given over a period of two to 20 months. Of the 13 patients seven showed an objective response (31). Similarly 21 patients with stage D prostatic carcinoma were treated by Sogani et al (32): eight of ten patients with bone pain experienced relief from pain lasting from three to 16 months. Remarkably, in three of four patients with measurable lymph nodes the lesion disappeared. Altogether 19 patients responded with an average duration of 10-1/2 months. Although none of the 21 men enrolled into the trial had previous hormonal therapy, 13 had had irradiation (Table 7).

Airhart et al (33) compared 1.5 mg DES plus 0.75 g flutamide with 1 mg DES in 20 patients with previously untreated stage D prostatic carcinoma. Objective response was noted in 3/6 patients receiving

Table 7. Phase II Trials of Flutamide in M1-PCA:

	No	PR	Side Effects *	
Stoliar (48)	18	7	?	
Prout et al (31)	13	7	thromboembolism	2
			GI-tract	1
Airhart et al (33)	6	4	?	
Sogani + Whitmore (32)	21	19	cardiovascular	4
			hepatic	2
Total	58	37	(63%)	

* Gynecomastia regularly:
Impotence rarely.

DES and 6/14 men on flutamide. 50% of the patients on flutamide survived one year and 43% two years. A similar study comprising 15 patients was reported by Jacobo et al (34), who concluded that neither DES nor flutamide displayed significant superiority (Table 8).

In the light of these investigations, the side effects are crucial: Sogani et al (32) noticed gynecomastia in 18 of his 21 patients, two developed abnormal liver function tests, and four suffered from cardiovascular toxicity (with one death). Fukushima et al (35) observed markedly altered cortisol metabolism in patients with prostatic carcinoma, presumably due to intrahepatic cholestasis or hepato-cellular damage. In the continuation of this study reversible cirrhosis-like disturbances of steroid metabolism were found (36). A positive effect of the drug is the apparent preservation of sexual potency (32).

Table 8. Phase III Trials of Flutamide in M1-PCA:

	Partial Remission		
	Jacobo et al (34)	Airhart et al (33)	Total
Flutamide 0.75-1.5 G	2/8	6/14	36%
vs.			
DES 1 mg	3/5	3/6	54%

3. The classical antiandrogen cyproterone acetate has been tested in more than 1000 patients with prostatic carcinoma (37,38). The literature contains reliable information of 101 patients treated with cyproterone acetate who had no previous therapy; from these patients the parameters best documented by Scott et al (39) and Wein et al (40) are the decline of acid phosphatase in 14/23 patients, reduced local tumor mass in 23/35 and relief of bone pain in 12/21 subjects. Geller et al (41) noted an objective remission lasting from four to 14 months in all five patients with T3-4, M0-1 lesions. The first phase III-trial stems from Jacobi and Altwein (38): cyproterone acetate (300 mg IM/week) was compared with estradiol undecylate (100 mg IM four-weekly). The former drug proved to be more effective, as the castration effect as judged by plasma testosterone was more pronounced, the objective voiding pattern and tumor response was superior and the side effects were fewer.

Deferred Orchiectomy. Whereas primary hormone therapy shows a response rate of 83% (42), secondary endocrine manipulation is of equivocal value. Although the substitution of subcapsular orchiectomy for estrogens or cyproterone acetate after hormonal escape of the tumor is common in urological practice, its effectiveness has never been investigated properly. In a retrospective study Stone et al (42) evaluated the effectiveness of deferred orchiectomy in 21 patients with carcinoma of the prostate following the diagnosis of estrogen escape. Regression was seen in one patient and stabilization of disease in four. Subjectively three patients improved and six remained unchanged. Biorn et al (43) in 29 patients with estrogen escape (defined differently however) noted a higher number of subjective responses with regression in five of the 29 patients. Denis et al (44) considered the testosterone level of critical value in predicting which patient will benefit from deferred orchiectomy. Further study of this topic appears to be desirable.

Hormones plus Chemotherapy. Primary chemotherapy would be the ideal modality for the patients with carcinoma a priori resistant to hormones; however, at the present time this cannot be reliably predicted. Among the few primary chemotherapy trials reported, Merrin (45) achieved a partial objective response to cis-platinum in 22/34 patients (64%). However, in these patients orchiectomy and estrogens were administered at the same time (!)

SUMMARY AND CONCLUSION

One is able to distinguish four therapeutic regimens for metastatic prostatic carcinoma:

1. Orchiectomy plus estrogens,
2. Orchiectomy first; other hormonal treatment later,
3. Hormone application first; orchiectomy later, or
4. Hormones plus chemotherapy.

The employment of orchiectomy plus estrogens has been seriously questioned by the VACURG (Study I) and it appears that the addition of orchiectomy to estrogen-treatment does not give better results than estrogen alone. Some authors prefer orchiectomy alone in asymptomatic patients with stage D cancer and reserve estrogens until the disease progresses. However, evidence has been provided that patients whose prostatic carcinoma has progressed despite castration do not respond to estrogens. Furthermore, it has been shown that estrogens are superior to orchiectomy in preventing cancer deaths. The argument that the rate of lethal cardiovascular complications more than outweighs the anticancer activity has been refuted by Bennett et al (46) and De Vere White et al (9).

Thus, hormones evolve as the primary treatment of choice in metastatic prostatic carcinoma, independent of the patient's symptoms, as long as the prostatic carcinoma is "virginal". Taking into account the phase III-trials dealing with hormonal therapy only, one is surprised to find neither estrogen nor the non-estrogen compounds superior. Among the latter, cyproterone acetate is clearly the most effective drug among the available hormones. Since the estrogen side effects are lacking, one would favor cyproterone acetate as the first choice in patients with disseminated prostatic carcinoma and a good performance status. Progression while on cyproterone acetate requires a change of the primary treatment; it appears reasonable to use delayed orchiectomy, and possibly, as a secondary treatment, estrogen.

Adjunctive application of antiprolactins serve to suppress the estrogen and cyproterone acetate induced hyperprolactinemia. The latter phenomenon was found to be partly responsible for the development of gynecomastia, the increment of ACTH-like action upon the adrenal gland, and a rise of prolactin-binding to the prostate in the non-castrated individual. It is tempting to speculate that prolactin may contribute to the development of hormone resistance in the patient suffering from metastatic carcinoma of the prostate.

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ESTROGENS IN THE TREATMENT OF PROSTATIC CANCER

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INTRODUCTION

Treatment with estrogens is the most commonly used modality for the management of advanced prostatic carcinoma (PCA). Their major mode of action is thought to be by the suppression of Lutenising Hormone (LH) and Follicle Stimulating Hormone (FSH) release from the pituitary to control the output of testosterone (1). Only chlorotrianisene (Tace™) does not exert an antigonadotrophic action. The decrease of plasma testosterone and testicular atrophy result primarily from the central gonadotrophin suppression; however, a direct inhibition of testosterone synthesis is also involved (2) (Table 1). There is evidence that estrogens inhibit the testicular

Table 1. Estrogen* Inhibition of Androgen Synthesis

Enzyme	Lowered Precursor	Author
17.20 Desmolase	Dihydroepiandrosterone	Samuels 1964 (48)
	Androstenedione	
3β OH Steroid dehydrogenase	Progesterone	Goldman 1968 (49)
	17 α Progesterone	Yanaihara 1972 (2)
	Testosterone	
17α Hydroylase	17 α OH Pregnenolone	Samuels 1964 (48)
	17α OH Progesterone	

* Estradiol - 17β and DES

Table 2. Testosterone Levels (ng/dl) in PCA After Treatment With Orchiectomy and Estrogens

Author	Orchiectomy	DES (mg q.d.) .2	1.0	3.0	5.0
Young 1968 (50)	50				50
Robinson 1971 (4)	30			10	
Mackler 1972 (51)	50 ± 50		80		190
Kent 1973 (5)		700 ±276	410 ±359		130 ±110
Shearer 1973 (6)	47 ± 23		66 -86	45 ±20	47 ±25

3β-hydroxysteroid dehydrogenase, thus blocking the degradation of pregnenolone, one step in the formation of testosterone in vivo (3). Since steroidogenesis inhibition requires a high dose of estrogen, this mechanism may only be operative with chlorotrianisene.

The drop in plasma testosterone is dose dependent (Table 2): 1 mg. diethylstilbestrol does not cause uniform testosterone suppression whereas 3 mg. is generally regarded as effective as 30 mg. (4-6) despite a recent study comparing testosterone levels in castrated males and intact males receiving 1 mg. diethylstilbestrol for prostatic carcinoma (7) in which the results showed little change in hormonal levels after six months in either group. The increase in sex hormone binding globulin (SHBG) which reduces the percentage of biologically active testosterone is a further beneficial effect of estrogen therapy (8).

Table 3. Five-Year Survival After Estrogen Treatment for PCA. The Influence of the Degree of Differentiation

Grade	Schirmer et al. 1965 (52)	Esposti et al. 1971 (53)	Faul et al. 1977 (54)
I	90%	67.9%	59.6%
II	71%	54.7%	65.5%
III	65%	11.0%	35.8%
IV	52%	./.	32.1%

Besides their "extraprostatic" action estrogens exert some kind of end organ antagonism at the prostate itself. In 1958 Franks (9) stated that estrogens will consistently produce cytologic damage to prostatic carcinoma cells, depending on the degree of differentiation (Table 3). Cosgrove et al (10) treated nine patients with stage T3-4 prostatic carcinoma with estrogens only, and carried out a further biopsy after 12 months. In four patients no tumor cells were found, confirmed by total prostatectomy in one patient. Furthermore, estrogens interfere with the 5α-reductase (11) and DNA polymerase (12) of the tumor cell. This direct nuclear site of estrogen action, however, requires high doses which cannot be achieved in man. Chronic estrogen treatment enhances the catabolism of the weak oxidized androgens (13). Furthermore, estrogen can serve as an effective competitor for dihydrotestosterone binding to the high affinity androphile, an action which coincides with the ability of systemically administered estrogens to block the binding of H3-androgens to their receptors (14).

Recently specific estradiol receptor protein has been demonstrated in prostatic carcinoma (15), the presence of which is, according to Sidh et al (16), a guide to estrogen-sensitive tumors.

CLINICAL APPLICATION OF ESTROGENS IN PROSTATIC CARCINOMA

Since Strohm's attempts to ease pain with estrogens in patients with cancer of the prostate (17), a multitude of reports dealing with a variety of estrogenic preparations (Table 4) have appeared in the literature. The experience accumulated to date may be summarized as follows:

Estrogens in Early Carcinoma of the Prostate

The five-year survival rates using different estrogens vary considerably but in retrospective studies a compound-related effect does not emerge. More interestingly the 10 and 15-year survival rates are remarkably similar in patients with and without total prostatectomy (Table 5).

The prospective studies (Table 6) do not show a significant difference at five and 12 years. Even though a word of caution is necessary, the effectiveness of estrogens in controlling intracapsular tumor is worth stating. At any rate, one should realize that the response rate of the tumor to estrogen application is dependent upon the degree of differentiation (Table 3). Recently, Sinha et al (18) have shown that the ultrastructural features of prostatic cancer cells permit the distinction of two basal cell types: type I (light) cells are estrogen-sensitive, whereas type II (dark) cells are refractory to hormones.

Table 4. Estrogens Used for the Treatment of Prostatic Carcinoma

Class	Drug	Dose	Remarks
Conjugated estrogens	Premarin	2.5 - 7.5 mg/d p.o.	used in VACURG III
Stilbene derivatives	DES	1 - 3 mg/d p.o.	reference estrogen
	Dienestrol	5 mg/d p.o.	very active
	Hexestrol	5 mg/d p.o.	somewhat less active
	Diethylstilbestrol diphosphate (Honvan)	360 mg/d p.o. 1.2 g/d i.v. (10 days)	active excellent palliation
	Chlorotrianisene (TACE)	12 - 24 mg/d p.o.	no LH suppression, no PRL release
Ester of estradiol	Ethinylestradiol	0.6 mg/d p.o.	very active; used in combination with a long acting estrogen
	Estradiol undecylate	100 mg/month i.m.	long acting
	Polyestradiol phosphate	40 - 80 mg/month i.m.	long acting
Combination with nitrogen mustard	Estramustine phosphate	560 mg/d p.o.	secondary treatment
Organosilicone	Cisobitan	900 mg/d p.o.	active, rather toxic

Table 5. Estrogens in Early (T1-2 NO,x MO) PCA: Retrospective Studies

Author	No.	Treatment	Survival
Barnes, 1969 (55)	108	TUR + Stilbestrol and/or Orchiectomy	57% (10 years) 33% (15 years)
Belt, 1942 (56)	160	Stilbestrol + Prostatectomy	46% (10 years) 21% (15 years)
Bennett, 1970 (57)	30	Stilbestrol	56% (5 years)
	34	Tace	67% (5 years)
	37	Ethinyl estradiol	81% (5 years)
Belt and Schroeder, 1971 (58)	222	Stilbestrol + Prostatectomy	78.6% (5 years)

Estrogens in Advanced Carcinoma of the Prostate

The first convincing evidence of the efficacy of estrogens was provided by Nesbit and Baum in 1950 employing historical controls for comparison (19). Further proof has been obtained by two large-scale retrospective studies in which classical "androgen control" therapy (orchiectomy plus estrogens) led to a 15.5% five-year and 3.9% 10-year survival rate (20). Results of orchiectomy alone were somewhat inferior (five-year survival 11.6%) and estrogens alone slightly superior (five-year survival 21.4%). These retrospective findings were confirmed by a prospective trial conducted by the Veterans Administration Cooperative Urological Research Group (VACURG) in patients with locally advanced and disseminated prostatic carcinoma (21), from which the following facts emerged:

1. placebo as initial therapy was the worst treatment in terms of nine-year survival,

2. estrogen alone was superior, and

3. survival with orchiectomy plus estrogens was slightly better than with orchiectomy plus placebo (comparable to orchiectomy alone in Emmett's (20) retrospective study).

Thus, two independent trials demonstrated that estrogens are

Table 6. Estrogens in Early (T0-2 N0/NX,M0) PCA: Prospective Studies

Author	No.	Treatment	Survival	P	Remarks
Byar, 1972 (59)	148	Placebo	46% (5 years)		no cancer deaths
		Stilbestrol	59% (5 years)		
		Orchiectomy + Placebo	52% (5 years)		
		Orchiectomy + Stilbestrol	31% (5 years)	n.s.	
Madsen, 1980 (60)	120 Stage I	Stilbestrol + Prostatectomy	31% (12 years)		19 cardio-vasc. deaths
		Placebo + Prostatectomy	35% (12 years)		17 "
	179 Stage II	Stilbestrol + Prostatectomy	35% (12 years)		29 "
		Placebo + Prostatectomy	32% (12 years)	n.s.	26 "

somewhat effective in retarding the course of prostatic carcinoma. The first VACURG study, however, indicated that lethal cardiovascular side effects more than offset the retardation of tumor growth. The second VACURG study revealed a strong influence of the estrogen dose in stage III disease (patients with potential longevity) and a weak influence in stage IV prostatic carcinoma (21).

Even though, the action of "androgen control" treatment is cancerostatic rather than cancerocidal (22) it apparently prolongs life (23) and provides excellent palliation in advanced disease. There are some long term survivors even with prostatic carcinoma at this stage (24). A recent retrospective study by de Vere White et al (25) revealed a 40% (17/42 patients) five-year and 12% (5/42) 10-year survival following orchiectomy and estrogen therapy. Recently Lepor et al (26) have shown in a non-concurrent prospective study that estrogen treatment reduced the tumor death rate by 45% (62 patients without endocrine treatment vs 54 patients with estrogen treatment - $p < .004$).

DOSAGE OF ESTROGENS

The recommended dose of the standard estrogen diethylstilbestrol has ranged from .25 mg. q.d. (27) to 100 mg. q.d. (28). Occasionally even 1000 mg. q.d. has been given (29). Whether or not it is useful to start with doses between 30-100 mg. reducing to a maintenance dose between 1-3 mg. is not clear. In metastatic disease the optimal dose was investigated in the VACURG study II where 1 mg. diethylstilbestrol daily was found clinically sufficient and produced only a few adverse side effects. This also holds true for 3 mg. diethylstilbestrol daily (30). This appears to be the critical estrogen dose (31) to which the equieffective daily dose of other estrogen compounds is: 0.2 mg. ethinylestradiol, 360 mg. diethylstilbestrol diphosphate (Honvan™), 24 mg. chlorotrianisene (Tace™), and 7.5 mg. Premarin™, a natural conjugated water-soluble estrogen.

SIDE EFFECTS OF ESTROGENS

Although of great value in prostatic cancer estrogen therapy may also be accompanied by undesirable side effects. The most common include reversal of the normal masculine characteristics, decreased libido, impotence, gynecomastia, mastodynia, salt and water retention, and disturbance of the coagulation mechanism and of lipid metabolism. However, the use of estrogens has been particularly questioned in recent years for the following reasons:

Cardiovascular toxicity (myocardial infarction, cerebrovascular accidents, arteriosclerotic heart disease, pulmonary embolism) is a phenomenon to which Blanchot et al had already directed attention in 1952 (32). This observation passed more or less unnoticed until the famous first VACURG study (33) proved that the estrogen arms had a cardiovascular death rate of 42% each vs 33% and 35%, respectively in the non-estrogen arms for stage III (C) cancer and 36% and 27% vs 24% and 27%, respectively for stage IV (D) cancer. It is noteworthy that these differences are obvious only when comparing the estrogen with the arm in which placebo was given as the initial therapy. Further investigation on this subject has made it clear that patients with pre-existing cardiovascular disease are particularly at risk and that the cardiovascular side effects are dose-related. This toxicity was particularly distressing for patients with potential longevity, e.g. stage III (C) tumors, where 5 mg. diethylstilbestrol led to an excess of non-cancer deaths as compared to 1 mg. which still seemed to produce equal survival rates. The risk may be further reduced if those patients with elevated triglyceride- and plasminogen levels (34,35) are excluded or receive an inhibitor of platelet aggregation.

Hyperprolactinemia. Ratner et al in 1963 were the first to demonstrate the ability of estrogens to stimulate release of prolactin from the pituitary (37). Boyns et al in 1974 found a dose and drug-

related effect (38) and Jacobi (39) has summarized all the evidence regarding the positive influence of prolactin on the testis, adrenal gland and the prostate itself. Hanafy et al (40) brought up the point that the hyperprolactinemia may be responsible for the development of estrogen resistance of the tumor.

Impairment of the immune response. Estrogen and prolactin are active in this regard (41).

Hepatic toxicity and salt and water-retention.

Psychic alterations requiring psychiatric treatment have been observed in 40% of the patients (44).

SUMMARY AND CONCLUSION

Estrogens are active concerostatic drugs in early prostatic cancer producing survival rates which can match far more radical means of treatment. Their use in patients with low-stage cancer and potential longevity has been discredited mainly due to serious adverse side effects. However long term survivors have been reported even in metastatic tumors. In addition estrogens have been recognized as providing excellent palliation.

Simultaneous castration has not been proven to be superior to estrogen treatment alone. The value of delayed castration in estrogenized men is not yet clear (45). Delayed estrogen treatment in patients subjected to castration only as suggested by Menon et al (46) does not slow down the progression of cancer (47).

In the EORTC Urological Group studies (reported in this volume) none of the alternative treatments has yet been shown to be superior to Stilbestrol, though some may have less cardiovascular toxicity. Therefore it seems reasonable to replace estrogen treatment for metastatic disease in asymptomatic patients by other compounds, e.g. cyproterone acetate, and to reserve the palliative effect of estrogens for those patients presenting with serious symptoms related to their prostatic carcinoma.

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TREATMENT OF PROSTATIC CANCER WITH DIETHYLSTILBOESTROL - DETECTION AND EVALUATION OF THE VASCULAR RISK

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SUMMARY

A retrospective study of patients suffering from prostatic cancer and treated with estrogens has allowed us to develop a test to discriminate a population with a high risk of thrombotic complications due to estrogens from a population that can benefit without danger from this form of hormonal therapy. The so-called "estrogen tolerance test" relies on the presence of circulating immune complexes which can be precipitated from serum by 25% saturated ammonium sulphate.

In the group of patients in which immune complexes exceeded 700 µg/ml, 41% developed thrombo-embolic complications including embolism and arterial thrombosis. The group of patients in whom the values were under 300 µg/ml had a lower risk of arterial thrombosis (2.5%) and the observed incidents were benign and mostly venous thrombosis.

Test values above 700 µg/ml should preclude treatment with diethylstilboestrol.

INTRODUCTION

The treatment of prostatic carcinoma by estrogens is associated with an elevated risk of venous and arterial thrombosis (1). In women, oral contraception using combined therapy with estrogens and progestogens is also responsible for an increase in the incidence of vascular thrombosis (2,3,4).

The discovery of circulating immune complexes (C.I.C.) containing a monoclonal IgG with anti-ethinyl estradiol activity (5) which could be precipitated by ammonium sulphate at 25% saturation in the serum of a woman who had suffered a pulmonary embolism whilst taking the pill, led to the development of a simple test of serum precipitation which was shown to precipitate almost exclusively immunoglobulins and complement fractions, a finding consistent with precipitation of C.I.C. (6). These C.I.C. were later shown to contain anti-ethinyl estradiol antibodies in several cases (7).

The test was used to detect the vascular risk in women on oral contraceptives.

The immune complex precipitation test was positive in 95% of women who had suffered vascular thrombosis while using the pill. In those who had not experienced such a complication, there were two different groups, one in which the test was found to be negative, even after years of use and a second group in which the test was positive. The latter group could be considered "at risk" (8). This paper presents the results of this test applied to patients suffering from prostatic cancer who were or were not treated by estrogens.

MATERIAL AND METHOD

Three groups of patients were selected for this study, a group of 65 patients with prostatic cancer treated with varying doses of diethylstilboestrol (D.E.S.) ranging from 1 - 25 mgs per day, a second group of 19 patients with untreated prostatic cancer, and a third group of 25 men of about the same age, in apparently good health and with no evidence of any cancer.

The detection of circulating immune complexes was achieved by precipitation of serum in 25% saturated ammonium sulphate. The proteins were measured by the method of Lowry and expressed in μg/ml (6,8).

RESULTS

Thrombosis

Thrombotic complications were found in 12 of 84 patients (14.2%) before treatment with D.E.S., and in 18 of 65 (22.7%) patients treated with D.E.S. The difference is significant ($p < 0.05$).

In patients treated with D.E.S. the observed venous thrombotic complications were nine cases of venous thrombosis and two cases of pulmonary embolism. In addition, seven cases of arterial thrombosis occurred including four cases of coronary thrombosis and one cerebral thrombosis and two patients with thrombosis in a limb.

The influence of D.E.S. dosage in the 57 cases in which dosages are known with precision is shown in Figure 1. Of the patients, seven were treated with 1 mg per day. In this group one case of venous thrombosis was observed, the incidence of complications being similar to the group without estrogens. Twenty-seven patients received doses of 2 - 3 mg. of D.E.S. per day. In this group eight cases of thrombosis were seen (30%) of which four were venous thrombosis, two were pulmonary embolism or pulmonary artery thrombosis and two developed systemic arterial thromboses. Twenty-three patients received at least 5 mg. of D.E.S. per day. In this group eight cases of thrombosis were seen (35%), three cases of venous thrombosis and five of systemic arterial thrombosis. The mean duration of estrogenic treatment was no different in patients with and without thrombosis, being 17.6 $\pm$ 33.1 months during the first group and 16 $\pm$ 13.9 months in the second ($p > 0.1$). The thrombosis appeared at the earliest 12 dyas and at the latest 12 years after starting treatment.

Immune complex precipitation test

The average quantity of proteins precipitated in 25% saturated ammonium sulphate expressed in μg of protein per ml of serum was the same in the control group and in the group with untreated prostatic cancer (Table 1). These values were 387 $\pm$ 197 μg and 396 $\pm$ 294 μg respectively, with extreme values between 0 and 800 μg. The distribution curve was unimodal (Fig. 2A).

In the group treated with D.E.S., the mean quantity of precipitated proteins was 746 $\pm$ 698 μg/ml with extreme values ranging from 94 to 2,400 μg. The distribution was bimodal with two subpopulations as shown in Figure 2B - a subgroup similar to the control group and a further group above the normal values. This second subgroup represented 27 of the 65 patients treated by D.E.S.

These two sub populations did not differ statistically with regard to mean age (71 and 75 years respectively), length of treatment (12.1 and 17.3 months), or dosage (4.7 and 4.6 mg of D.E.S. per day).

Relation between increase in immune complexes and thrombosis

Among the 65 treated subjects, 47 suffered no thrombosis on treatment. In this group the mean value of precipitated immune complexes was 687.9 $\pm$ 676 ug/ml. In the 18 patients who suffered thrombosis the mean value was 839.2 $\pm$ 716 ug/ml. This is not significantly different from the above group.

The distribution of the values in these two groups however is not homogeneous as the distribution was bimodal, as it was in the group suffering thrombosis (Fig. 3). However, when the frequency of

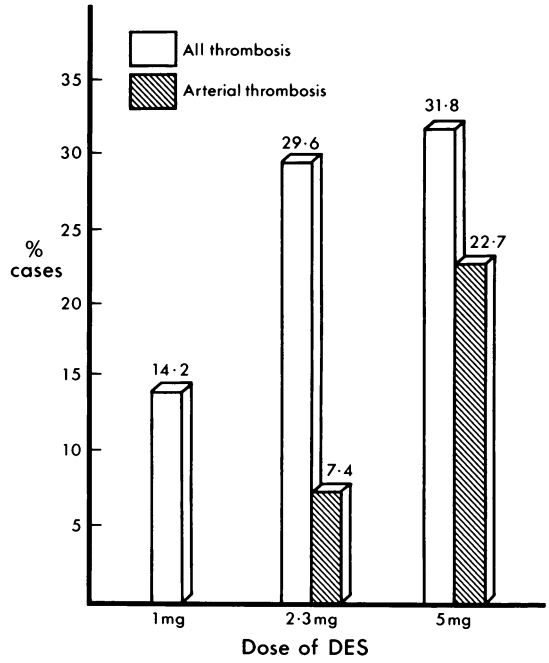

Fig. 1. Frequency of vascular thromboses in relation to the dose of D.E.S.

Table 1. Precipitation test of serum proteins in 25% saturated $(NH_4)_2SO_4$ solution

Group	No. of patients	Proteins ug/ml	T
Controls	24	387.7	197.7
Untreated cancers	19	396.6	294.0
Treated cancers	65	746.1	698.0

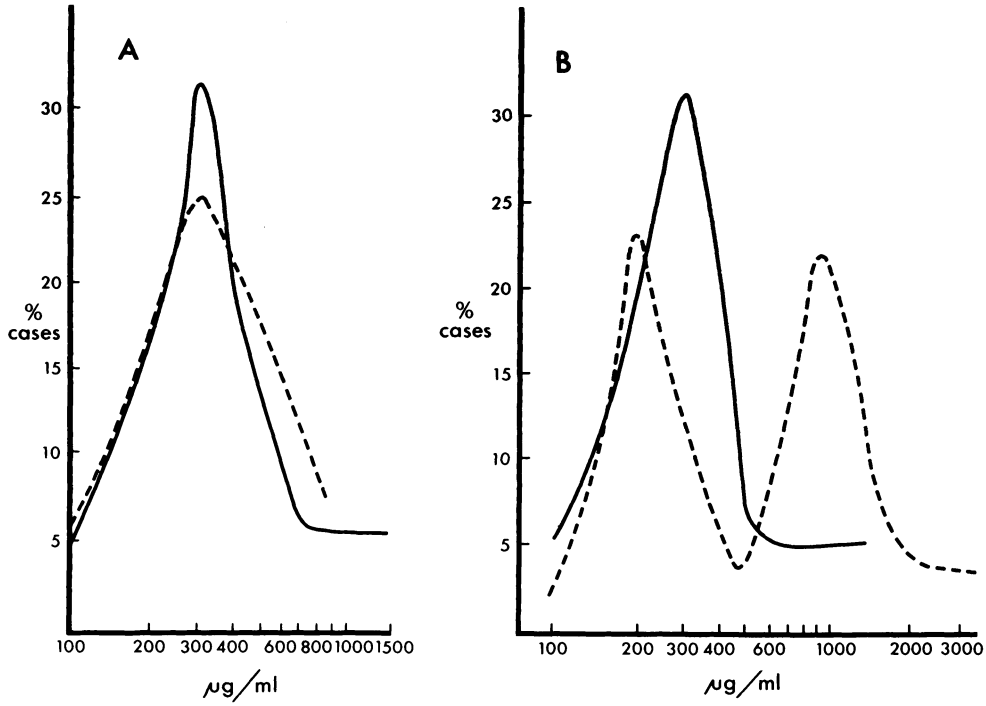

Fig. 2. Distribution of patients based on the quantity of immunoglobulins precipitated by 25% ammonium sulphate solution.
A: dotted line - controls. solid line - patients with untreated prostatic cancer. The distribution is normal for these two populations.
B: solid line - patients with an untreated prostatic cancer. dotted line - patients with prostatic cancer treated with D.E.S. The treated patients exhibit a bimodal distribution.

thrombosis in the total treated population was analysed as a function of the critical value of 700 µg/ml, it may be seen from Table 2 that thrombotic complications were more frequent in the group with high values (nine of 27 patients) than in the group with low values (nine of 38 patients), and that they were of a different type, nearly all venous thrombosis occuring in the group with low values (eight out of nine cases, $p < 0.05$) whereas arterial thrombosis (six out of seven cases, $p < 0.02$) were seen in the group with high values.

The two cases of pulmonary embolism which also had high values were classified separately. The lack of signs suggesting either pelvic or lower extremity phlebitis in these two cases suggests the possibility that they were due to pulmonary arterial thrombosis and not to embolism.

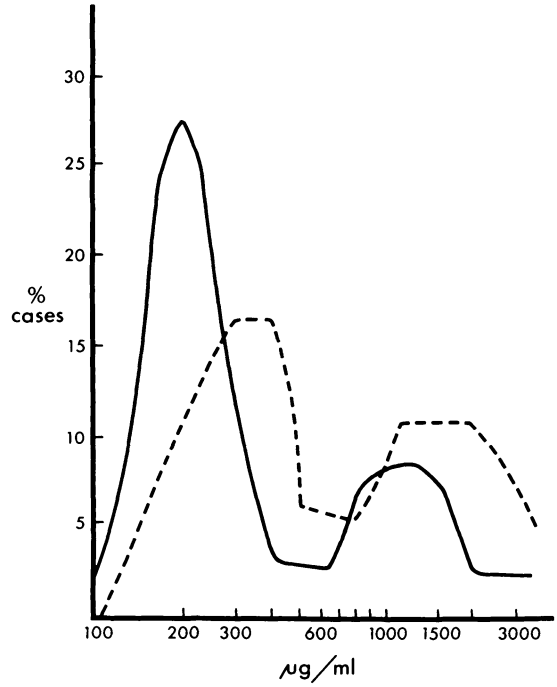

Fig. 3. Distribution of patients treated with D.E.S., based on the values of precipitated immunoglobulins. solid line - patients without, and dotted line - patients with vascular thromboses.

Table 2. Thromboses and precipitation test of serum proteins in 25% saturated $(NH_4)_2SO_4$ solution

Protein precipitates in µg/ml of serum	< 700	>700	p
No. of patients	38	27	
No. of thromboses	9 (23.6%)	9 (33.3%)	
- venous thrombosis	8	1	<0.05
- arterial thrombosis	1	6	<0.02
- pulmonary embolus or pulmonary artery thrombosis	0	2	

DISCUSSION

After reviewing the pertinent literature one is inclined to revise the classical notion of the benignity of estrogen treatment of prostatic cancer. In TO cancers, the global mortality rate is greater in the group treated with D.E.S. as a result of thrombo-embolic accidents. In cases where the tumor is clinically perceptible and metastases are present, treatment with D.E.S. does not significantly modify the survival rate, for the increased mortality due to thrombo-embolic complications more than compensates for the decreased mortality due to the drug's anti-cancer effect (1,9.10).

If it became possible to identify patients in whom the estrogen related thrombo-embolic risk was minimal, it would be tempting to continue to offer them the opportunity of treatment with D.E.S., whose anti-tumoral effectiveness has been clearly established (11-16).

The test that has been used here relies on the detection of circulating immune complexes containing antibodies to estrogens. It was developed to study the vascular risk in women using oral contraceptives and has been responsible for the discrimination of a group of women at special risk, evaluated as being about 30% of users. This test is positive in 95% of women who have suffered a thrombosis.

In prostatic cancer, the same test used in patients treated with D.E.S. allows one to distinguish two populations, (i) a group with values less than 300 μg/ml in which the probability of arterial thrombosis is small (2.5%) and the observed cardiovascular complications are those of venous thrombosis and (ii) a group with values above 700 μg/ml in which the thrombo-embolic risk is probably above 30% with serious complications such as pulmonary embolism and arterial thrombosis. In our study this group represents 41% of treated subjects.

These findings are the basis for an argument in favor of a therapeutic attitude based upon the evaluation of the tumoral risk, the iatrogenic risk of D.E.S. and the eventual benefits that the patient may gain from such treatment. Small doses of D.E.S. 2 mg per day or less should be recommended, while, at the same time, monitoring C.I.C. and plasma testosterone values (17). Patients treated with D.E.S. for three months, whose C.I.C. levels are above 300 μg/ml, should ideally be treated by other means. However, when other forms of therapy are impractical and the C.I.C. levels are between 300 and 700 μg/ml, continuing treatment with D.E.S. is probably acceptable.

A test value above 700 μg/ml should exclude D.E.S. treatment no matter what the observed tumoral situation is. Moreover, therapeutic safety could possibly be enhanced through study of the blood coagulation factors.

In fact, the physiopathologic mechanisms responsible for the arterial and venous thrombosis seen in prostatic cancers treated with D.E.S. may not be univocal.

As we have seen, a number of thromboses, essentially those due to arterial thrombosis, appear to be associated with the presence of circulating immune complexes. In one case it was demonstrated that these complexes contained an immunoglobulin that reacted specifically with D.E.S. (18) in the same way that immunoglobulins found in women using oral contraception reacted with ethinyl-estradiol (7). These estrogen-antiestrogen complexes could be a cause of endothelial alteration, leading to thromboses (19) and may be compared to the experimental vasculitis due to immune complexes (20). In these cases the lesions increase with the quantity of foreign proteins injected. Even though a dose related effect was observed in our study, it must be stressed that immune complexes appear even for small doses of D.E.S. and that vascular thromboses are also seen in women taking low dose oral contraceptives.

Concerning the fact observed in our study that venous thromboses do not seem to be correlated with the presence of immune complexes, it may be due to the fact that many other factors predisposing to venous thrombosis exist in these aged men suffering from a disease which is thrombogenic in itself.

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PROGESTINS IN PROSTATIC CANCER

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Progestins are synthetic compounds with progestational activity. One group of these compounds related to 17-alpha-hydroxyprogesterone has mainly progestational effects while another group exerts more anti-androgenic activity. This paper reviews the effect of treatment of the first group of compounds in patients with advanced prostatic cancer.

The first favourable and preliminary results with the then available progesterone compounds were reported more than thirty years ago (1,2). Subsequent reports in a small number of patients utilizing a series of progesterone derivatives - hydroxyprogesterone caproate (3), chlormadinone acetate (4) and megestrol acetate (5), have also yielded favourable results. The synthesis of a new semi-synthetic compound, medroxyprogesterone acetate (MAP), in 1958 (6,7) with high progestational activity and active by the oral route brought a well tolerated drug that has been widely used in clinical oncology especially in endocrine related tumors (8).

REPORTED RESULTS

MAP was used in low doses for long periods in two studies. Ferulano et al (9) treated thirty-four patients with locally advanced tumor of whom eleven also had metastases. Roughly half of the patients were previously treated by estrogens. The treatment scheme provided a total dose of MAP of 900 mg IM every five months with an interval rest of two months. Good objective and subjective results

were reported with no side effects over a period of twenty months in all patients.

Kondo and Saito (10) treated ten patients with advanced tumor and no previous therapy with oral doses of MAP of 15 to 50 mg/day for one to eleven months. Objective improvement based on prostatic size was noted in all patients. All patients had subjective improvement. No side effects were noted.

In the VACURG study III, MAP in low doses was compared to Stilboestrol (DES), conjugated equine estrogens and the combination MAP-DES. No significant differences in survival were noted in patients with advanced prostatic cancer (11). Higher doses were used by Rafla and Johnson (12). They treated twelve evaluable patients with 300 mg/day orally. A satisfactory response was noted in nine out of twelve patients with an average duration of four months.

Bouffioux (13) reported on the response of forty untreated prostatic cancers which were randomly given MAP or estrogens. MAP was given at a dose of 500 mg IM 2 - 4 times a week for one month, followed by 100 mg/day orally. A 40% objective remission rate was observed with a mean duration of thirteen months. The results with estrogens were somewhat better. Twelve patients in relapse on estrogens were also treated with MAP. A good subjective result was obtained in seven and an objective response in two.

Our own experience goes back to 1961 when we treated nine intact patients with advanced prostatic cancer in a pilot trial with 1 g/day orally for one month. No objective responses according to the protocol were noted but three patients showed subjective response. Seven cases were interrupted by a switch to bilateral orchiectomy in the third week of the study (14). Another schedule was applied in a second trial (15) where MAP was given in a loading dose of 3 x 500 mg IM weekly for two weeks, followed by an oral dose of 3 x 100 mg for two weeks and a maintenance of a daily oral dose of 2 x 100 mg for two months. In eleven patients with clinically advanced previously untreated prostatic cancer a partial remission was obtained in six patients with a fair subjective response. In nine patients with prostatic cancer in relapse only three patients showed some subjective response and no objective response was noted.

DISCUSSION

It is difficult to give a clear view on the activity of MAP as a single agent in patients with prostatic carcinoma due to the contradiction of data and the variability of the patients. It is however safe to say that MAP treatment in new untreated cases of prostatic cancer will bring objective partial and/or subjective remission in some patients. However few subjective remissions have been noted in

patients with prostatic cancer in relapse and almost no objective remissions.

In a preliminary study where the activity of MAP was compared to diethylstilboestrol (DES) Bouffioux was able to conclude that MAP seems less efficient than DES expressed in terms of duration of remissions and survival (16). The decrease in plasma levels of testosterone is notably slower than with bilateral orchiectomy or DES treatment (15,16).

This decrease is a reflection of the pituitary inhibitory activity of MAP which has been reported (17). The overall net benefit could however be attributed to a variety of pharmacological actions demonstrated by MAP: interactions with circulating hormones, competitive binding to 5 alpha-reductase, competitive binding to the androgenic receptor sites and a direct toxic effect on tumor cells (8). The role of progestins in prostatic cancer will relate to the net result on the metabolic pathway of the androgens which is obtained by a FSH-LH blockade, a competitive binding to 5 alpha-reductase and the enhanced catabolism of circulating androgens (18) (Fig. 1). This may explain the varied effects of progestins in selected cases. Concerning its activity on prostatic cancer we hope that the EORTC Urological Group's Protocol 30761 comparing the effect of cyproterone acetate, medroxyprogesterone acetate and stilboestrol in patients with advanced prostatic cancer stage T3 and T4 will allow a final conclusion on the effect of MAP in prostatic cancer as a single agent.

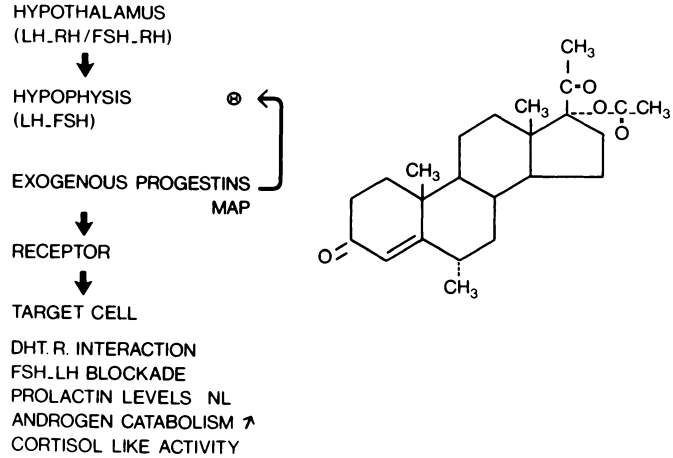

Fig. 1. Pathways of progestin activity in patients with prostatic cancer.

The rationale for the use of progestins in prostatic cancer has already been outlined by Scott in 1973 (19). At the moment we know that androgen, estrogen, progestin and prolactin receptors have been demonstrated in prostatic tissue with collateral activity of other hormones (20). It is possible that overall results will be improved by combination hormonal therapy eventually coupled to chemotherapy in earlier stages of the diseases (21). Combination hormonal treatment will be studied in a randomized way by the EORTC Urological Group in its protocol 30805 in which bilateral orchiectomy versus stilboestrol versus bilateral orchiectomy plus cyproterone acetate is to be evaluated in patients with metastatic prostatic cancer.

CONCLUSIONS

From the published data and our own clinical experience we conclude that MAP teatment as a single agent in patients with advanced prostatic cancer may show objective and subjective responses mainly in untreated cases.

The results obtained are probably inferior to those of standard treatment and future application of this drug may be in combination with other hormonal treatment.

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NOLVADEX (TAMOXIFEN) AN ANTIOESTROGEN OF POTENTIAL USE IN THE MANAGEMENT OF CARCINOMA OF THE PROSTATE

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Well-differentiated prostatic cancer cells, like normal prostatic cells, remain for a time, androgen-dependent. Oestrogens in sufficient dosage reduce circulating androgens, and this has been the rationale for conventional oestrogen therapy. At least 3 mg. daily of Stilboestrol, in divided doses, are needed to suppress plasma testosterone levels throughout 24 hours (1,2), but clinically 1 mg. per day is as effective in controlling the disease (3). This therapeutic evidence for an additional direct action of oestrogens upon the prostate is supported by the classical animal experiments of Huggins and Clark in 1940 (4) and by the tissue culture studies of Earnsworth (5).

Oestrogen receptors occur in normal, hyperplastic and malignant prostatic tissue (6,7) and if these receptors are functional it can be postulated that antioestrogens will also influence prostatic metabolism and growth. There is increasing experimental and clinical evidence to support this concept.

Nolvadex (Tamoxifen) is an active antioestrogen which is a non-steroidal derivative of triphenylethylene. It specifically binds to oestrogen receptors in target tissues and is effective in the management of post-menopausal women with hormone dependent breast cancer (8-10). Animal and in vitro studies demonstrate that this drug also influences the growth and metabolism of prostatic cancer.

Using male Copenhagen X Fischer F1 rats bearing the androgen dependent well-differentiated R3327 Dunning rat carcinoma, Ip, Milholland and Rasen (11) have shown that 0.5 mg/Kg Nolvadex given five times a week suppresses tumour growth in 91% of rats. Plasma testosterone levels in the rats are also reduced. However, in young

human males treated with Nolvadex for oligospermia, continual administration of the antioestrogen results in an elevation of gonadotrophins and a secondary rise of plasma testosterone (12).

Habib et al (13) have shown in vitro that Nolvadex displaces plasma androgens from sex hormone binding globulin. In the tissue it inhibits 5 α-reductase and 17-β-hydroxysteroid dehydrogenase activity with a corresponding reduction of 5α-dihydrotestosterone (to 13% of total metabolites) and androstenediol totally. 3 α(β) hydroxysteroid dehydrogenase activity is markedly increased with a large increase (75%) of androstendione levels.

This experimental data suggests that the reduction of 5α-reductase activity and the diminished conversion of testosterone to its most active metabolite dihydrotestosterone, by Nolvadex, may be beneficial in the treatment of human prostatic cancer. This is supported by the objective response observed in antioestrogen treated rat prostatic carcinoma. The displacement of bound testosterone from sex hormone binding globulin and the secondary rise of plasma testosterone levels could, however, be detrimental and may explain the exacerbations of bone pain which have been observed in patients with prostatic cancer and bony metastases treated with Nolvadex (14).

The results reported from two clinical studies of Nolvadex in the management of advanced progressive disease are encouraging. Morgan et al (15) noted seven "responders" and eight "disease stabilisation" in 28 patients. In a phase II trial, reported by Glick (16) which recruited 31 patients and used National Prostatic Cancer Project criteria of response, three patients showed objective partial response and four objectively stable disease, an overall response rate of 23%. In this series no patient experienced exacerbation of bone pain. These results suggest that Nolvadex has some activity and that it deserves further study.

Nolvadex is not excessively toxic. Although major untoward effects which have been reported include nausea and vomiting, hot flushes, mild thrombocytopenia and leukopenia, skin rashes, retinopathy and corneal opacities, and hypercalcaemia, all are rare when this agent is used in the management of breast cancer (17). In a personal toxicity study of 11 patients with non-metastatic prostatic cancer and one with metastases treated with Nolvadex 10 mg. b.d. for a mean period of 26 months, no toxicity was observed. It is clear that further phase II studies and controlled clinical trials are needed to establish the possible role of Nolvadex in the management of carcinoma of the prostate.

SUMMARY

Experimental and clinical evidence suggests that the antioestrogen Nolvadex directly influences prostatic metabolism. Clinical trials are needed to evaluate its role in the management of prostatic cancer.

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CARCINOMA OF THE PROSTATE: ADRENAL INHIBITORS

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SUMMARY

Adrenal androgens may continue to stimulate prostatic carcinoma after the suppression of testicular function by orchidectomy or oestrogen therapy. The production of adrenal androgens may be prevented by adrenalectomy, pituitary ablation or adrenal inhibitors. This paper discusses the therapeutic potential and complications of these techniques with special reference to the adrenal inhibitors.

Until recently, primary conventional endocrine therapy for carcinoma of the prostate gland has been orchidectomy or oestrogen administration. The principle objective of both methods has been to suppress the major androgen, testosterone, which stimulates this hormone dependent tumour. Oestrogens may have an additional direct action on the gland. Treatment failure may be because cancer cells which remain hormone dependent are secondarily stimulated by adrenal androgens or because clones of non-hormone dependent cells develop and cause tumour progression. Non-hormone dependent cells will only respond to cytotoxic chemotherapy whereas hormone dependent cells may respond to secondary hormonal therapy.

There is evidence for increased androgen production by the adrenal gland following conventional endocrine therapy. During oestrogen treatment there may be a small rise in plasma testosterone levels (1,2). This may be due in part to increased adrenal cortical activity and in part due to increased binding of testosterone by sex hormone binding globulin. Oestrogens do stimulate adrenal cortical activity. Doe et al (3) found that serum non-protein bound cortisol levels were higher in untreated carcinoma of the prostate patients

at 9 a.m. and 9 p.m. The 9 p.m. levels were again significantly higher in oestrogen treated patients. Peeling and Griffiths (2) found that the stimulation of the adrenal gland with Tetracosactrin does not significantly elevate plasma testosterone levels in untreated carcinoma of the prostate, but may do so after several months of oestrogen treatment.

Following orchidectomy Sciarra et al (4) found that in 17 of 28 patients with carcinoma of the prostate plasma testosterone fell to very low levels. In 10, however, they did not fall below 100 ng/100 ml. Dexamethasone treatment reduced these higher levels. Inadequate reduction of plasma testosterone by orchidectomy in their experience was not associated with clinical improvement.

The principal androgens produced by the adrenal cortex are Androstenedione and dehydroepiandrosterone (Fig. 1). Compared with testosterone and dihydrotestosterone, they only weakly stimulate the prostate (5). They are, however, converted in peripheral target organs, including the prostate, to testosterone and dihydrotestosterone. Adrenal androgen production is stimulated by adrenocorticotrophic hormone (ACTH) but the adrenal androgens do not themselves suppress ACTH production by a feedback mechanism.

The rationale for secondary endocrine therapy is to suppress the adrenal androgens. This may be achieved by pituitary ablation or by surgical or medical adrenalectomy. The pituitary may be ablated by surgery, irradiation (6) or alcohol injection (7). By removing the source of ACTH, there is secondary hypoplasia of the adrenal cortex.

As long ago as 1945, before the advent of corticosteroid replacement therapy, Huggins and Scott (8) reported a series of patients subjected to bilateral total adrenalectomy. Adrenalectomy, even after replacement steroids became available, has not been widely practised. This is because the best results achieved have only been a temporary subjective improvement in 50% of patients (9) and in addition there are major surgical complications of adrenalectomy in elderly men with advanced malignant disease.

Pituitary ablation is less traumatic and also results in a 50% subjective response (10).

For many years alternative methods of suppressing adrenal cortical activity without subjecting the patients to surgery or irradiation have been suggested. In 1950 Sprague et al (11) observed that corticosteroid without adrenalectomy, reduced adrenocortical activity by suppressing ACTH production. Several workers have reported various response rates to corticosteroid therapy (12,13,14). In general there is temporary subjective improvement manifest by less metastatic pain, improved appetite and increased weight. Only occasional

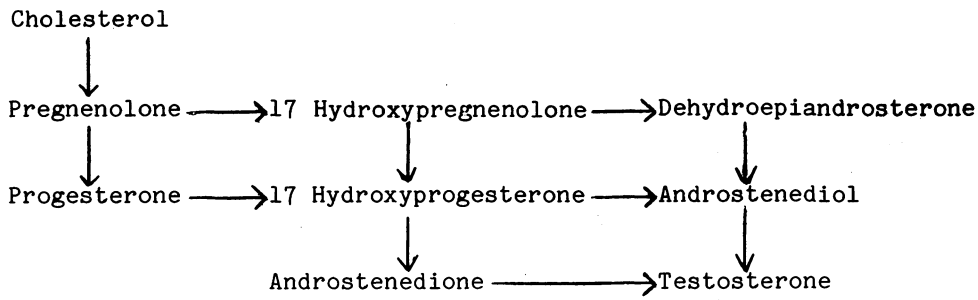

Fig. 1. Production of Androgens by the Adrenal Cortex.

objective responses have been claimed. Complications include fluid and sodium retention, potassium loss, hypertension, gastro-intestinal irritation and peptic ulcer, glycosuria and osteoporosis.

Drugs which specifically inhibit adrenal androgen synthesis include Aminoglutethimide, Spironolactone, Cyproterone acetate, Cyanoketone, Estradiol 17β, Hydroxymethylene and Medrogesterone (5). Cyproterone acetate is currently being investigated by the EORTC and other clinical groups because of its direct antiandrogen activity on the prostate gland, but it also acts on the adrenal gland by inhibiting the enzyme 17,20 desmolase (responsible for the conversion of 17 hydroxypregnenolone to dehydroepiandrosterone) and 17β hydroxy steroid dehydrogenase (responsible for conversion of Pregnenolone to Progesterone and 17 Hydroxypregnenolone to 17 hydroxyprogesterone - see figure 1). Two other adrenal inhibitors have been investigated. These are Aminoglutethimide which inhibits 20,21 desmolase and suppresses the conversion of cholesterol to pregnenolone, and Spironolactone (an aldosterone inhibitor) which suppresses 17,20 desmolase activity and the conversion of 17 Hydroxypregnenolone to dehydroepiandrosterone.

Walsh and Siiteri (15) reported that spironolactone produced a subjective remission in three of seven orchidectomised patients with progressive prostatic cancer. Plasma levels of testosterone, androstenedione and dehydroepiandrosterone were significantly reduced.

Aminoglutethimide is a derivative of glutarimide which was used in the USA as an anticonvulsant until it was withdrawn because of its endocrine side effects. It inhibits adrenal production of aldosterone, cortisol and androgens (16) by inhibiting the conversion of cholesterol to delta 5-pregnenolone (17). There is no evidence to indicate that it inhibits testicular function (18). In a personal series reported in 1974 (19) we administered Aminoglutethimide 500-1000 mg in divided doses daily, together with Stilboestrol 1 mg three times a day, cortisone 20-25 mgs daily and Fludrocortisone 0.1 mg

daily to 27 patients with progressive metastatic carcinoma of the prostate. Eight patients (30%) experienced complete relief of pain and nine (35%) partial relief of pain. Objectively there was a fall of acid phosphatase in four of 13 patients with elevated levels (31%), decreased metastatic bone scan activity in one, reduction of prostatic size on rectal examination in one and resolution of ureteric obstruction on an intravenous urogram in one patient. Complications included severe hypotension and an Addisonian crisis (acute adrenocortical failure) in four patients given Aminoglutethimide before steroid replacement therapy. The other complications are listed in Table 1.

Sanford et al (20) gave Aminoglutethimide with steroid replacement therapy to seven patients. They reported three responses (two reductions of acid phosphatase and one regression of bone metastasis). They observed that rising ACTH levels can reverse the effect of Aminoglutethimide unless suppressed by dexamethasone or hydrocortisone.

It would seem, therefore, that Aminoglutethimide may be of some value in the management of advanced prostatic cancer. This claim, however, can only be confirmed by a controlled Phase II trial using modern criteria of response. A second Phase II trial is also probably needed to determine the true objective response to the steroid "replacement" therapy given without the Aminoglutethimide, as this also suppresses adrenal cortical activity.

Table 1. Aminoglutethimide
Side Effects in 27 patients (19)

Side Effects	No. of patients
1. Before Steroid Replacement	
Hypotension, "Addisonian Crisis"	4
2. With Steroid Replacement	
Nausea and vomiting	8
Lassitude	11
Depression	5
Oedema	3
Skin rash	1

CONCLUSIONS

This paper has discussed the concept of adrenal inhibitors as a form of secondary hormonal therapy. Evidence has been given for increased production of androgens by the adrenal cortex during conventional endocrine therapy. Three clinical questions need to be answered:-

1. Is pituitary ablation as effective as adrenal ablation and inhibition with fewer complications?

2. Has the advent of cytotoxic chemotherapy obviated the need for further consideration of secondary endocrine therapy?

3. Should adrenal inhibitors ever be combined with conventional endocrine therapy as primary treatment (e.g. orchidectomy and cyproterone acetate as proposed by Bracci in 1975) (21)?

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CARCINOMA OF THE PROSTATE - ADRENALECTOMY AND HYPOPHYSECTOMY

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Today we must ask ourselves whatever happened to adrenalectomy and hypophysectomy for carcinoma of the prostate. Those of us who are old enough, will remember the busy days, 20 years ago, when we took adrenals out for carcinoma of the breast and prostate, and when that did not work, we would refer the patient for removal of the pituitary by one method or another. Today, we have altogether stopped taking out the adrenals, and we refer the patient for hypophysectomy less and less often. But it is timely to think why, especially since there appears to be a new vogue of treatment by 'medical adrenalectomy' about to be launched upon us.

Adrenalectomy was introduced by Huggins and Scott in 1945 (1) with the intention of removing the extratesticular androgens that were thought to be keeping the prostate cancer cells stimulated. It was found that relapse of the disease was mirrored by the rise in urinary 17-ketosteroids and that this could be diminished by bilateral adrenalectomy (2). Occasionally there were quite remarkable cures. Most of us can remember cases such as:-

> M.J. aged 61. Relapse of carcinoma of prostate after previous stilboestrol and orchidectomy 1958. Bilateral adrenalectomy 1958. Complete relief of pain. Equivocal changes in x-rays. Back at work until 1967. Readmitted with carcinoma of sigmoid colon. Anterior resection, liver found full of metastases. Died 1968.

Equally, when the operation was performed on a large scale, all surgeons agreed in the finding that about 50% had a subjective relief of pain - often quite dramatic. In Murphy's review (3) of his own experience and that from the literature, he noted subjective improve-

ment that was "good" or was maintained for longer than 3 months in 58 out of 126 patients (46%). But the snag was that the objective response was seldom so convincing: indeed in the same series only 6 (5%) were thought to have objective evidence of healing of metastases. Moreover the improvement in symptoms, however subjective, and however suspect to objective criteria - was never long-lived. In Murphy's experience (3) the survival in 26 such patients was, on average, between 6.8 and 20.8 months.

The failure of adrenalectomy in practice to produce a lasting result made us turn away from an operation that was not without morbidity in the old men who had to undergo it. It seemed as if we could obtain just as good results by giving them cortisone, without the discomfort and morbidity of surgery. We were reinforced in our reluctance to undertake the operation when laboratory workers discovered that after castration the adrenal glands only produced a tiny fraction, some 0 - 3%,of the normal testosterone production. It hardly seemed worth while removing the adrenals to remove this tiny additional source of stimulation to the cancer which had probably in any event escaped from hormonal control.

And so we turned to hypophysectomy. This seemed more promising, once our colleagues in neurosurgery had developed a technique for removing the hypophysis either by transnasal introduction of Yttrium rods, or the cryosurgical probe, or with the combined transethmoid-transsphenoid approach. Hypophysectomy would remove the source of adrenal stimulation and decrease the supply of prolactin which was believed to assist the prostate in its handling of androgen. The procedure was introduced for the prostate by Scott in 1952 (4) and at first the operative and post operative complications were so great as to make the procedure unjustifiable. When techniques were simplified, and as a method for measuring whether or not the hypophysectomy had been completely done was developed - the measurement of the rise in growth hormone output to a hypoglycaemic challenge - the operation won itself an established position as a useful measure. It was specially useful in controlling pain: here it was quite remarkable. Most series, whatever the technique used to ablate the pituitary, claimed a 70% incidence of pain relief. At the same time, it was disappointingly short-lived; survival after hypophysectomy being measured in months, not in years. The mean survival reported by Murphy in 27 patients (3) was 4.8 months and the longest survival was 13.6 months.

In our hospital such patients have been referred to A.W. Morrison, an otorhinolaryngologist, who uses the combined transnasal transethmoid approach and has allowed me to quote his unpublished figures from a much larger series of hypophysectomy, most of which were performed for carcinoma of the breast. There were 21 patients. There was no operative mortality. All experienced subjective relief of pain, and there were no cases of blindness. Three patients

needed treatment for meningitis and in 2 there were persistent CSF leaks that required a second operation. There was however no suggestion in his series that life was prolonged, even though the relief of pain often lasted for up to a year. The pain relief was so striking that one patient at least rose from his bed in his euphoria only to sustain a pathological fracture.

In my own practice hypophysectomy is still requested for the occasional patient, otherwise reasonably well, and with a reasonable life-expectancy, who is in uncontrollable pain despite appropriate radiotherapy and orchidectomy. Given a trustworthy technique, relief of pain is almost certain even if it may not last very long. Other methods of obtaining pituitary ablation, such as the transnasal introduction of Yttrium, have been equally successful (5).

As with adrenalectomy, so with ablation of the pituitary experience forces us to ask the question - why do these patients experience such striking, and often such sudden relief of pain? And in the context of adrenalectomy more specially, we must ask why the occasional patient has experienced a prolongation of life. If we could find out more about the answers to these 2 questions we might find it easier to be enthusiastic about techniques that offer medical adrenalectomy with newer agents that try to improve upon the effects of cortisone and aminoglutethimide.

We must not forget these 2 procedures, obsolete though they may have become, for they hold out a hope for the future. It is possible (and laboratory studies already suggest that it may be feasible) that we may be able to re-educate those wild clones that have abandoned their responsiveness to oestrogens and their dependence upon androgens. If this proves to be so, then we may need to look again at the timing of the use of chemotherapy and hormone treatment: if chemotherapy can convert anaplastic clones back towards their parent tissue - and it seems that it sometimes can - then perhaps in the future we shall reverse the order of battle, start off with chemotherapy, and follow with stilboestrol, orchidectomy and perhaps - who can say - even with adrenalectomy and hypophysectomy again.

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ESTRAMUSTINE AND PREDNIMUSTINE

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Estramustine and prednimustine are both hormone-cytostatic agents where a steroid hormone compound known to have antitumour effect has been linked to an alkylating agent.

ESTRAMUSTINE

The intention with estramustine was to carry the cytostatic agent to the target organ where the drug is accumulated due to the action of specific receptors. Originally estramustine was considered for the treatment of mammary carcinoma but its main use has been in cancer of the prostate. Estramustine phosphate or estracyt is an estradiol phosphate which is esterified with a nor nitrogen mustard derivative in the form of a carbamate. In recent years there has been much discussion of estramustine. This presentation will not report details but will give an updated review.

Estramustine strongly inhibits the pituitary production of gonadotrophic hormones and in vitro studies have shown that it also has a local effect on the prostate. Høisaeter (1) demonstrated that estramustine phosphate inhibits DNA synthesis in the ventral rat prostate in vitro and induces cell degeneration of the ventral rat prostate in organ culture. He also showed that conversion of testosterone to dihydrotestosterone by 5α- reductase is inhibited by estramustine phosphate (2).

Recently Hartley-Asp and Gunnarsson (3) studied in tissue culture the effect of estramustine on a cell line of human prostate cancer that was lacking androgen and estrogen receptor and unresponsive to hormone stimulation either by testosterone or estradiol. Exposure of the cells to estramustine caused inhibition of cell

growth and cell destruction which was dependent on dose and on exposure time. The inhibitor effect of estramustine was higher than that of the nor nitrogen mustard moiety indicating that the unmetabolized compound has the strongest cytotoxic effect. It could also be shown that the effect of estramustine is due to interactions between estramustine and intracellular cell components.

A direct cytotoxic effect of estramustine on human prostate cancer in vivo was reported by Osafune et al in 1978 (4) who treated a 46 year old man with poorly differentiated prostatic cancer with estramustine and subsequently performed a total prostatectomy. The tumour was found to be necrotic. In the remaining part of the prostate there were hormonally induced changes but no necrosis.

Initially estramustine was mainly used in advanced prostate cancer, resistant to ordinary hormone therapy but in recent years it has been evaluated even in other patient categories. Table 1 gives a survey of the effect of estramustine phosphate therapy in 868 patients reported in the literature. Of 597 patients resistant to previous hormone therapy, 48% responded favourably. In those 271 cases where estramustine was given as the initial treatment there was a good effect in 84%.

Since estramustine has a good effect even in a number of cases resistant to ordinary hormone treatment we decided to investigate whether the drug, when given as initial therapy, would be more effective than the ordinary estrogenic regime. We performed a prospective randomized multi-centre trial in the Stockholm area in which all patients with well and moderately well differentiated prostate carcinoma and needing treatment were given either estramustine or our conventional estrogenic regimen, polyestradiol phosphate + ethinyl estradiol. Patients with poorly differentiated tumours were excluded from this trial as they were involved in another study.

Table 1. Clinical Results of Estracyt in Prostatic Carcinoma.

Route of Administration	Previous Estrogen Therapy		Previously Untreated		Total	
	No. of Patients	Positive Responses	No. of Patients	Positive Responses	No. of Patients	Positive Responses
Intravenous	252)		45)		297)	
	)	288	)	228	)	516
Oral	345)		226)		571)	
Total	597	48%	271	84%	868	59%

Of 182 patients observed for two years or more, 88 received estramustine and 94 the standard hormonal regimen. In both groups reduction of the local tumour occurred in about 80% of the cases and elevated acid phosphatase activity as a rule was reduced to normal. There was no significant difference between the two groups with respect to the duration of remission, incidence of cytologic remission and the frequency of cardiovascular and other complications.

Patients with advanced and poorly differentiated prostatic carcinoma were treated with estramustine phosphate in a non-randomized trial. Of 90 patients, 76 had verified metastases. In 73 cases the disease was progressive in spite of previous hormone and/or irradiation therapy and in 17 cases estramustine phosphate was given as primary therapy (Table 2). In Table 3 it is seen that the treatment had better effect in those patients given no previous hormone therapy. However, even some estrogen-resistant cases had benefit from estramustine.

Leistenschneider and Nagel (5) performed a randomized study of estramustine in poorly differentiated prostate carcinoma, previously untreated. Ten patients were given estramustine and ten ordinary estrogenic hormones. Following 3-6 months observation, cytologic remission was observed in seven of ten cases on estramustine and one of ten cases on conventional estrogen medication.

From the findings mentioned above we have concluded that in the primary treatment of well and moderately well differentiated prostate carcinoma, estramustine has no advantage over ordinary hormone therapy. On the other hand the drug has a favourable effect in a number of hormone resistant cases. There is also evidence that estramustine is superior to conventional estrogen therapy in disseminated poorly differentiated prostate cancer and should probably be the treatment of first choice in this category of patients.

Table 2. Estramustine Therapy in 90 Cases of Poorly Differentiated Prostate Carcinoma.

Previous estrogenic therapy (estrogen + irradiation 39)	64
Previous irradiation	9
Previously untreated	17

Table 3. Effect of Estramustine Phosphate in Patients With Poorly Differentiated Prostate Carcinoma Who Had Previous Estrogen Therapy, Compared With the Effect in Patients Given No Previous Hormone Therapy (Improved/Number of Cases Observed).

	Previous Estrogen	No Previous Estrogen
Regression of local tumour	8/16	11/14
Cytologic remission	12/22	5/8
Reduction of soft tissue lesions	5/8	5/7
Reduction of bone tissue lesions	3/28	2/14
Reduction of elevated acid phosphatase activity	10/29	8/14

(This table only includes those cases with evaluation of the respective parameter both before and after treatment)

PREDNIMUSTINE

Prednimustine is another antitumour drug where a steroid hormone group is linked with a nitrogen mustard derivative. In 1954 Valk and Owens (6) reported that high dose corticosteroid therapy provides palliation in many cases of advanced prostate carcinoma and such therapy has since been frequently used in the treatment of this disease. The antitumour effect of corticosteroid compounds is incompletely elucidated but it is believed that they affect cell division by inhibiting the number of cells entering the S-phase.

Prednimustine is a chlorambucil ester of prednisolone. In experimental tumour systems the drug was found to have a high therapeutic index. The drug has been used mainly in hematological diseases, mammary carcinoma and carcinoma of the ovary but has also been evaluated in carcinoma of the prostate in the United States by members of the National Prostatic Cancer Project (NPCP).

In NPCP protocol 400 patients who were progressing despite hormonal therapy and pelvic irradiation were randomized to receive either the combination of estramustine and prednimustine or to prednimustine alone. If prednimustine was ineffective they were crossed over to estramustine. Although objective response was minimal and approximately equal in both groups there was a relatively good subjective response in each group with pain relief in 34% and improved performance status in 39% (7).

Prednimustine has a certain antitumour effect but does not appear to be superior to estramustine. It may be worth trying in cases where the tumour does not respond to estramustine. Its main indication, however, is in malignant disease of the hematopoietic system.

SUMMARY

Estramustine and prednimustine are hormone-cytostatic agents with antitumour properties. Both drugs have a low myelotoxic effect. Estramustine phosphate has two-fold effect in prostate cancer, inhibition of gonadotropin production and, at least in certain cases, a local effect in the prostate by inhibiting cell growth. A number of hormone resistant patients have responded favourably to estramustine therapy. The drug has also been found useful in disseminated and poorly differentiated prostate cancer. So far there is limited experience of prednimustine therapy in prostate cancer. The main indication of the latter drug is malignant disease of the hematopoietic system.

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Editorial Note (P.H.S.)

The 868 patients mentioned in Table 1 represent cumulative figures from the literature and as Professor Andersson has observed 48% previously treated with estrogens responded favourably.

Unfortunately the criteria of response in the 51 publications from which these patients come are not always clearly defined and in the absence of the accompanying references it is difficult to draw firm conclusions. The data in Table 3 refer to the 90 patients with poorly differentiated cancer treated in a non-randomized study. Unfortunately not all patients were evaluated with respect to all parameters before and after treatment. This accounts for the relatively small numbers for which observations are available.

PEPLEOMYCIN, INTERFERON AND ESTRACYT IN THE TREATMENT OF POORLY DIFFERENTIATED PROSTATIC CARCINOMA

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PEPLEOMYCIN

An investigation using Pepleomycin in the treatment of prostatic carcinoma was reported at the WHO centre meeting on prostatic carcinoma in March 1979 in Stockholm (1). Some degree of objective and subjective improvement was observed in a series of six patients with stage C and D tumors. Side effects including gastro-intestinal disturbances, fever and dermatological symptoms were noted.

A pilot study has also been performed in Stockholm, including 12 patients with poorly differentiated prostatic carcinomas, initially treated by a full course of irradiation to the local tumor. In seven patients hormone therapy had been given after the finding of persistent cancer cells in the local tumor. When this therapy failed, Pepleomycin was given in a dose of 5 mg i.m., three times weekly to a total dose of 90 - 200 mg. In the remaining five patients Pepleomycin was given immediately after the irradiation therapy failed, without any hormone therapy.

RESULTS

In eight patients the serum acid phosphatases levels were raised before Pepleomycin was given. In four of these, they returned to normal, in one the acid phosphatases level remained high and in the other three patients the levels increased markedly. A decrease in the size of the prostate was observed in four patients, no change in three and an increase in size in one. In three patients who received 200 mg of Pepleomycin, the cytological smear showed regressive cells compared with the smear prior to therapy.

The duration of remissions was from two to ten months (mean five months) but most of the patients have ongoing therapy.

Subjective response with pain relief was observed in four of five patients with pain before the therapy. The remaining patient had increasing pain during treatment.

The side effects of Pepleomycin included loss of hair and skin changes in all and fever after the injections in six patients. No lung toxicity was observed.

INTERFERON

Interferon has been given to three patients with disseminated poorly differentiated prostatic carcinomas. All were previously untreated.

Daily doses of 3 million I.U. of Interferon were given and treatment has now continued for 6, 7, and 9 months.

The preliminary results indicate a stabilization of the disease with no progression of the bone metastases. All have had total pain relief and no infection of any kind has been observed during the treatment.

Cytological smears taken after 1, 3, 6 and 9 months are unchanged. The serum acid phosphatase levels are all above normal.

One patient with a bulky tumor of the head had an almost complete remission (nine months), his neurological symptoms have disappeared, his weight has increased and he feels better in himself.

RANDOMIZED TRIAL WITH INTERFERON, PEPLEOMYCIN AND ESTRACYT

In patients with poorly differentiated prostatic carcinoma, verified with fine needle aspiration biopsy, a phase III study has been started to compare Interferon vs Pepleomycin vs Estracyt. All patients have disseminated tumors, verified with scintigraphy, skeletal X-rays and computerized tomography. An increased serum acid phosphatase is not regarded as an indication of tumor spread. Immunological tests are performed during the treatment.

TREATMENT

Pepleomycin is given at a dose of 5 mg i.m. three times weekly to a total dose of 150 mg. Interferon is given in daily doses of 3 million I.U. for at least three months and Estracyt 280 mg is given orally twice daily for an unlimited time period.

So far only 12 patients have been randomized - five to Estracyt, four to Pepleomycin and three to Interferon.

In this preliminary report it is only possible to observe that the serum acid phosphatase level has become normal in the group of patients treated with Estracyt but is unchanged in the other two groups and that prostatic size has decreased slightly in patients receiving Pepleomycin and Interferon but markedly in those receiving Estracyt. Pain relief has been observed in all three groups.

REFERENCES

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Editorial Note - P.H.S.

The information on the activity of Pepleomycin in prostatic cancer is limited and that on Interferon is even more so. The introduction of a randomized study at this time seems premature. Treatment is limited to three months in those receiving Pepleomycin. This is a very short period of time in a patient with cancer of the prostate and will make assessment of objective response a difficult task.

CHEMOTHERAPY OF PROSTATE CANCER: THE NATIONAL PROSTATIC CANCER PROJECT EXPERIENCE

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USA

ABSTRACT

Since 1972 the Treatment Subgroup of the National Prostatic Cancer Project has accessioned 1748 patients with various stages of prostatic cancer to 13 different treatment protocols. Seven of these protocols have been closed and the results in the initial studies have been published. Six protocols are active and continue to accrue patients. Principal investigators have a prominent role in protocol design as well as in the evaluation and publication of data emanating from these protocols. Active agents identified to date include cyclophosphamide, 5-fluorouracil, methyl-CCNU, imidazole-carboxamide, estramustine phosphate and streptozotocin. Agents chosen for their presumed activity, but currently under investigation, include methotrexate and cis-platinum.

ORGANIZATION AND RESPONSIBILITIES

The Treatment Subgroup of the National Prostatic Cancer Project was organized in 1972. The original membership consisted of five institutions (M.D. Anderson Hospital and Tumor Clinic, University of Iowa, the Virginia Mason Clinic, Massachusetts General Hospital and the Johns Hopkins Hospital) whose representatives agreed to design protocols directed at the treatment of specified groups of patients with prostatic cancer, accession patients into such protocols as well as share the results of such clinical evaluations. In the nine years since its inception, the Treatment Subgroup has been expanded by the addition of several institutions either on active or provisional status. Only one institution is no longer active in the Subgroup.

Table 1. United States National Prostatic Cancer Project

Director - Gerald P. Murphy, M.D., D. Sc.

Treatment Subgroup Members

Institution	Principal Investigator
M.D. Anderson*	Douglas Johnson, M.D.
University of Iowa	Stefan Loening, M.D.
Mason Clinic	Robert Gibbons, M.D.
Massachusetts General Hospital	George Prout, Jr., M.D.
Johns Hopkins	William Scott, Ph.D., M.D.
University of Tennessee	Mark Soloway, M.D.
University of California, San Diego	Joseph Schmidt, M.D.
Tulane University	Stuart Bergman, M.D.
Wayne State University	James Pierce, M.D.
Roswell Park	Sunmolu Beckley, M.D.
Baylor College of Medicine	Peter Scardino, M.D.
Walter Reed**	David McLeod, M.D.
Rush-Presbyterian-St. Luke's**	Charles McKiel, Jr., M.D.
University of California, Los Angeles**	Jean De Kernion, M.D.

* No longer active member

** Provisional status

Table 2. United States National Prostatic Cancer Project Protocol Chairmen

Protocol Number	Principal Investigator
100	Dr. William W. Scott
200	Dr. William W. Scott
300	Dr. Joseph D. Schmidt
400	Dr. Gerald P. Murphy
500	Dr. Joseph D. Schmidt
600	Dr. Robert P. Gibbons
700	Dr. Stefan A. Loening
800	Dr. Mark S. Soloway
900	Dr. Joseph D. Schmidt
1000	Dr. Robert P. Gibbons
1100	Dr. Stefan A. Loening
1200	Dr. Mark S. Soloway
1300	Dr. J. Edson Pontes

The professional and scientific activities of each member institution's participation in the clinical trials is the responsibility of the principal investigator (Table 1). Each principal investigator heads his institutional team for screening, accession, treatment and evaluation of patients with prostatic cancer. Although there is some variation among institutions, this team consists of the principal investigator, one or more co-investigators, a research assistant or project coordinator, an oncology nurse and either a urology fellow or resident.

Again starting in 1972, responsibility for each specific protocol was assigned to one individual generally from among the principal investigators (Table 2). Each Protocol chairman has responsibility not only for the design of the protocol but for evaluation of data collected regarding efficacy and toxicity of agents, presentation of such data at national meetings and eventual

publication of the data in urology or cancer-related journals.

SPECIFIC PROTOCOLS

The initial studies executed by the Treatment Subgroup were protocols 100 and 200 (Figures 1 and 2). Protocol 100 was designed to study two single agents, cyclophosphamide and 5-fluorouracil, compared to standard or conventional treatment in patients with relapsing advanced prostatic cancer who had not had excessive prior radiotherapy to the lumbosacral spine and/or pelvis (1). On the other hand, Protocol 200 was designed to study two single agents, estramustine phosphate and streptozotocin, each with minimal myelosuppression, compared to standard therapy in patients with stage D prostatic cancer who also had relapsed to endocrine manipulation but who had extensive irradiation therapy (2).

As data on toxicity and response rates were accumulated, active agents were selected from existing protocols and then used as indicator agents for successive protocols. Thus Protocol 100 was succeeded first by Protocol 300 (figure 3) which in turn was followed by protocols 700 and 1100 (Figures 7 and 11) (3,4). Again, the common denominator for these clinical trials has been the patients with metastatic prostatic cancer who have not had extensive irradiation therapy but who have failed endocrine manipulation.

Similarly, Protocol 200 was succeeded by Protocol 400 (Figure 4) which in turn was succeeded by Protocol 800 and later Protocol 1200 (Figures 8 and 12) (5,6). All patients entered into this sequence of studies had relapsed to endocrine manipulation and had had extensive irradiation.

In 1976 because of the results in the protocols extant, the Treatment Subgroup initiated a study of chemotherapy and conventional endocrine manipulation in newly diagnosed patients with stage D disease (7). These patients had a common background of having had no prior hormonal manipulation. Protocol 500 (Figure 5) was the prototype. This study has recently been closed to patient entry and has been succeeded by Protocol 1300 (Figure 13). At about the same time, the Treatment Subgroup initiated a study of hormonal therapy and chemotherapy in patients with stage D prostatic cancer who had been considered stable on diethylstilbestrol (DES) therapy, 1 mg t.i.d. for a minimum of three months. Protocol 600 (Figure 6) is still open.

In 1978 the Treatment Subgroup, again based on the efficacy and acceptable toxicities of agents used in patients with advanced disease, embarked on studies evaluating adjuvant chemotherapy, either with cyclophosphamide or estramustine phosphate, in patients with earlier and potentially curable prostatic cancer. Protocol 900

(Figure 9) has been designed for patients having their primary disease treated either with radical surgery alone, with radionuclide implantation or with cryosurgery. Protocol 1000 (Figure 10) employs the same chemotherapeutic agents but in patients who have been

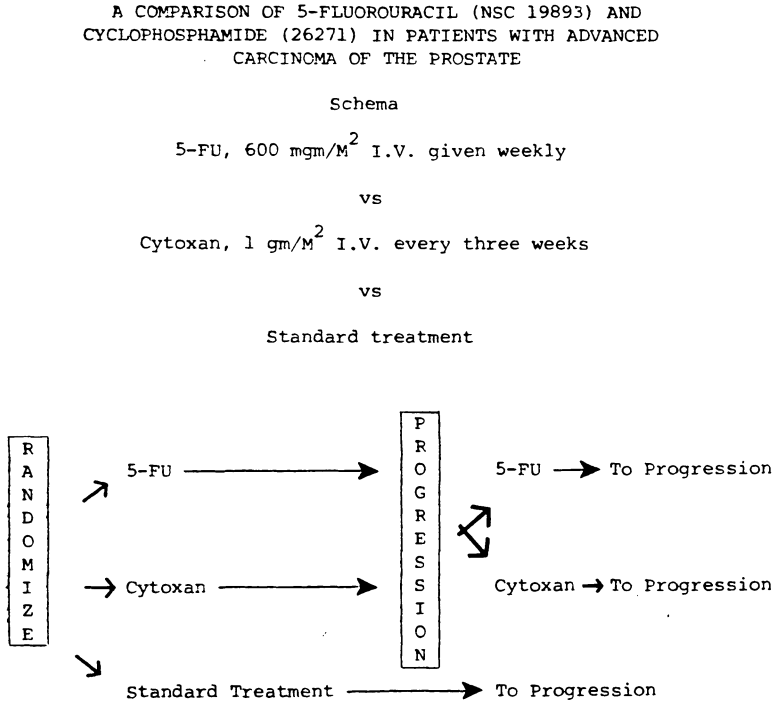

Fig. 1. National Prostatic Cancer Project. Protocol 100.

treated primarily with external beam radiotherapy either to the prostate alone or to the prostate and the pelvis as based on either preliminary pelvic lymphadenectomy or lymphangiogram followed by needle aspiration biopsy. Both protocols 900 and 1000 continue to accession patients.

A COMPARISON OF ESTRACYT AND STREPTOZOTOCIN (NSC 85998)
IN PATIENTS WITH ADVANCED CARCINOMA OF THE PROSTATE
WHO HAVE HAD EXTENSIVE IRRADIATION

Schema

Estracyt, 600 mg/M^2 p.o. daily in
three divided doses

vs

Streptozotocin 500 mg/M^2 I.V. daily
for five days every six weeks

vs

Standard therapy

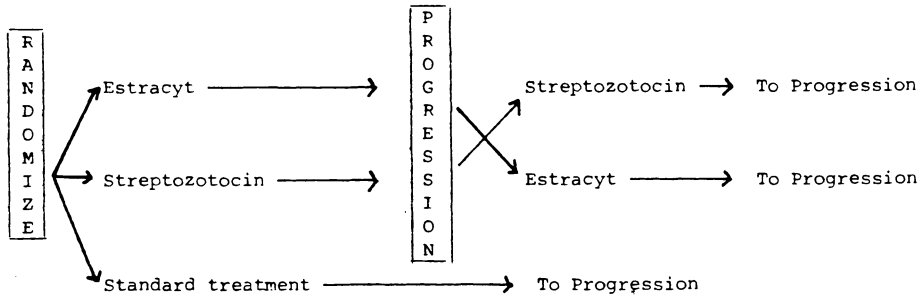

Fig. 2. National Prostatic Cancer Project. Protocol 200.

A COMPARISON OF PROCARBAZINE (NSC 77213), DTIC (5- (3,3 DIMETHYL-1-TRIAZENO) IMIDAZOLE-4-CARBOXAMIDE) (NSC 45388) AND CYCLOPHOSPHAMIDE (NSC 26271) IN PATIENTS WITH ADVANCED CARCINOMA OF THE PROSTATE

Schema

DTIC, 200 mg/M^2 I.V. days 1-5, 21 days rest, then repeat the cycle

vs

Procarbazine, 100 mg/M^2 p.o. daily days 1-22, 3 weeks rest. Rx days 44-65, 3 weeks rest, etc.

vs

Cytoxan, 1 gm/M^2 I.V. every three weeks

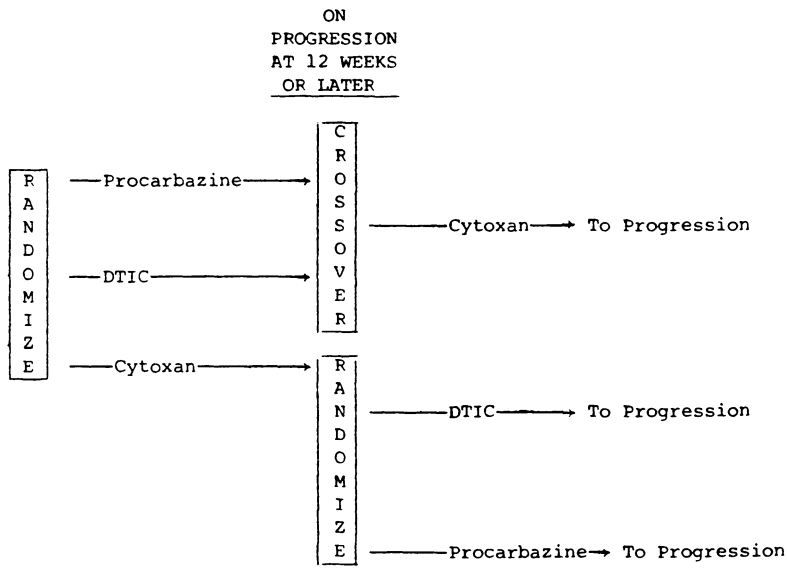

Fig. 3. National Prostatic Cancer Project. Protocol 300.

A COMPARISON OF ESTRACYT PLUS LEO 1031 (NSC-134087)
AND LEO 1031 ALONE IN PATIENTS WITH ADVANCED CARCINOMA
OF THE PROSTATE WHO HAVE HAD EXTENSIVE IRRADIATION

Schema

Estracyt, 600 mg/M^2 p.o. daily in three divided doses + LEO 1031, 24 mg/day (total dose) p.o. in three divided doses

vs

LEO 1031, 24 mg/day (total dose) in three divided doses

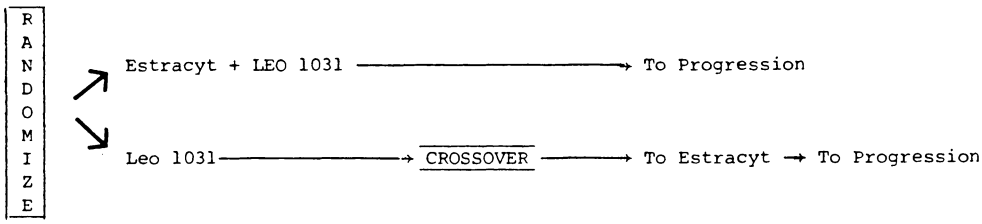

Fig. 4. National Prostatic Cancer Project. Protocol 400.

A COMPARISON OF CYCLOPHOSPHAMIDE (NSC-26271) PLUS ESTRACYT (NSC-89199) VS CYCLOPHOSPHAMIDE (NSC-26271) PLUS DIETHYLSTILBESTROL VS DIETHYLSTILBESTROL OR ORCHIECTOMY IN NEWLY DIAGNOSED PATIENTS WITH CLINICAL STAGE D CANCER OF THE PROSTATE WHO HAVE NOT HAD PRIOR HORMONAL THERAPY

Schema

Cytoxan, 1 gm/M^2 I.V. every three weeks
plus
Estracyt, 600 mg/M^2 p.o. daily in three divided doses

vs

Cytoxan, 1 gm/M^2 I.V. every three weeks
plus
Diethylstilbestrol, 1 mg t.i.d. p.o.

vs

Diethylstilbestrol, 1 mg t.i.d. p.o.
or
Orchiectomy
(Investigator's Option)

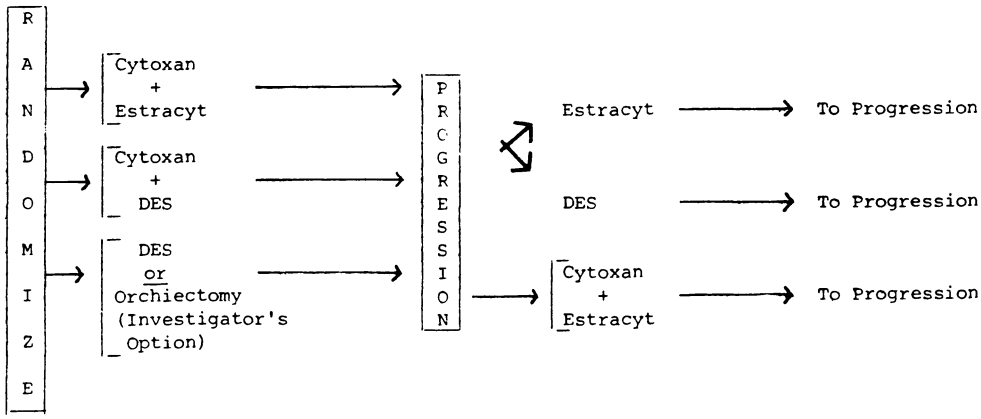

Fig. 5. National Prostatic Cancer Project. Protocol 500.

A COMPARISON OF COMBINATION CHEMOTHERAPY-HORMONAL THERAPY WITH HORMONAL THERAPY ALONE IN PATIENTS WITH CLINICAL STAGE D PROSTATIC CARCINOMA

Schema

Diethylstilbestrol, 1 mg t.i.d. p.o.

vs

Diethylstilbestrol, 1 mg t.i.d. p.o.
plus
Cytoxan, 1 gm/M^2 I.V. every three weeks

vs

Diethylstilbestrol, 1 mg t.i.d. p.o.
plus
Estracyt, 600 mg/M^2 p.o. daily in three divided doses

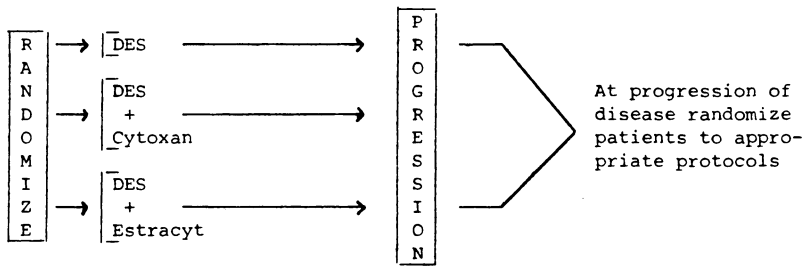

Fig. 6. National Prostatic Cancer Project. Protocol 600.

A COMPARISON OF HYDROXYUREA (NSC 32065), METHYLCCNU (NSC 95441), AND CYCLOPHOSPHAMIDE (NSC 26271) IN PATIENTS WITH ADVANCED CARCINOMA OF THE PROSTATE

Schema

Hydroxyurea, 3 mg/M^2 p.o. every three days in three divided doses

vs

MECCNU, 175 mg/M^2 p.o. every six weeks

vs

Cytoxan, 1 gm/M^2 I.V. every three weeks

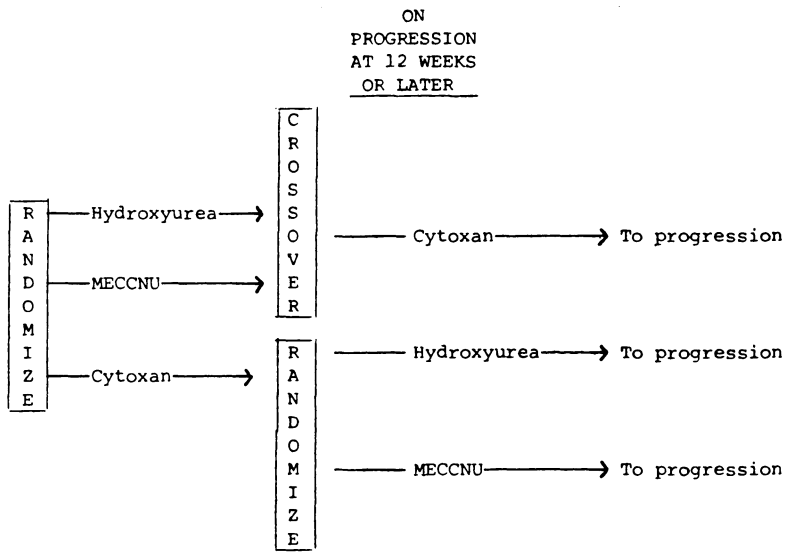

Fig. 7. National Prostatic Cancer Project. Protocol 700.

A COMPARISON OF ESTRACYT (NSC 89199), VINCRISTINE (NSC 67574)
AND ESTRACYT PLUS VINCRISTINE IN PATIENTS WITH
ADVANCED CARCINOMA OF THE PROSTATE WHO HAVE HAD EXTENSIVE IRRADIATION

Schema

Estracyt, 600 mg/M^2 p.o. daily in three divided doses

vs

Vincristine, 1 mg/M^2 (2 mg maximum dose) I.V. once every two weeks

vs

Estracyt, 600 mg/M^2 p.o. daily in three divided doses

plus

Vincristine, 1 mg/M^2 (2 mg maximum dose) I.V. once every two weeks

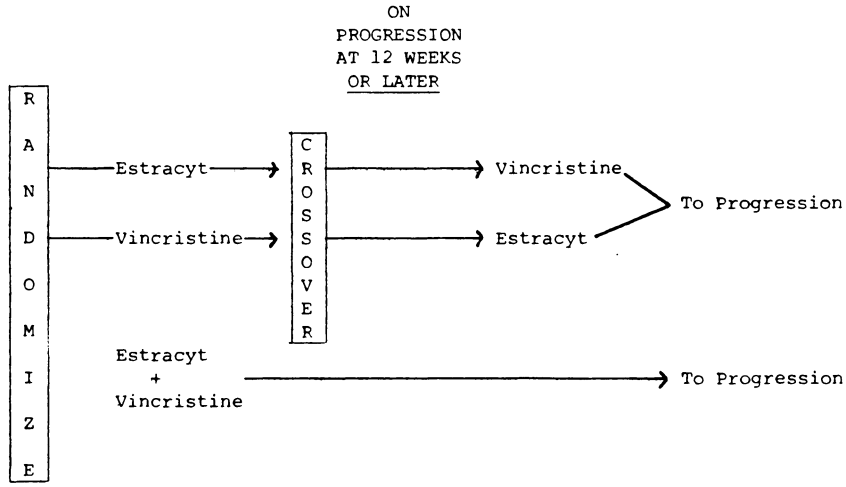

Fig. 8. National Prostatic Cancer Project. Protocol 800.

A COMPARISON OF LONG-TERM ADJUVANT CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE (NSC 26271), ESTRACYT (NSC 89199), OR NO-ADDITIONAL-TREATMENT IN PATIENTS WITH DEFINITIVE SURGICAL TREATMENT FOR ADENOCARCINOMA OF THE PROSTATE

Schema

Prior total prostatectomy alone or with ^{198}Au seed implants or cryosurgery and lymph node evaluation by pelvic lymph node dissection or lymphangiogram with fine needle biopsy followed by randomization for adjuvant chemotherapy to receive either:

Estracyt, 600 mg/M^2 p.o. daily in three divided doses

or

Cyclophosphamide, 1 gm/M^2 I.V. every three weeks

or

No treatment

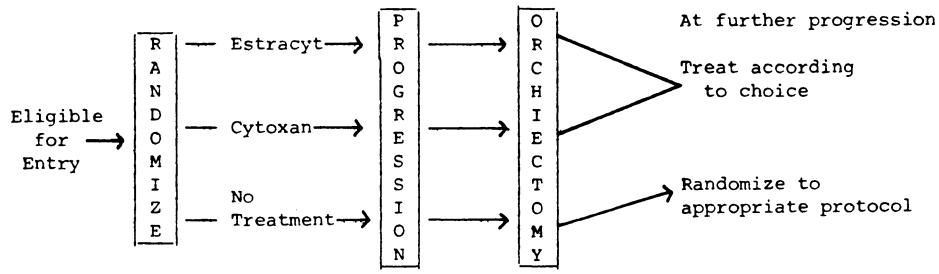

Fig. 9. National Prostatic Cancer Project. Protocol 900.

A COMPARISON OF LONG-TERM ADJUVANT CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE (NSC 26271), ESTRACYT (NSC 89199) OR NO-ADDITIONAL-TREATMENT IN PATIENTS WHO HAVE HAD DEFINITIVE EXTERNAL BEAM OR INTERSTITIAL RADIOTHERAPY FOR ADENOCARCINOMA OF THE PROSTATE

Schema

Prior definitive radiotherapy and lymph node evaluation by pelvic lymph node dissection or lymphangiogram with fine needle biopsy, followed by the randomized adjuvant chemotherapy to be either:

Estracyt, 600 mg/M^2 p.o. daily in three divided doses

or

Cyclophosphamide, 1 gm/M^2 I.V. every three weeks

or

No treatment

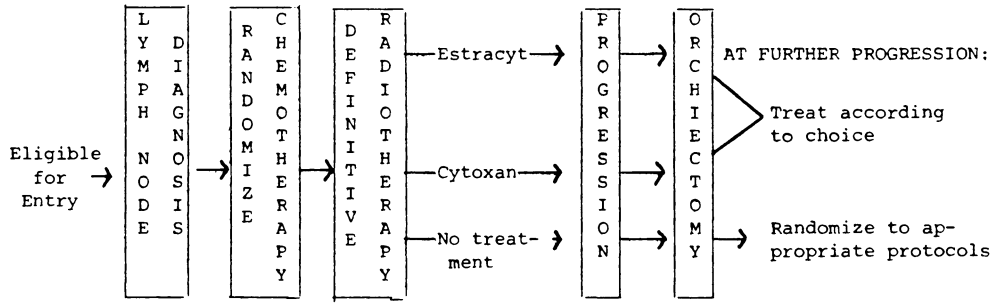

Fig. 10. National Prostatic Cancer Project. Protocol 1000.

A COMPARISON OF METHOTREXATE (NSC-740), CIS-DIAMMINEDICHLOROPLATINUM (II-NSC-119875), AND ESTRACYT (NSC-89199) IN PATIENTS WITH ADVANCED CARCINOMA OF THE PROSTATE

Schema

Methotrexate, 40 mg/M^2 I.V. on day 1,
60 mg/M^2 I.V. on day 8, q 7 days thereafter

vs

DDP - prehydrate the patient over 60 minutes prior to DDP with 500 ml of 5% dextrose/½ normal saline (D5/½NS). Place 60 mg/M^2 of DDP in 100 ml D5/½NS for I.V. infusion over 15 minutes. Follow the DDP with 3 hours of I.V. infusion with 1000 ml of D5/½NS + 40 meq KCl + 37.5 gm mannitol. Give Compazine at 10 mg I.M. q 4 hours around the clock on the day of DDP therapy then q 4 hours PRN. DDP will be given on days 1, 4, 21, 24, and then once every month. Prevent exposure of DDP to light and do not bring DDP into direct contact with equipment containing aluminum.

vs

Estracyt, 600 mg/M^2 p.o. daily in three divided doses.

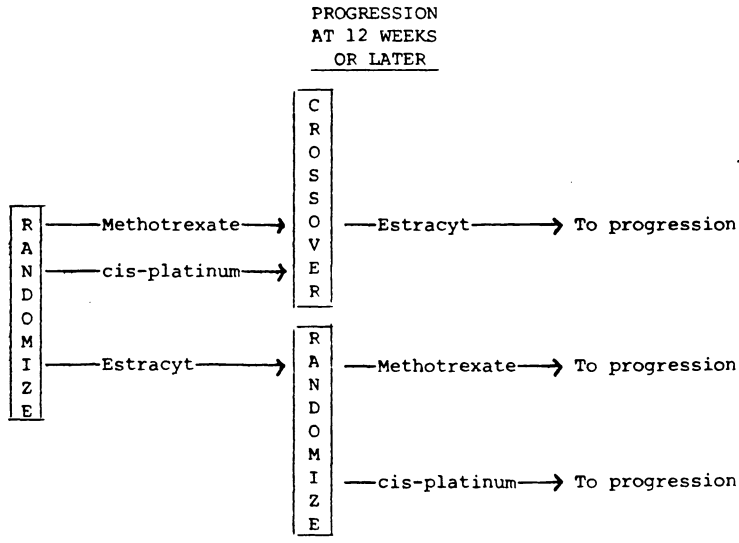

Fig. 11. National Prostatic Cancer Project. Protocol 1100.

A COMPARISON OF ESTRACYT (NSC 89199) VERSUS CIS-DIAMMINEDICHLOROPLATINUM (DDP) (II NSC 119875) VERSUS ESTRACYT PLUS DDP IN PATIENTS WITH ADVANCED CARCINOMA OF THE PROSTATE WHO HAVE HAD EXTENSIVE IRRADIATION TO THE PELVIS OR LUMBOSACRAL AREA

Schema

Estracyt, 600 mg/M^2 p.o. daily in three divided doses

vs

DDP - prehydrate the patient over 60 minutes prior to DDP with 500 ml of 5% dextrose/½ normal saline (D5/½NS). Place 60 mg/M^2 of DDP in 100 ml D5/½NS for I.V. infusion over 15 minutes. Follow the DDP with 3 hours of I.V. infusion with 1000 ml of D5/½NS + 40 meq KCl + 37.5 gm mannitol. Give Compazine at 10 mg I.M. q 4 hours around the clock on the day of DDP therapy then q 4 hours PRN. DDP will be given on days 1, 21 and then once every month. Prevent exposure of DDP to light and do not bring DDP into direct contact with equipment containing aluminum.

vs

Estracyt plus DDP, administer both agents as noted above.

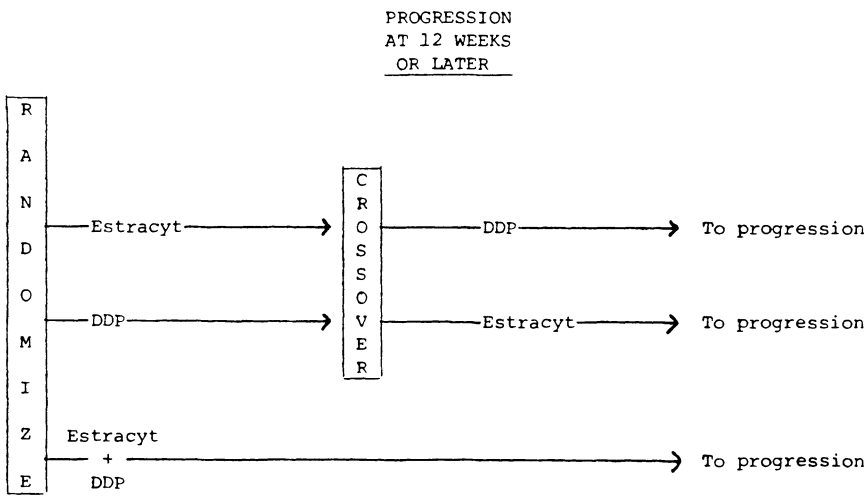

Fig. 12. National Prostatic Cancer Project. Protocol 1200.

A COMPARISON OF DIETHYLSTILBESTROL (DES) OR ORCHIECTOMY VS CYCLOPHOSPHAMIDE + 5-FLUOROURACIL (5-FU) + DES OR ORCHIECTOMY VS ESTRACYT ALONE IN NEWLY DIAGNOSED PATIENTS WITH CLINICAL STAGE D CANCER OF THE PROSTATE WHO HAVE NOT HAD PRIOR HORMONAL TREATMENT OR CHEMOTHERAPY

Schema

Diethylstilbestrol (DES), 1 mg t.i.d. p.o.
or orchiectomy

vs

Estracyt, 600 mg/M^2 p.o. daily

vs

DES, 1 mg t.i.d. p.o. or orchiectomy
plus
5-fluorouracil (5-FU), 350 mg/M^2 I.V. weekly
plus
Cytoxan, 650 mg/M^2 I.V. q 3 weeks

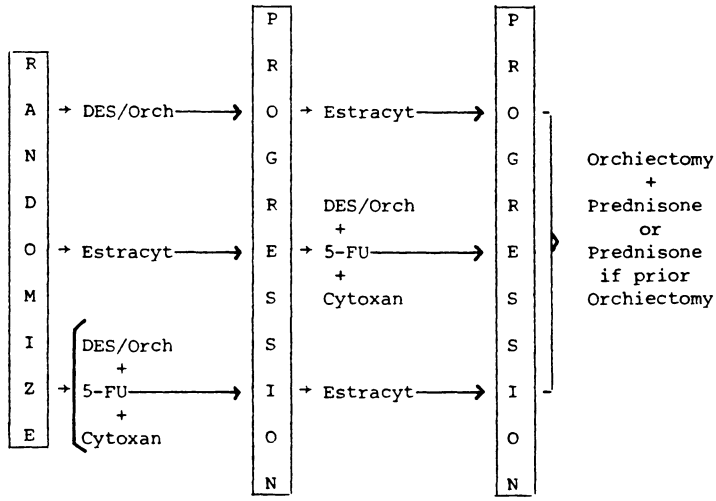

Fig. 13. National Prostatic Cancer Project. Protocol 1300.

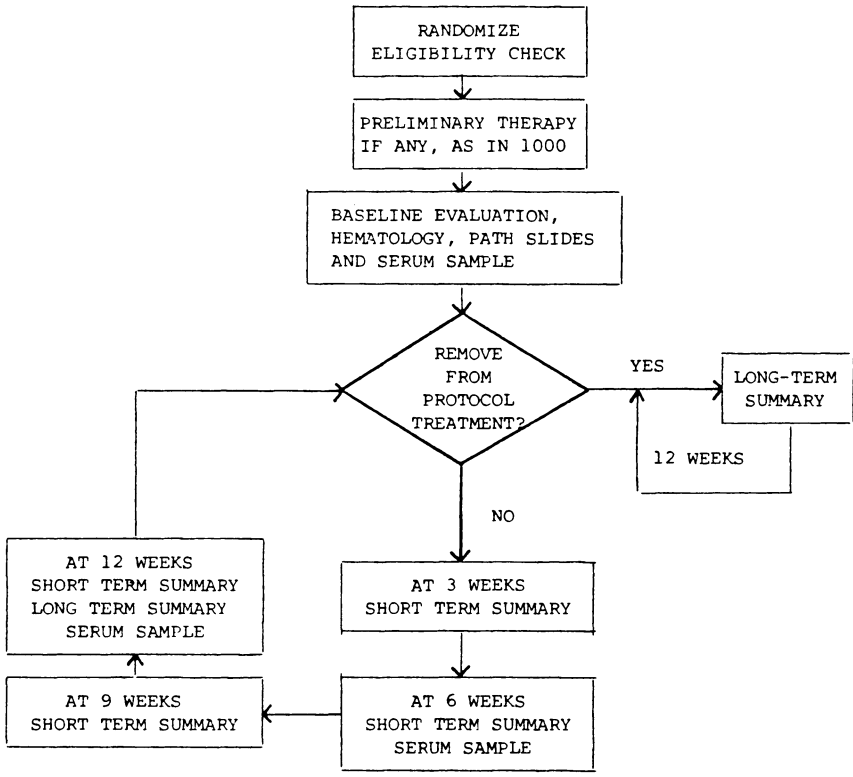

Fig. 14. National Prostatic Cancer Project. Information Flow Chart.

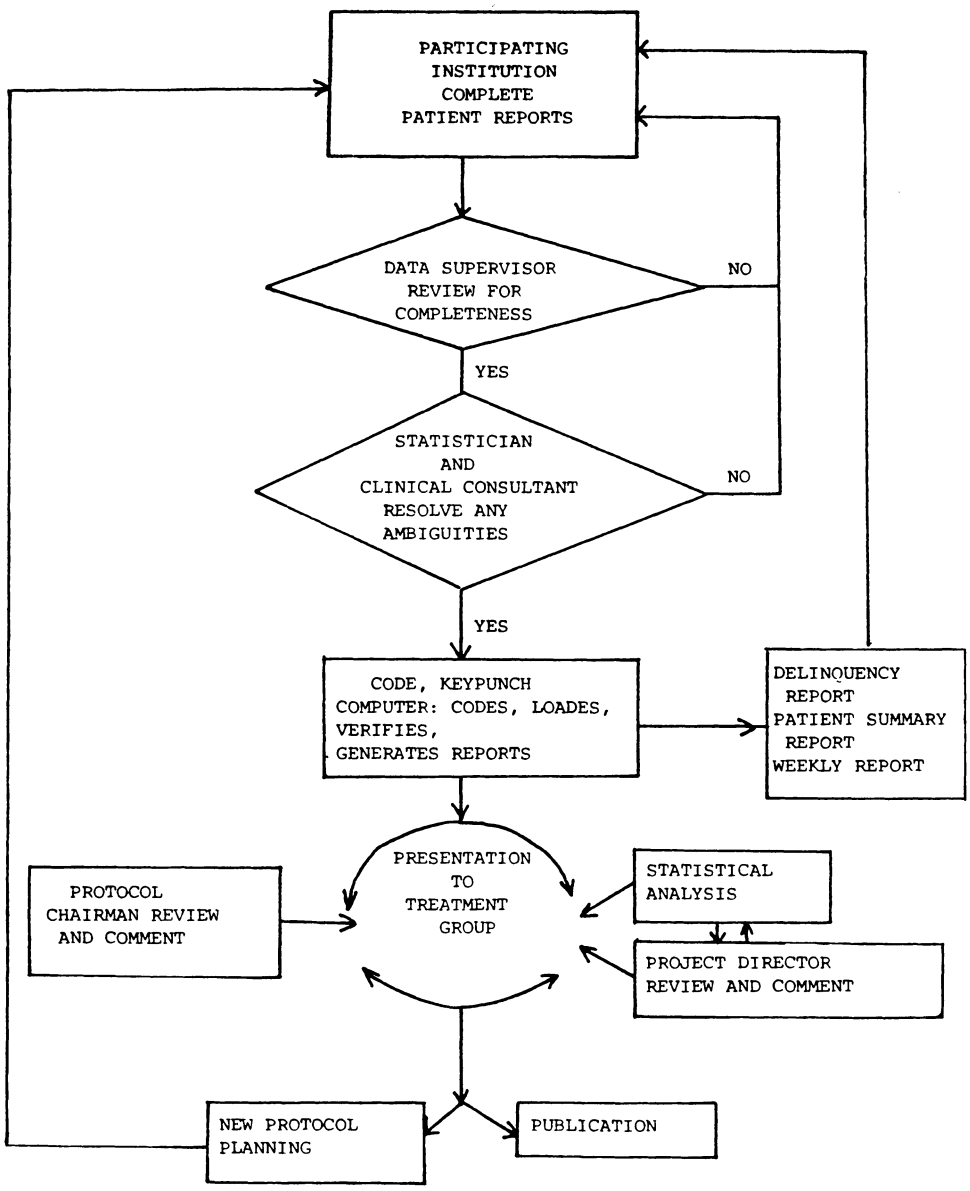

Fig. 15. National Prostatic Cancer Project Treatment Group Information Flow.

METHODS OF PROCEDURE AND PATIENT ACTIVITY

The information flow chart (Figure 14) diagrammatically summarizes each member institution's procedure in the accession, treatment and evaluation of patients in the various protocols. The information flow diagram in Figure 15 demonstrates the input of information into the headquarters statistical office as well as the flow of that data to the Subgroup and the specific protocol chairmen. The Treatment Subgroup meets regularly at least three times per year for presentation, discussion and evaluation of the data on existing protocols as well as to discuss the future clinical trials using chemotherapy. As of April, 1981, the Treatment Subgroup of the National Prostatic Cancer Project has accessioned a grand total of 1,748 patients. As seen in Table 3, this number includes 1097 patients entered into protocols now closed whereas 651 patients have been entered into active protocols (Table 4).

The current status of patients entered into the closed protocols is seen in Table 5. Eighty-one percent of this total are evaluable with only 4% considered protocol violations. The total exclusion rate in this group is 18%. Of this group, 12% are still receiving randomized therapy (either initial or crossover) whereas 22% are currently being followed.

The current status of patients entered into active protocols is seen in Table 6. Here the exclusion rate overall is 9% with protocol violations accounting for about one-half. The majority of patients entered into these protocols are either still receiving initial or crossover treatment and are being followed.

The current overall patient activity in the clinical trials program is seen in Table 7. It is apparent that approximately one-third of patients initially entered into Protocol 500 are still active, partly because of the protocol only being recently closed and partly because these patients were entered at an earlier phase in the natural history of their disease. The same is true for patients accessioned into protocols 900, 1000, and 1300.

RESULTS

Patients with advanced hormonally-refractory stage D prostatic cancer, treated with cyclophosphamide, 5-fluorouracil, methyl-CCNU or imidazole-carboxamide (DTIC) have demonstrated objective and subjective response rates higher than similar patients treated with standard therapy (8). Procarbazine and hydroxyurea were associated with increased toxicity yet response rates were no better than standard therapy. Agents currently under investigations in this category include methotrexate, estramustine phosphate and cis-platinum (Table 8).

Table 3. National Prostatic Cancer Project

Patient Entry for Each Participating Institution to Closed Protocols

	Protocol Number							
Institution	100	200	300	400	500	700	800	Total
M. D. Anderson	41	42	37	27	-	9	30	186
Univ. of Iowa	19	10	38	13	55	11	7	153
Mason Clinic	26	42	49	57	21	15	31	241
Mass. General	20	8	13	6	46	14	8	115
Johns Hopkins	19	23	22	18	15	22	13	132
Univ. Tennessee	-	-	2	10	28	22	8	70
U. C. San Diego	-	-	4	4	49	16	20	93
Tulane Medical	-	-	-	-	28	7	3	38
Wayne State	-	-	-	-	46	9	1	56
Roswell Park	-	-	-	-	6	-	-	6
Baylor College	-	-	-	-	6	-	-	6
Walter Reed	-	-	-	-	1	-	-	1
Total	125	125	165	135	301	125	121	1097

Table 4. National Prostatic Cancer Project

Patient Entry for Each Participating Institution to Current Protocols as of April, 1981

Institution	Protocol Number 600	900	1000	1100	1200	1300	Total	Grand Total
M.D. Anderson	-	-	-	-	-	-	-	186
Univ. of Iowa	28	21	7	18	8	8	90	243
Mason Clinic	8	-	5	109	31	2	65	306
Mass. General	8	-	-	-	3	10	21	136
Johns Hopkins	20	-	4	21	17	5	67	198
Univ. Tennessee	51	-	11	27	11	12	112	182
U. C. San Diego	13	2	18	9	7	2	51	144
Tulane Medical	15	28	11	11	1	5	71	109
Wayne State	8	7	15	6	1	9	46	102
Roswell Park	4	6	8	14	6	3	41	47
Baylor College	2	-	-	3	3	9	17	23
Walter Reed	2	3	7	8	9	5	34	35
Rush-Presbyterian	-	2	1	3	-	5	11	11
U. C. Los Angeles	8	1	-	2	8	6	25	25
Total	167	70	87	141	105	81	651	1748

Table 5. National Prostatic Cancer Project

Status as of September 26, 1980 Closed to Patient Entry

	Protocols							
	100	200	300	400	500	700	800	Total
Entered	125	125	165	135	301	125	121	1097
Exclusions								
Protocol Violations	3	2	2	5	19	9	8	48
No Treatment (TRT)	4	6	7	6	7	7	5	42
Received < 3 Wks TRT	8	12	25	8	24	11	18	106
Insufficient Information For Evaluation	0	0	2	0	14	0	0	16
Evaluable	110	105	116	129	237	98	90	885
Currenty on TRT*	0	0	1	0	123	0	3	127
Currently Being Followed	2	2	7	3	200	13	15	242

* May Include Crossover TRT

Table 6. National Prostatic Cancer Project

Total Patients Entered and Status of Current NPCP Protocols as of April, 1981

	Protocol						
	600	900	1000	1100	1200	1300	Total
Entered	167	70	87	141	105	81	651
Exclusions							
Protocol Violations	14	7	1	-	2	-	24
No Treatment (TRT)	1	2	2	9	1	-	15
Received < 3 Wks TRT	7	-	-	5	6	-	18
Evaluation Pending	26	9	14	40	31	54	174
Evaluable	119	52	70	87	65	27	420
Currently on TRT*	58	41	62	63	40	74	338
Currently Being Followed	94	70	87	93	71	78	493

* May Include Crossover Treatment

Table 7. National Prostatic Cancer Project

Clinical Trials Program - Patient Activity

May, 1981

Closed Protocols	Patients Active	Total Accessioned
500	113	301
700	0	125
800	3	121
Open Protocols		
600	58	169
900	42	72
1000	61	90
1100	64	148
1200	39	108
1300	99	86

Table 8. Average Response Rates and Major Toxicities of All Patients Randomized to Date in Each Drug Category (Endocrine-Refractory Patients - No Significant Irradiation Therapy).

April, 1981

Agent (No. Patients)	Response Rate Objective/Subjective	Toxicity
Cyclophosphamide (119)	35%/41%	35%
5-Fluorouracil (33)	36%/47%	35%
Methyl-CCNU (27)	30%/41%	56%
DTIC (55)	28%/35%	26%
Standard (36)	19%/22%	14%
Hydroxyurea (28)	15%/19%	46%
Procarbazine (38)	13%/21%	30%
Methotrexate (34)	50%/15%	41%
Cis-platinum (27)	26%/12%	32%

Table 9. Average Response Rates and Major Toxicities of All Patients Randomized to Date in Each Drug Category (Endocrine-Refractory Patients with Prior Significant Irradiation Therapy).

April, 1981

Agent (No. Patients)		Response Rate Objective/Subjective	Toxicity
Streptozotocin	(38)	32%/28%	23%
Estramustine phosphate	(86)	26%/23%	35%
Standard	(21)	19%/19%	22%
Vincristine	(34)	15%/31%	32%
Leo-1031	(62)	13%/35%	17%
Cis-platinum	(22)	23%/ 8%	35%
Cis-platinum plus estramustine phosphate	(20)	45%/22%	46%

In those patients with advanced relapsing stage D prostatic cancer who have had extensive pelvic irradiation, objective and subjective response rates have been higher with treatment using stretozotocin or estramustine phosphate compared to patients receiving standard therapy (8). Prednimustine (Leo-1031), either alone or in combination with estramustine phosphate, and the agent vincristine were not as effective as standard therapy. Cis-platinum alone and combined with estramustine phosphate is currently under study in this patient population (Table 9).

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COMBINATION OF CHEMOTHERAPY AND HORMONES IN PROSTATIC CANCER

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ABSTRACT

The National Prostatic Cancer Project in a four-year period has accessioned 301 patients with newly diagnosed Stage D-2 prostatic cancer into a treatment protocol comparing cyclophosphamide (Cytoxan) plus estramustine phosphate (Estracyt) versus Cytoxan plus diethylstilbestrol (DES) versus either DES or bilateral orchiectomy. The three treatment arms appear comparable in terms of entry criteria with the exception of elevated acid phosphatase for one arm which as yet has not influenced the response rates. Thus far, toxicities to the chemotherapy combination treatment arms have not been excessive although more prevalent than in the DES/orchiectomy arm. Major toxicities have been anemia, leukopenia and nausea and vomiting. Preliminary data show that the treatment arm consisting of DES plus Cytoxan is showing a slight but non-significant advantage in delaying progression of the disease compared to the other two treatment arms at the initial 12-week evaluation. Patient follow-up and data collection is still not complete for the assessment of survival and response durations. Approximately one-third of patients entered into the study are still on original or crossover therapy with 75% of patients still in that category. More complete and meaningful data should be available in another 18 months.

INTRODUCTION

In early 1973, the National Prostatic Cancer Project (NPCP) initiated a series of four chemotherapy protocols designed exclusively for those patients with metastatic carcinoma of the prostate (Stage D-2) who had failed to respond or who no longer

responded to endocrine therapy (1). One of the initial and ongoing objectives of the program was to determine the efficacy and safety of single agents in the treatment of advanced prostatic carcinoma resistant to endocrine manipulation, with the hope that this in turn would lead to effective combination treatment for all stages of prostatic cancer.

From the results of protocol 100, "A Comparison of 5-Fluorouracil and Cytoxan in Patients with Advanced Carcinoma of the Prostate," it was determined that Cytoxan (cyclophosphamide) is a useful chemotherapeutic agent in relapsing Stage D prostatic carcinoma. From Protocol 200, "A Comparison of Estracyt and Streptozotocin in Patients with Advanced Prostatic Cancer who have had Extensive Irradiation," sufficient data was gathered underscoring the efficacy of Estracyt (estramustine phosphate) in this group of patients. Based on these data and pilot studies at Roswell Park Memorial Institute and elsewhere documenting the activity as well as relative lack of hematologic toxicity of Estracyt, the use of both these agents in earlier phases of Stage D prostatic cancer seemed justified.

Treatment utilizing exogenous estrogens, generally diethylstilbestrol 1 mg t.i.d., has been accepted for approximately 40 years (2,3). Estrogens can arrest the progress of prostatic cancer and in many cases reverse the progression of Stage D disease for variable intervals. Similarly, bilateral orchiectomy has been widely utilized for patients with advanced prostatic cancer (4).

Based on the above information, the Treatment Subgroup of the NPCP felt the need to design chemotherapy trials for patients who had not had prior endocrine therapy and for whom earlier treatments with combination chemotherapy and endocrine therapy might be of greater benefit (5,6). This protocol (Protocol 500) was designed to compare two combinations of agents in a randomized fashion with either diethylstilbestrol or bilateral orchiectomy. A crossover design was included to allow a second episode of treatment for patients removed from initial treatment because of disease progression and/or toxicity.

SCHEMA AND DOSAGE

Protocol 500 compares the efficacy of Cytoxan + Estracyt versus Cytotoxan + diethylstilbestrol (DES) versus either DES or bilateral orchiectomy alone in patients with newly diagnosed Stage D-2 prostatic cancer who have not had prior endocrine therapy. Cytoxan is administered intravenously at a dose of 1 g/m^2 every three weeks, Estracyt is given at a dose of 600 mg/m^2 orally every day in three divided doses, and DES is given in the standard dose fashion 1 mg. t.i.d. orally. Patients randomized to the

endocrine therapy only arm receive either DES or bilateral orchiectomy based on the option of the investigator and patient (Appendix 1).

PATIENT SELECTION

Criteria for eligibility in this study include (a) patients diagnosed within 30 days of entry with histologically confirmed prostatic cancer with bone or soft tissue metastases; (b) an expected survival of at least 90 days; (c) an entry white blood cell count of $4,000/mm^3$ or greater and platelets of $100,000/mm^3$ or greater; (d) freedom from significant infection and satisfactory recovery from any recent surgery; and (e) fully informed written consent for the study.

Ineligibility criteria include (a) history of prior treatment with either Cytoxan, Estracyt, DES or orchiectomy. (A history of radiation therapy does not in itself exclude a patient from entry into this study); (b) presence of another malignancy except non-melanomatous skin cancer; and (c) patients who, in the opinion of the investigators, have other significant medical diseases making the patient a poor risk for possible chemotherapy.

BASELINE LABORATORY STUDIES

All patients entered into this study have been required to have the following tests performed within two weeks of initiation of treatment. These include complete history and physical examination with special attention paid to the prostatic findings, urinalysis, hemoglobin, leukocyte count, platelet count, differential count, serum creatinine or BUN, serum bilirubin, acid and alkaline phosphatases, serum glutamic oxaloacetic transaminase (SGOT), prothrombin time, chest x-ray, either radionuclide bone scan or skeletal survey, excretory urogram (IVP) and documentation of the histologic diagnosis by appropriate slide material which in turn is sent to the NPCP headquarters.

Following the initiation of treatment, patients receive follow-up blood studies at three-week intervals and all baseline studies are performed at 12-week intervals to assess both response to treatment and toxicities.

EVALUATION OF RESPONSE TO THERAPY

In this study, patients are evaluated for their response to treatment according to the National Prostatic Cancer Project criteria for objective reponses (1). These include complete objective regression, partial objective regression, objective stability and objective progression. The criteria for each of these response categories are included in Appendix 2. In addition,

Table 1: NPCP Chemotherapy Protocol - 500

Status of Patients Entered
As of April 10, 1981

Category	Treatment			Total
	DES or Orchiectomy	DES + Cytoxan	Estracyt + Cytoxan	
Patients on study after 3 or more weeks of treatment	82	75	80	237
Protocol Violations	10	6	3	19
No Treatment	4	1	2	7
Removed before 3 weeks	3	10	11	24
Insufficient data for Evaluation	2	4	8	14
Total	101	96	104	301

the duration of response is tabulated for those patients so designated.

CROSSOVER DESIGN

As indicated in the schema (Appendix 1), the initial treatment may be discontinued either because of objective disease progression or toxicity. Patients initially treated with the combination of Cytoxan and Estracyt may then be crossed over to receive DES. Patients initially treated with the combination of Cytoxan and DES are crossed over to receive Estracyt. Lastly, patients initially treated with either DES or bilateral orchiectomy can then be crossed over to receive the combination of Cytoxan and Estracyt. Following discontinuation of the second treatment because of either progression or toxicity, all patients are followed indefinitely and are treated at the option of the investigators.

CURRENT RESULTS

Protocol 500 was activated on July 1, 1976, and closed to patient entry on August 31, 1980. Since that time, patients with similar eligibility criteria have been entered into a new protocol (Protocol 1300).

A total of 301 patients have been accessioned to Protocol 500 (Table 1). Information of on-study variables is now available for 287 patients, with response and toxicity data available for 237 patients. The exclusion rate has been approximately 17% in each of the three arms. Approximately one-third of all patients are still being treated with either the initial or crossover therapies.

As shown in Figure 1, age distributions are similar in all three treatment arms. All other on-study variables seem to be equal for the three arms with the exception of a greater proportion of patients with increased acid phosphatase activity in the Estracyt plus Cytoxan arm.

At this time 19 patients have been reported as complete objective regressions (Table 2). These include seven patients treated with either DES or bilateral orchiectomy (9%), five patients treated with a combination of DES and Cytoxan (7%) and seven patients treated with a combination of Cytoxan and Estracyt (9%). Partial responses have been reported for 58 patients or 24% of the total. These include 24 patients treated with either DES or bilateral orchiectomy (29%), 20 patients treated with DES plus Cytoxan (27%) and 14 patients receiving Cytoxan and Estracyt (18%). Objective stability has been recorded for 121 patients or 51% of the total. Included are 35 patients in the endocrine therapy arm (43%), 41 patients receiving Cytoxan plus DES (55%) and 45 patients receiving Estracyt plus Cytoxan (56%). Objective

Table 2: NPCP Chemotherapy Protocol - 500

Objective Response According to Treatment, April 1981

	Treatment							
	DES or Orchidectomy		DES + Cytoxan		Estracyt + Cytoxan		Total	
Response	No.	%	No.	%	No.	%	No.	%
Complete	7	9	5	7	7	9	19	8
Partial Regression	24	29	20	27	14	18	58	24
Stable	35	43	41	55	45	56	121	51
Progression	16	20	9	12	14	18	39	16
Total	82		75		80		237	

Table 3: NPCP Chemotherapy Protocol - 500

Response and Evaluation Status of Patients in the DES/Orchiectomy Treatment Arm

	DES		Orchiectomy		
Category	No.	%	No.	%	Total
Complete	5	9	2	8	7
Partial	16	29	8	31	24
Stable	26	46	9	35	35
Progression	9	16	7	27	16
Total	56		26		82
Exclusions	11	16	5	15	16
Incomplete	1	1	2	6	3
Total	68	(67)[a]	33	(33)[a]	101

[a] Percentage of 101 entered to this treatment arm

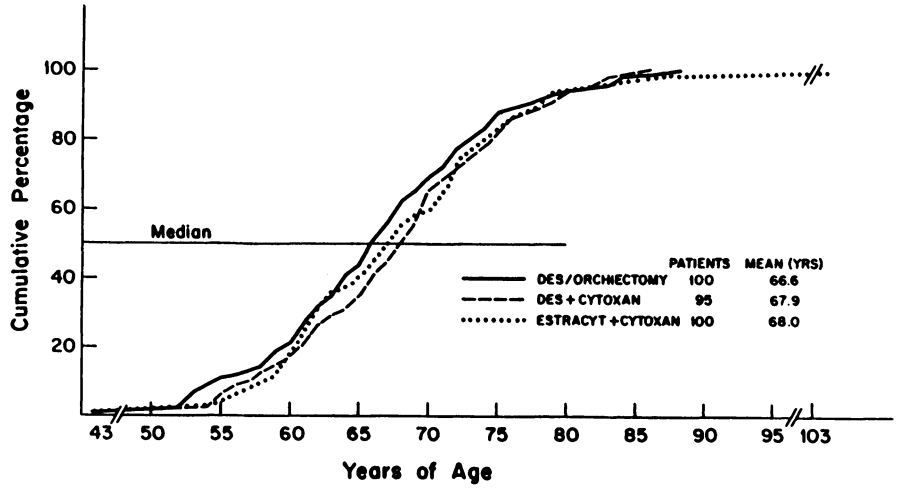

Fig. 1. Age Distribution According to Treatment

progression on the initial randomized treatment has been reported for 39 patients or 16% of the total. These include 16 patients on endocrine therapy (20%), 9 patients on DES plus Cytoxan (12%) and 14 patients on Estracyt plus Cytoxan (18%).

Crossover responses have been reported in 36 of 63 patients so treated (50% response rate). These include patients who progressed on their initially randomized therapy as well as patients who initially responded then progressed and responded to the crossover treatment. Most of the crossover responses to date have been in the stable category.

The response rates to either DES or bilateral orchiectomy are similar to date, namely 84% for DES and 73% for orchiectomy (Table 3). In a small subset of patients with only soft tissue metastases, the response rates to date are greater but not statistically significant compared to the vast majority of patients with bony metastases alone or the combination of skeletal and soft tissue metastases. Subjective responses such as improvements in performance status and pain are relatively the same in all three treatment arms.

Major toxicities thus far include the tendency to lowering of hemoglobin in the patients treated with Estracyt and Cytoxan, more leukopenia in patients treated with the combination of DES and

Table 4: NPCP Chemotherapy Protocol - 500

Reasons for discontinuing randomized treatments for evaluable patients

	Treatment							
	DES or Orchiectomy		DES + Cytoxan		Estracyt + Cytoxan		Total	
Reasons	No.	%	No.	%	No.	%	No.	%
Still on Treatment	45	55	25	33	27	34	97	41
Progression	33	40	22	29	25	31	80	34
Nausea - Vomiting	-		7	9	10	12	17	7
Other Toxicities	4	5	11	15	11	14	26	11
Death	-		6[a]	8	3[b]	4	9	4
Other	-		4	5	4	5	8	3
Total	82		75		80		237	

[a] Five of the six were still in response at death, one due to auto accident, two to myocardial infarction, and one to congestive heart failure, and one to erythroleukemia.

[b] One was stable at death from heart failure, one to non cancer causes that were unspecified and one of unknown cause.

Cytoxan, and a very few instances of thrombocytopenia, mostly in patients treated with DES plus Cytoxan.

Nausea and vomiting have been predominant (66% incidence) in the two arms employing Cytoxan but have been infrequent (20%) in the patients receiving only endocrine therapy.

Patients initially treated with either DES or orchiectomy have been crossed over to Cytoxan and Estracyt mainly because of disease progression, with a smaller subset crossed over because of cardiovascular toxicity of DES (Table 4). On the other hand, both drug toxicity and disease progression have been prominent reasons for discontinuation of treatment in the two arms utilizing Cytoxan.

At this early date, 75% of patients responding to treatment are still responding (Figure 2). Only 24% of patients entered into the study have died (Figure 3). At this time there is no advantage for either DES or orchiectomy regarding survival for patients treated in the endocrine arm (Figure 4). Survival in general has been equal for all three arms of the study with a transient advantage in the second year of treatment for those patients receiving DES plus Cytoxan (Figure 3). Based on the experience to date with patients accessioned, another 18 months of patient follow-up and data collection will be necessary to lead to meaningful results and conclusions.

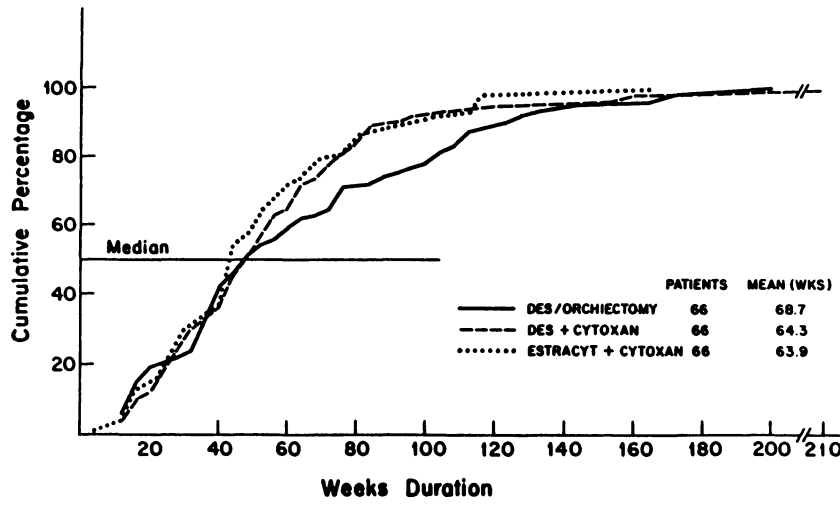

Fig. 2. Distributions of Response Duration for Responders (Complete, Partial Regression) According to Treatment.

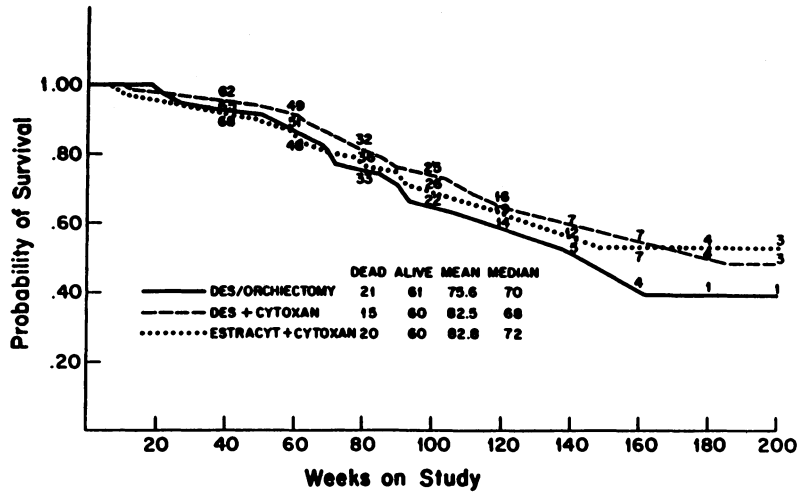

Fig. 3. Probability of Survival for Randomized Treatment Groups (April 1980).

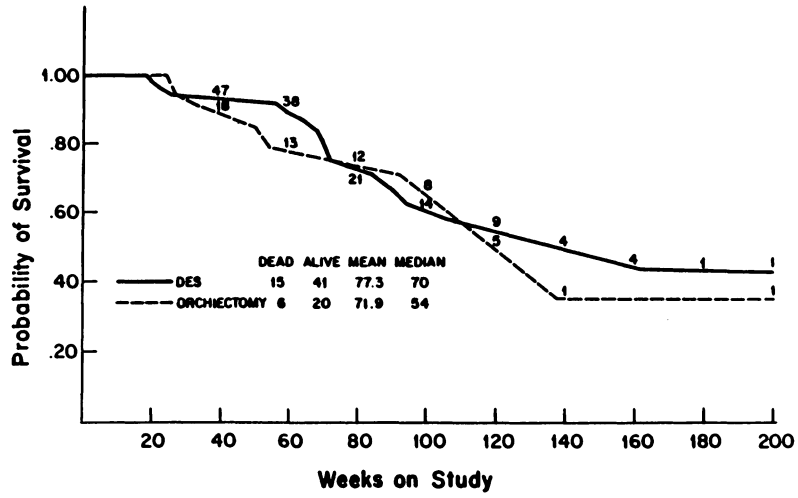

Fig. 4. Probability of Survival for Patients Electing DES or Orchiectomy in the DES/Orchiectomy Arm.

OTHER CURRENT STUDIES

Protocol 1300 compares endocrine therapy alone (DES 1 mg. t.i.d. or bilateral orchiectomy) to estramustine phosphate alone (60 mg./m^2 orally in three divided daily doses) to the combination of endocrine manipulation (DES or orchiectomy), 5-fluorouracil (350 mg./m^2 IV weekly) and cyclophosphamide (650 mg./m^2 IV every three weeks). Accession to this protocol began in September, 1980, 81 patients are currently enrolled but no data are yet available.

Similarly on the hypothesis that combination therapy may be more effective in patients whose prostatic cancer contains both hormonally sensitive and insensitive cell populations, the NPCP began a trial in 1976 aimed at those patients with stage D disease clinically stable on DES therapy (Protocol 600). One hundred and sixty seven patients have been entered and randomized to either: a) continuation of DES 1 mg. t.i.d., b) the addition of cyclophosphamide 1 gm./m^2 IV every three weeks to DES, or c) the addition of estramustine phosphate 600 mg./m^2 orally in three divided daily doses to DES therapy. Although the study is still in progress and results are not complete, preliminary data suggest that the addition of cyclophosphamide to DES reduces the rate of progression compared to DES alone.

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APPENDIX 1

Schema

National Prostatic Cancer Project

Protocol 500

Cytoxan, 1 gm/m^2 I.V. every three weeks
plus
Estracyt, 600 mg/m^2 orally daily in three divided doses

vs

Cytoxan, 1 gm/m^2 IV every three weeks
plus
Diethylstilbestrol 1 mg t.i.d. orally

vs

Diethylstilbestrol 1 mg t.i.d orally
or
Orchiectomy (Investigator's Option)

APPENDIX 2

Response Criteria

National Prostatic Cancer Project

Protocol 500

OBJECTIVE REGRESSION

All of the following criteria:

Complete

Absence of any clinically detectable soft tissue tumor mass. This may include the primary tumor.

Return of an elevated acid phosphatase to normal level.

The recalcification of all osteolytic lesions if present.

No evidence of progression of osteoblastic lesions, if any are present.

If hepatomegaly is a significant indicator, there must be a complete reduction in liver size, and normalization of all pretreatment abnormalities of liver function.

Partial

A 50% reduction in measurable or palpable soft tissue tumor mass when present.

Return of an elevated acid phosphatase to normal.

The recalcification of some osteolytic lesions if present.

If hepatomegaly is a significant indicator, there must be a reduction in liver size and at least a 30% improvement of all pretreatment abnormalities of liver function.

There must be no increase in any other lesion and no new areas of malignant disease may appear.

No significant deterioration in weight (greater than 10%), symptoms, or performance status (one score level).

OBJECTIVELY STABLE

All of the following criteria:

Insufficient regression of primary indicator lesion to meet criteria above.

Less than 25% increase in any measurable lesion.

No significant deterioration in weight (greater than 10%), symptoms or performance status (one score level).

OBJECTIVE PROGRESSION (or also Relapse after Adequate Therapy)

Any of the following criteria:

Significant deterioration in symptoms related to prostatic cancer, decrease in weight, decrease in performance status, or pain requiring medication.

Development of recurring anemia, secondary to prostatic cancer (not chemotherapy).

Development of ureteral obstruction.

Appearance of new areas of malignant disease.

Increase in any previously measurable lesion (soft tissue and lung, excluding bone) by greater than 50% in two perpendicular diameters.

Increase in osseous metastases as shown by scan.

An increase in acid or alkaline phosphatase alone is not to be considered an indication of progression. These should be used in conjuction with other criteria.

STRATEGY OF TREATMENT IN THE ADVANCED STAGES - ROUND TABLE REPORT

J. P. Blandy[1] and M. Pavone-Macaluso[2]

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Although cancer of the prostate is completely preventable by castration before puberty, prevention at such a price is obviously out of the question. Equally, in later life, it is necessary to weigh up the cost to the patient, in terms of quality of life, of any slight extension of its duration. Denis reminded us that in autumn it is not the length of days but their freedom from distress which should be our primary concern.

Advanced local disease without detectable metastases (T3. M0).

1. The value of lymph node staging

We discussed at length the necessity for lymph node staging. Several proponents took the view that there was no value (to the patient) of knowing the precise N staging, since such knowledge would not alter treatment, and Bagshaw, speaking from his considerable experience, had reached the conclusion that when the acid phosphatase was elevated and the tumour grade was undifferentiated, then nothing was to be gained by node dissection since the nodes were bound to be involved by tumour. After discussion it was felt that perhaps there was a place still remaining for node dissection as a preliminary step in exploration prior to an attempt at a "salvage prostatectomy", if such an operation were to be contemplated.

2. Local radiotherapy for T3 tumours

Most surgeons would agree, it seemed, that radical prostatect-

omy was out of the question for T3 tumours, and Bagshaw was asked whether he thought these growths were suitable for radiotherapy. He replied that in his hands they seemed to do reasonably well, and added that in many of these cases, local extension beyond the normal limits of his small field of irradiation, or the demonstration in CAT or lymphangiogram of involvement of pelvic lymph nodes, could be included in the area to be irradiated by adjustment of the treatment field.

Since radiotherapy was so dependent upon the bulk of the tumour, Altwein asked whether we should not try to shrink the bulky T3 tumours by preliminary estrogens or cyproterone acetate prior to radiation, particularly in patients whose grossly swollen prostates were accompanied by outflow obstruction. Bagshaw agreed that this was useful in practice, and from the discussion that followed, it seemed clear that the optimum combination of radiotherapy and hormones was still an area worth studying in clinical trials.

The comparable use of hormones to shrink the "inoperable" prostate in order to render it suitable for radical perineal prostatectomy was referred to by Schmidt, and Dr Goodwin reminded us of the earlier attempts by Dr Winfield Scott to achieve just this goal, but added that he thought most surgeons had abandoned this procedure.

3. Salvage prostatectomy after "failed" radiotherapy

The discussion then turned naturally to the place of "salvage prostatectomy" after a course of radiotherapy which had been followed by persistence or return of tumour in the irradiated field. Jacobi's important finding, that when cancer cells were found in fine-needle aspiration smears from prostates two years after completion of radiotherapy, such patients nearly always were found to grow wide-spread metastases within the next few years, emphasised the real risk that these "silent" cancer cells posed for the patient. With this view Andersson agreed, particularly when the cytological features of these tumours were less than well-differentiated. The place of salvage prostatectomy remained in question, and again the question of the price the patient would have to pay in terms of operative morbidity would need to be submitted to carefully controlled trials before it could be taken up with any enthusiasm.

Metastatic Disease

1. Timing for therapy

Nothing raised so much argument among the participants as the timing of treatment of metastases. Many favoured immediate treatment, arguing classically that those that were smallest should respond best; others took the view that treatment ought to be

deferred until symptoms occurred. On a show of hands (in the absence of hard evidence) the room was equally divided.

2. Significance of a raised acid phosphatase

Byar's interesting observations that showed such a close relationship between very small elevations of serum acid phosphatase (measured by the old enzymatic method) and the subsequent fate of patients in long term follow up, led to a stimulating discussion as to the role of acid phosphatase, and in particular, to a renewed respect for the traditional enzymatic method. If Byar's observations were confirmed, then one should perhaps regard even a very small elevation of acid phosphatase as a sinister harbinger of occult metastases.

3. Orchidectomy or estrogens?

Having dodged the question of when to start to treat metastases, we turned to the choice of which method of hormone treatment - and in particular, what was the place of orchidectomy? In the VACURG studies it was clear that those assigned to the orchidectomy group in the initial randomisation did less well than those offered estrogen or estrogen plus orchidectomy. Despite a few rather curious claims that orchidectomy was somehow cheaper (for whom?) than estrogen therapy, the consensus of the discussion was that orchidectomy ought to be restricted to those patients who were not reliable pill-takers or for whom the cardiovascular complications of estrogens made them contraindicated.

4. Which estrogen?

None of the alternatives to cheap, simple, old-fashioned DES seemed, from the results of the endless clinical trials that we had listened to, to be any better. Unfortunately, it appeared from the discussion that because DES was so cheap, no manufacturer had much interest in making it, and worse, the risk of stilboestrol being added to cattle food had led to it being banned in certain European countries. Other "natural" estrogens, if pregnant mares' urine can be regarded as natural for mankind, gave equally good, but no better results. There was no advantage in using TACE or cyproterone acetate in the first instance.

5. Chemotherapy

When the claims of the US National Prostatic Studies were critically evaluated, it was pointed out that none of the results justified the use of any chemotherapeutic agent in the first line treatment of metastases. The estrogen-mustard combinations might however have a place, Andersson argued, in the initial treatment of metastases when the tumour grade was especially anaplastic. The

proper place for chemotherapy, at this time, seemed to belong to the treatment of patients whose tumours had "escaped" from estrogen therapy. Even here, as emerged very clearly from Schmidt's reports on the clinical trials, some of these "escapers" were, in fact, men who had failed to take estrogen therapy as prescribed. At this stage the discussion came near the brink of the treatment of "failed estrogen therapy" which was the proper subject of the next round table, as a result of which this one came to its natural conclusion.

STRATEGY OF TREATMENT IN PROSTATIC CANCER - A CONTRIBUTION TO THE DISCUSSION

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Appropriate therapy of patients with prostatic cancer with the multitude of existing modalities depends on three important factors - the general condition of the patient, the stage of the cancer and the biological potential of the tumor.

The general condition of the patient should be judged on age, index of vitality, and the presence or absence of associated disease especially cardio-vascular disease; the interest of the patient in sexual activity should also be noted.

The stage of disease should be based on physical findings, laboratory tests, medical imaging techniques and surgical evaluation in some instances. These examinations should result in reliable and reproducible parameters to measure existing or progressive disease.

The biological potential of the tumor should currently be based on a satisfactory tissue specimen and a consultation with the pathologist. A Gleason scale of 9 or 10 seems to be an accurate prognostic factor (1).

Based on this information we should be able to classify patients with prostatic carcinoma as having local or systemic disease. A reasonable effort to collect information on the possible invasion of the regional lymph nodes includes the study of prognostic factors in lymphatic spread, lymphangiography and fine needle biopsy and/or lymphadenectomy (1,2). However, accumulated data would indicate that no local treatment be it radical surgery, interstitial radiotherapy or external beam radiation brings disease control when cancer is present in the local regional nodes (3,4,5).

An international pathology reference system and a referee pathologist are essential to accurate statistical evaluation of any trial concerning prostatic cancer. Another problem of the pathology is that with the exception of the poorly differentiated lesions prostatic microcarcinoma should be eliminated from our clinical data.

True localized cancers can be treated by total or radical prostatectomy, external beam irradiation or interstitial radiotherapy. Total prostatectomy seems the best treatment for the localized nodule (6).

Systemic disease is a problem whose natural history varies according to the different characteristics of the tumor and its host. Hormonal treatment in its diverse forms aims to abolish the physiologic effect of circulating androgenic hormones. Bilateral orchiectomy is indicated in patients with prostatic cancer in relapse where serum testosterone is not reduced to anorchic levels (7). The debate on late or early hormonal treatment is by no means completely resolved (8). Finally, the value of multi-modal hormonal treatment and adjunctive chemotherapeutic treatment can best be determined by future randomized trials and seem to offer the only solution to the dilemma of the management of prostatic cancer.

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ON THE METHODOLOGY OF CONTROLLED CLINICAL TRIALS

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INTRODUCTION

A wide and varied literature exists on the appropriate methodology that one should employ in the design and analysis of clinical trials. As many concepts are somewhat controversial in nature and not widely accepted or understood by many researchers, this paper will present a very general overview of the underlying principles involved in the design and analysis of clinical trials.

THE PROTOCOL

The most important document pertaining to any trial is the protocol, a self contained description of the rationale, objectives and logistics of the study. The protocol provides the scientific basis for the study and describes how the trial is to be carried out. Staquet, Sylvester and Jasmin (1) have published practical guidelines for the preparation of cancer clinical trial protocols. They provide precise suggestions concerning the contents of the chapter headings found in Table 1 along with a large bibliography of papers related to the design and analysis of cancer clinical trials.

PUBLISHING TRIAL RESULTS

One of the main problems encountered in interpreting the results of a clinical trial is due to the wide variability that exists in the methods used to assess treatment efficacy and in the manner in which one publishes trial results. It is often difficult to place a trial in its proper perspective due to an inconsistent or incomplete reporting of the patient population under study, the

Table 1: Protocol contents

1. Background and Introduction
2. Objectives of the Trial
3. Selection of Patients
4. Design of the Trial (Including a Schema)
5. Therapeutic Regimens and Toxicity
6. Required Clinical Evaluations, Laboratory Tests and Follow-Up
7. Criteria of Evaluation
8. Registration and Randomization of Patients
9. Forms and Procedures of Collecting Data
10. Statistical Considerations
11. Administrative Responsibilities
12. References

Appendices: Performance Status Scales

TNM or Other Classifications

treatment administered, toxicities observed, or the statistical methods employed to assess treatment efficacy. In addition there may be important differences from one trial to another in defining what constitutes a response to treatment, especially in advanced disease when there are multiple lesions. Some standardization in the reporting of treatment results in cancer patients is required if the results of published trials are to be properly interpreted.

The World Health Organization has attempted such a standardization by publishing the WHO Handbook for Reporting Results of Cancer Treatment (2). This handbook proposes a minimum data set which should be reported and provides definitions for terms such as response, duration of response and duration of survival. In addition a grading system for the reporting of toxicity is provided. As the WHO Handbook deals with the reporting of cancer treatment results in general rather than in the specific setting of a clinical trial, Kisner and Sylvester (3) have proposed a set of guidelines based on the WHO Handbook but adapted specifically to cancer clinical trials. These works should be consulted not only at the time when the trial is to be analyzed and the results published, but also when designing new protocols since the definitions proposed in these papers should be incorporated into the protocol and data forms at the design stage.

PHASE I, II AND III TRIALS

In cancer research one encounters Phase I, Phase II and Phase III trials. What is the purpose of each type of trial and how do they differ?

In its simplest terms a Phase I trial may be thought of as a toxicity screening trial where after testing in animals, the drug is administered for the first time in man in order to determine the maximum tolerated dose. Several patients are treated at each of a series of increasing dosage levels in order to determine which level should be tested in a Phase II trial. It should be emphasized that the assessment of treatment efficacy is not an endpoint of interest in Phase I trials as we are at this point only dealing with the initial human clinical pharmacological evaluation of the drug.

Once a maximum tolerated dose is determined from a Phase I study, the drug is screened for potential anti-tumour activity in a Phase II trial in a limited number of patients with advanced measurable disease. The purpose of a Phase II trial is to see whether or not the drug has sufficient activity to warrant further testing in a larger number of patients. A Phase II study is not an absolute test of the drug's efficacy but rather a screen of the drug at a given dose in a given group of patients. For example, a drug may initially be tested in 20 patients and rejected from

further study if no responses are observed.

One problem encountered in Phase II trials is that patients entered in such a trial have usually failed on a number of previous treatments and may be in such poor condition that the chances of their responding to any treatment, even an active one, are very low. Thus a negative result in a Phase II trial does not exclude the possibility that the drug might be active in previously untreated patients or in patients with less advanced disease.

Once a drug has shown some degree of activity in a Phase II trial at an acceptable level of toxicity, the next step is to introduce the drug into a Phase III trial. The purpose of a Phase III trial is to determine the relative effectiveness of the new treatment by comparing the new drug or perhaps a combination containing the new drug to the classical or best available treatment in a large number of patients. The rest of this paper will deal specifically with the design and analysis of Phase III trials.

DESIGNING PHASE III STUDIES

Randomization

When one wishes to compare two or more different treatments in a clinical trial, the treatment groups to be studied should be as similar as possible with respect to all factors, whether known or not, which might affect a patient's response to treatment. Randomization is used as a method of assigning treatment to patients that permits valid statistical comparisons to be drawn without making any special assumptions (4).

Randomization has three primary advantages over using historical controls:

1. An investigator cannot either consciously or subconsciously assign more patients of a certain type to receive a particular treatment. Thus bias is eliminated in the assignment of treatments.

2. The process of randomization tends to balance the distribution of the various prognostic factors, whether known or not, in the various treatment groups. Thus the treatment groups to be compared will tend to be truly comparable.

3. The process of randomization allows one to calculate the so called P-value, the probability of obtaining a difference at least as large as that actually observed in the trial if in fact the treatments are equivalent.

While there are many other advantages to randomization they will not be discussed here. The interested reader is referred to

Byar et al (4) for further details. In any multicenter study a centralized randomization where participating institutions contact a central office by telephone or telex is to be preferred to a system of envelopes for the following reasons:

1. A centralized randomization ensures that the randomization is done correctly. A system of envelopes is to be discouraged as this method can easily be abused.

2. With a centralized randomization one knows at all times exactly how many patients have been entered on study and who they are.

3. With a centralized randomization it is possible to request overdue forms for all patients entered on study.

Stratification

When one analyzes in a statistical model the effect that various prognostic factors and treatment may have on a patient's response to treatment, it is often found that even if there is a significant treatment effect, several of the prognostic factors may be more important than the treatment itself. Performance status for example is an important prognostic factor in determining the duration of survival in prostatic cancer patients as shown in figure 1. You will not often find treatment differences of this magnitude in a clinical trial. It is clear that any imbalance in the distribution of the performance status in the various treatment groups to be compared will seriously bias the treatment comparisons unless an adjustment for the performance status is made. In the case of a retrospective study, data on prognostic factors such as the performance status are not usually available for each patient so that no correction for such biases is possible.

In a randomized prospective trial it is possible to take into account the effect of various prognostic factors by means of stratification, either at the time of randomization or at the time of analysis. Stratification at the time of randomization for the one or two most important prognostic factors ensures that no major imbalances in the number of patients receiving each treatment will occur in these prognostic subgroups.

While stratification at randomization is beneficial, overstratification may actually turn out to provide a worse balance than if no stratification at all had taken place. After a certain point, overstratification becomes equivalent to no stratification at all and may by chance turn out to be worse.

In determining the number of strata one should estimate the expected number of patients that will fall into each stratum and combine those strata which are expected to contain only a few

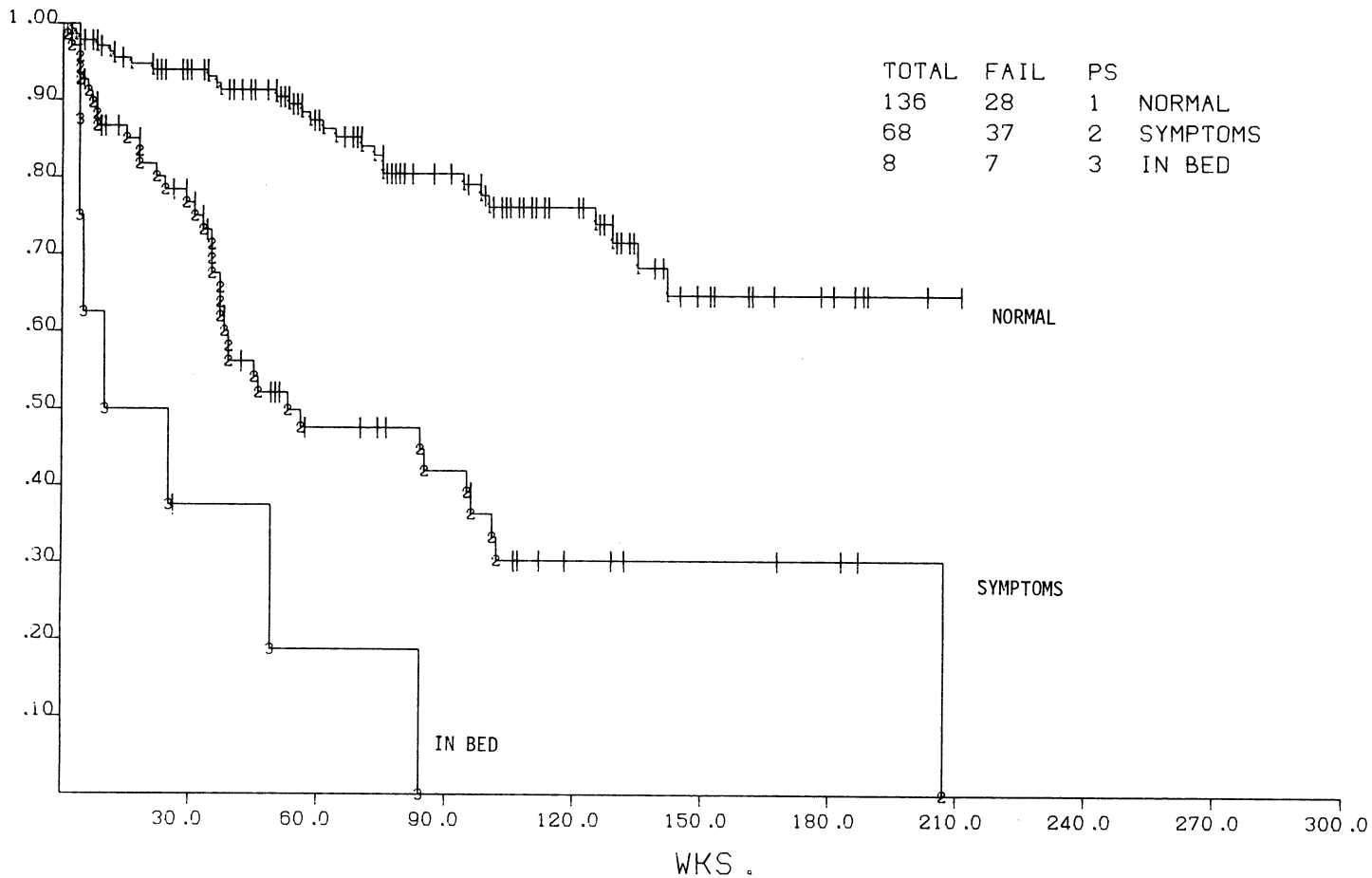

Fig. 1. Duration of Survival of T3, T4 Prostatic Cancer Patients According to Performance Status (PS).

patients. In a multicenter study I would recommend stratifying by institution and then generally by the one variable thought to be the most important.

Independently of whether or not one has stratified at the time of randomization, it is important to stratify at the time of analysis for those variables which prove to be of prognostic importance (5). In this manner you adjust for the effect that a variable might have on the endpoint of interest. While a moderate imbalance in the distribution of a prognostic factor in the various treatment groups is not a serious problem, stratification at randomization guards against any large imbalances that might arise and for which adjustment procedures may no longer be adequate.

ANALYZING PHASE III STUDIES

Excluding Patients from the Analysis

Perhaps one of the most controversial and misunderstood concepts in the analysis of comparative Phase III trials is the question of excluding non-evaluable patients from the treatment results. When analyzing clinical trial data exclusion of patients for any reason other than ineligibility may no longer permit an unbiased evaluation of the results since the reason for exclusion may be correlated with the treatment group and the endpoints under study.

In the case of an adjuvant study suppose patients are randomized to receive either adjuvant chemotherapy or no adjuvant treatment after removal of the primary tumour. One is tempted to exclude from the statistical analysis those patients who for one reason or another did not receive chemotherapy in accordance with the protocol or who received a reduced number of cycles due to early death or toxicity. On the other hand the same patient, if he had been randomized to the no treatment control group, would have been considered to be fully evaluable and included in the statistical analysis. Excluding patients from the chemotherapy group will result in comparing all the patients of the control group to only a selected subset of the patients randomized to receive chemotherapy, i.e. the chemotherapy patients with the best prognosis. Such a practice will lead to biased results since the two treatment groups will no longer be comparable. In this case it is not surprising that a significant difference may be obtained in favour of adjuvant chemotherapy even if the treatment is of no real benefit. In order to avoid this situation all eligible randomized patients should be included in the statistical analysis. In this manner the balance established by the randomization will be maintained and you are answering the more realistic question: "Is the policy of giving adjuvant chemotherapy

whenever possible better than the policy of giving no adjuvant treatment?" It is unrealistic to think that in practice all patients will receive chemotherapy exactly as specified in the protocol. While this example involved a non treated control group, the same principles would apply in the comparison of two or more treatments.

In the analysis of studies of advanced disease the same problem exists. However here it might be stipulated in the protocol that a patient must receive a certain minimal amount of treatment before the patient can be evaluated for tumour response. Even in this case care should be taken to determine whether or not the reason for exclusion is related to the treatment group or the disease in question. If more patients are excluded in one treatment group than in the other, the results may be subject to bias. When publishing trial results it is of utmost importance to account for every patient entered in the trial.

Analysis of Survival Data

A survival curve or a survival distribution is simply a graph or table giving an estimate of the proportion of patients in a specified group who are still alive at different times after randomization or start of treatment. Any technique used in the calculation of survival curves must take into account the possibility that a particular patient's death may not have been observed yet at the time of analysis. For patients who are still alive or lost to follow up at the time of analysis, the only information that is available is the date that the patient was last known to be alive. This is usually referred to as the date of censoring. In constructing a survival curve, the Kaplan-Meier Product Limit method is to be preferred (6).

If we want to compare the effect on survival of two different treatments, it is not sufficient to simply count the number of deaths in each treatment group, but it is also necessary to take into consideration the time of death and for those patients who are still alive, the duration of follow up for each patient.

In testing whether or not there is a significant difference between two or more survival curves, tests which utilize the entire curve should be employed rather than tests which just compare the curves at a specific point or points in time.

Two tests to be preferred are the Log Rank (Mantel-Haenszel) Test and the Gehan Generalized Wilcoxon Test, more fully considered by Peto et al in 1977 (5).

CONCLUSION

A wide number of topics pertaining to the methodology of controlled clinical trials have been discussed in this paper but only in a very general and somewhat superfical manner. Some very basic guidelines have been presented but it is now up to the reader to consult the references provided in order to obtain a fuller understanding of the appropriate techniques to employ in the design and analysis of such trials.

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THE E.O.R.T.C. PHASE II STUDIES IN ADVANCED PROSTATIC CANCER

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The E.O.R.T.C. Urological Group has had many difficulties in the evaluation of the treatment results of their phase II and III trials in prostatic cancer.

In phase II studies, the main problem in the assessment of the response to treatment, is the rarity of measurable metastases in this disease. The majority of the patients present with only evaluable lesions, such as bone metastases, hepatomegaly, ureteric obstruction, lymphangitic spread to the lungs, lymphoedema, and other evaluable features such as elevations of alkaline and acid phosphatase, weight loss, anemia and pain.

The available literature on the efficacy of chemotherapy in prostatic cancer is confusing as a result of poorly defined response criteria that differ from one study to another. For example, Yagoda (1) and Merrin (2) have independently examined the value of cisplatin in advanced prostatic cancer. Yagoda reported 3/25 (12%) partial remissions, whereas Merrin achieved partial remissions in 13/45 (29%).

What criteria did they use? Merrin observed remissions at the following sites: disappearance of lymphoedema of the legs (5 patients), decrease of hepatomegaly (1 patient), recalcification of a lytic bone metastasis or disappearance of a lesion on bone scan (3 patients), disappearance of lung and mediastinal lesions and ureteric obstruction (1 patient), disappearance of malignant effusions (2 patients) and disappearance of supraclavicular lymph nodes (1 patient). With the exception of the supraclavicular lymph nodes, all parameters used are evaluable rather than measurable. Yagoda observed remissions at the following sites: skin and subcutaneous

lesions, lymph nodes, and nodular lung metastases. All these metastases were measurable. A partial remission denotes a decrease of 50% or more in the sum of the products of the cross-diameters of measurable metastases. Obviously the results of Merrin and Yagoda are not comparable due to the different response criteria. This example reflects in a nutshell the problems of the interpretation of the literature on chemotherapy of prostatic cancer.

Several other sets of response criteria are being used. Each one depends at least partly on evaluable instead of measurable indicator lesions. N.P.C.P.'s parameters (3) may include measurable soft tissue lesions, but usually the indicator lesions are only evaluable. E.C.O.G.'s criteria (4) are based on measurable lesions, but if no measurable lesions are present, a 50% reduction of the serum acid phosphatase is considered a response. This is suprising since E.C.O.G. itself (5) has shown that there is no good correlation between the response in measurable metastases and the reduction in acid phosphatase. The ancillary scoring system (A.S.S.) of Kvols (6) does not take measurable lesions into consideration at all.

The goal of phase II studies is to measure the anti-tumor effect of a cytotoxic or a drug combination. The E.O.R.T.C. Urological Group has recognized this and accepts for their phase II studies only patients with bidimensionally measurable indicator lesions. These are superficial lymph node metastases, para-aortic and mediastinal lymph nodes, nodular lung metastases, skin and subcutaneous lesions and demarcated liver metastases. Apparently, CT scanning and ultrasonography are frequently necessary in the diagnostic work-up.

A recent analysis of a phase III study of the E.O.R.T.C. Urological Group has shown a close correlation between the response of the primary prostatic tumor and survival (7). Therefore, the Group feels justified in accepting the prostate as an indicator lesion provided that it can be measured by intrarectal ultrasonography.

In addition to this set of study parameters, E.O.R.T.C. has adopted the W.H.O. definitions of response for bidimensionally measurable tumors (8).

Presently, the Group is executing a study to examine the anti-tumor effect of Vindesine. It is expected that the results of this study will become available in 1981. The successive study will examine the value of Mitomycin-C in prostatic cancer.

In conclusion, the use of the strict criteria and study methods mentioned will lead to the collection of unequivocal data that meet scientific requirements for clinical research. CT scanning and ultrasonography should be used more frequently to demonstrate measurable metastases.

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PRELIMINARY RESULTS OF TWO EORTC RANDOMIZED TRIALS IN PREVIOUSLY UNTREATED PATIENTS WITH ADVANCED T3 - T4 PROSTATIC CANCER

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INTRODUCTION

At the end of 1976 and the beginning of 1977 respectively, the EORTC Genito-Urinary Tract Cancer Cooperative Group started two randomized phase III protocols in previously untreated patients with advanced T3-4 prostatic cancer (1-3). The first trial, 30762, compared Stilboestrol and Estracyt, while the second, 30761, was a randomized comparison of Stilboestrol, Cyproterone Acetate and Medroxyprogesterone Acetate. Both trials have now been closed to patient entry and the principal endpoints of the studies analyzed. This paper will present the interim results which have emerged from these two studies.

PROTOCOL 30762 (Study Coordinator: P.H. Smith, St. James's University Hospital, Leeds)

MATERIAL AND METHODS

Protocol 30762 is a randomized phase III study comparing Estracyt (estramustine phosphate) and Stilboestrol (DES) with respect to the objective response rate, duration of response, duration of survival, and the toxicity observed. Estracyt was taken at an initial dose of 280 mg. twice a day for the first eight weeks followed by 140 mg. twice daily while the dose of DES was 3 x 1 mg/day.

Patients were selected for the study according to the inclusion and exclusion criteria listed in Table 1 and treated for a minimum of

Table 1. Protocols 30761 and 30762 - Selection Criteria

CRITERIA FOR INCLUSION :

1. NO PREVIOUS TREATMENT FOR PROSTATIC CANCER (except for non-radical prostatectomy)

2. HISTOLOGICALLY PROVEN CARCINOMA OF THE PROSTATE

3. CATEGORY T3 : TUMOUR EXTENDING BEYOND THE CAPSULE WITH OR WITHOUT INVOLVEMENT OF THE LATERAL SULCI AND/OR SEMINAL VESICLES

   CATEGORY T4 : TUMOUR FIXED OR INVADING NEIGHBOURING STRUCTURES

4. ALL N M P AND G CLASSIFICATIONS

CRITERIA FOR EXCLUSION :

1. LIFE EXPECTANCY OF < 90 DAYS

2. CARDIOVASCULAR DISEASE WHICH IN THE OPINION OF THE INVESTIGATOR PRECLUDES THE ADMINISTRATION OF DES.

3. EXPECTED DIFFICULTIES OF FOLLOW-UP RELATED TO-PSYCHIATRIC DISORDERS, MARKED SENILITY OR TOO LARGE A DISTANCE HOME-HOSPITAL

4. PRESENCE OF ANOTHER NEOPLASM EXCEPT NON-MELANOMA SKIN CANCER

eight weeks before being evaluated for tumour response. If a response was observed, treatment was to be continued until progression. In the case of no change, treatment was to be continued or crossed over at the investigator's discretion while patients who progressed were to be crossed over to the other protocol therapy and reevaluated after eight more weeks. However the number of patients in whom treatment was actually crossed over after eight weeks was so small (5/248 patients) that this information was disregarded.

The protocol was started in November 1976 and closed to entry in June 1980. Patients are still being followed for duration of remission and duration of survival. A total of 336 patients were entered by 13 institutions. Unfortunately six centers were excluded due to institutional problems which reduced the total number of patients under study to 248, 125 and 123 on Estracyt and DES respectively. Table 2 shows that 90% of the patients were entered by four British institutions. Patient characteristics at entry on study are presented in Table 3; 51% of the patients were between 71 and 80 years old and 65% had a normal performance status. A T3 tumour was present in 71% of the patients while 49% had distant metastases.

Table 2. Protocol 30762 - Patient Distribution by Treatment and by Institution

INSTITUTION	ESTRACYT	DES	TOTAL
C. BOUFFIOUX, LIEGE	7	8	15
B. LARDENNOIS, REIMS	6	4	10
PH. SMITH, LEEDS	27	25	52
M. ROBINSON, CASTLEFORD	39	36	75
B. RICHARDS, J.R.G.BASTABLE, YORK	36	39	75
R.W. GLASHAN, HUDDERSFIELD	10	11	21
T O T A L	125	123	248

RESULTS

As of April 1981 a total of 45 and 57 patients were still being treated with Estracyt and DES respectively as indicated in Table 4. The remaining 146 patients went off study mainly due to progression (43%), excessive toxicity (16%) and non cancer related deaths (15%).

Side effects

Side effects can be broken down into three principal categories: painful gynecomastia, gastro-intestinal and cardiovascular. Overall there was no significant difference between the two treatments with respect to the number of patients who developed painful gynecomastia. Gastro-intestinal side effects were however reported more frequently by the patients treated with Estracyt than those treated with DES: 26% against 11% respectively. In addition they were more severe on Estracyt which had to be stopped in 8% of the patients(against none taking DES) due to severe abdominal and epigastric pain, gastro-intestinal distress, diarrhea and nausea and vomiting.

The incidence of cardiovascular side effects during treatment was 32%, namely 28% on Estracyt and 36% on DES (Table 5). Out of the 13 cardiovascular deaths which occurred during treatment, three were reported on Estracyt against 10 on DES. If we look separately at the patients according to whether or not they had a history of cardio-vascular disease at entry on study, no difference has been observed between the treatments in the incidence of cardiovascular side effects for those patients without previous cardiovascular history. However, the percentage of patients with a previous history of cardiovascular disease who developed cardiovascular complications during treatment is higher on DES (52%) than on Estracyt (26%). This difference is not statistically significant (p= .10) but nevertheless suggests that those patients with a previous history at entry on study tend to

Table 3. Characteristics at entry on study

	PROTOCOL 30761	PROTOCOL 30762
	PERCENTAGES	
METASTASES		
YES	52	49
NO	44	50
UNKNOWN	4	1
PAIN		
YES	24	32
NO	76	68
HISTOLOGY		
G1	23	23
G2	48	41
G3	24	34
GX	5	2
T CLASSIFICATION		
TO-2	1	2
T3	79	71
T4	20	27
SIZE OF PROSTATIC TUMOUR		
1 - 9 cm^2	46	28
10 - 19 cm^2	26	27
20 - 29 cm^2	19	31
> 30 cm^2	9	14
PERFORMANCE STATUS		
NORMAL ACTIVITY (1)	40	65
SYMPTOMS BUT AMBULATORY (2)	50	31
IN BED < 50% OF TIME (3)	10	4
AGE		
40 - 60	7	10
61 - 70	34	31
71 - 80	49	51
80 +	10	8
CHRONIC DISEASES		
YES	62	53
NO	38	47
CARDIOVASCULAR DISEASES		
YES	32	25
NO	68	75

Table 4. Protocol 30762 - Reasons for going off study (stopping treatment)

REASON OFF-TREATMENT	ESTRACYT	STILBOESTROL	TOTAL
STILL ON TREATMENT	45	57	102
PROGRESSION (Including deaths due to prostatic cancer)	39	24	63
EXCESSIVE TOXICITY	15	9	24
DEATH (Not due to cancer)	8	14	22
INELIGIBILITY	8	9	17
TREATMENT AND/OR EXAMINATIONS REFUSED	4	4	8
LOST TO FOLLOW UP	-	2	2
PROTOCOL VIOLATION	-	2	2
OTHER	6	2	8
T O T A L	125	123	248

Table 5. Protocol 30762 - Cardiovascular side effects

TREATMENT	NO HISTORY OF CARDIOVASC. DISEASE		HISTORY OF CARDIOVASC.DISEASE		TOTAL		TOTAL
	SIDE EFFECTS		SIDE EFFECTS		SIDE EFFECTS		
	NO	YES	NO	YES	NO	YES	
ESTRACYT	57	23(29%)	20	7(26%)	77	30(28%) +	107
STILBOESTROL	56	26(32%)	12	13(52%)	69	38(36%) ++	107
T O T A L	113	49(30%)	32	19(37%)	146	68(32%)	214

\+ 3 cardiovascular deaths
++ 10 cardiovascular deaths

develop more cardiovascular side effects when treated with DES than with Estracyt.

Of the cardiovascular side effects reported during treatment with Estracyt or DES, 52% consisted of fluid retention (heart failure, hypertension, oedema, dyspnoea and cramps), 25% of venous thromboembolism (deep venous thromboembolism, pulmonary embolism, cerebrovascular accidents and thrombophlebitis) and 21% of ischemic heart disease (infarctions, ventricular ectopic beats, S T segment and R.B.B.B.). No significanct difference in the type of cardiovascular complications was noted between the two treatments.

Tumour Response

The response of the prostatic tumour and the response of the bone metastases were analyzed separately. The response of the prostatic tumour is based on rectal palpation findings after a minimum of two months of treatment and is defined as complete remission (CR)- absence of any clinically detectable prostatic tumour mass; partial remission (PR)- 50% decrease of the product of the two maximum perpendicular diameters; no change (NC)- a change of less than 50%- and progression (PROGR)- a 50% increase of the product of the two maximum perpendicular diameters. All patients with follow up data at two months or for whom progression is known to have occurred prior to two months were taken into consideration.

The overall response rate (CR + PR) of the prostatic tumour is 42% (36% on Estracyt and 48% on DES Table 6) with no significant difference between the two treatments (P =0.13).

Table 6. Protocol 30762 - Overall best response of the prostatic tumour

TREATMENT	CR	PR	NC	PROGR.	TOTAL	% CR + PR
ESTRACYT	6	27	48	11	92	36
STILBOESTROL	2	44	44	6	96	48
TOTAL	8	71	92	17	188	42

P = .13

ADJUSTED FOR PERFORMANCE STATUS P = .18

ADJUSTED FOR G GRADE P = .17

Analyses of the potential factors related to the response of the prostatic tumour revealed that the size of the prostatic tumour, the performance status and the G grade are the most important prognostic factors. After adjustment for performance status and G grade, the P values are P =0.18 and P =0.17 respectively. Adjustment for tumour size gives P =0.05 in favour of DES although this does not take into account the imbalance of either the performance status or the G grade in the two treatment groups.

The response of the bone metastases is based on the results of an extramural review of all scans and/or X-rays. So far only 69 patients have been evaluated. The overall response rate is 30% (30% for Estracyt and 31% for DES). Adjustment for the performance status, the most important prognostic factor for the response of the bone metastases also reveals no difference (P =0.91) between the two treatments.

Since the presence or absence of pain was related to the patient's metastatic status, the percentage of patients with relief of pain at two months was also analyzed. Among the 65 patients who complained of pain at entry on study, 33 on Estracyt and 32 on DES, relief of pain at two months was observed in 61% of the patients treated with Estracyt and in 69% of the patients treated with DES.

Survival

Out of the 214 eligible patients for whom follow up has been received, 74 patients (35%) have died as of April 1981.

Figure 1 presents the duration of survival by treatment group taking into consideration all causes of death. Forty-three patients have died in the Estracyt group as opposed to 31 patients on DES. While there appears to be a difference between Estracyt and DES in favour of DES, adjustment for the most important diagnostic factor for survival - performance status, shows in fact that there is no significant difference between the two treatments (P =0.57, Logrank Test). If now one considers only those deaths due to malignant disease, the difference between Estracyt and DES is likewise non significant after adjustment for the performance status with P =0.39. This example shows very clearly how incorrect conclusions might be drawn even in a randomized trial if one does not adjust at the time of the analysis for those factors which are of prognostic importance.

If one considers now only those deaths due to cardiovascular disease, six in the Estracyt group and 11 in the DES group, there is no significant difference between the two treatments (P =0.28, Logrank Test) with respect to the duration of survival. An adjustment for the patient's age, the most important prognostic factor related to cardiovascular deaths, likewise did not reveal a significant difference between the two treatments (P =0.18, Logrank Test).

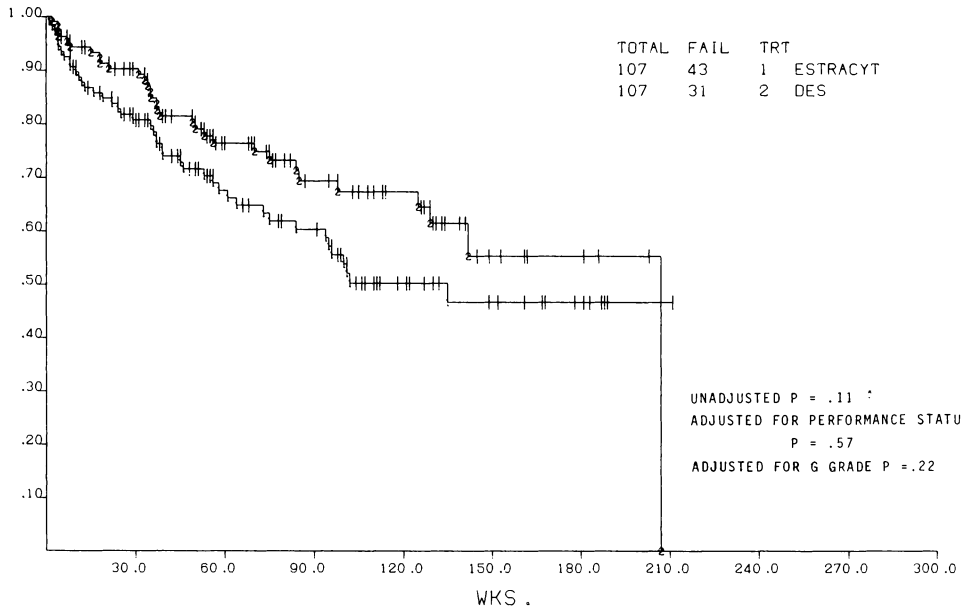

Fig. 1. Protocol 30762 - Duration of survival by treatment considering all causes of death.

CONCLUSIONS

Gastro-intestinal side effects were reported more frequently and were more severe in degree in the patients treated with Estracyt. On the other hand this trial suggests that patients with a previous cardiovascular history tend to develop more cardiovascular complications when treated with DES than with Estracyt.

So far comparisons of the response rates of the prostatic tumour and the bone metastases as well as the duration of survival have not shown any statistically significant difference between Estracyt and DES once one adjusts for the performance status.

PROTOCOL 30761 (Study Coordinator: M. Pavone-Macaluso, University of Palermo)

MATERIAL AND METHODS

Protocol 30761 is a randomized phase III study comparing Cyproterone Acetate (CPA) 250 mg/d., Medroxyprogesterone Acetate (MPA) 500 mg. I.M. 3 x weekly for the first eight weeks followed by 100 mg. orally twice a day and Stilboestrol (DES) 3 x 1 mg/d. The purpose of this trial, the patient selection criteria (Table 1) as well as the design are identical to protocol 30762 with the exception that there was no possibility of crossing over patients to another proto-

col arm. This protocol was started in February 1977 and was closed to patient entry in April 1981 at the time of this analysis.

A total of 314 patients were randomized by 29 institutions from eight countries. However four institutions representing 19 patients have thus far been excluded due to institutional problems, reducing the total number of patients under study to 295. The participating investigators with the number of patients entered are listed in Table 7. Almost half of the patients or 49% were entered by 10 Italian institutions.

Table 7. Protocol 30761 - Participating Institutions

INSTITUTIONS	TOTAL NUMBER OF PATIENTS RANDOMIZED
ITALY	
G. VIGGIANO, MESTRE	34
ZOLFANELLI, BARASOLO, VERCELLI	24
S. LEONE, GENEVA (S.M.NUOVA)	22
M. PAVONE, PALERMO	17
C. BONDAVALLI, MANTOVA	11
F. GASTALDI, GENOVA (OSP. CELESIA)	10
F. MERLO, BIELLA	8
M. PORENA, TERAMO	8
L. MARCO, TORINO	6
V. NADALINI, GENOVA (SAN MARTINO)	6
THE NETHERLANDS	
H. DE VOOGT, LEIDEN	31
J. ALEXIEVA-FIGUSCH, ROTTERDAM	21
FRANCE	
B. LARDENNOIS, REIMS	32
P. FARGEOT, DIJON	6
ENGLAND	
B. RICHARDS, YORK	11
M. ROBINSON, CASTLEFORD	9
D. NEWLING, HULL	7
Ph. SMITH, LEEDS	1
SPAIN	
M. PINEIRO, MADRID	12
L.R. ESTEVE, MADRID (CRUZ ROJA)	3
A.E. BARRILERO, MADRID (RAMON Y CAJAL)	2
BELGIUM	
C. SCHULMAN, BRUSSELS	4
C. BOUFFIOUX, LIEGE	3
PORTUGAL	
F.CALAIS DA SILVA, LISBON	5
AUSTRIA	
R. KOHLE, SALZBURG	2
T O T A L	295

Table 3 presents the distribution of patient characteristics at entry on study. Half of the patients entered the study with symptoms but were ambulatory (performance status 2), 49% were between 71 and 80 years old, 52% had metastatic disease, and 79% of the patients had a T3 tumour.

RESULTS

As of April 1981 a total of 42, 34 and 37 patients are still being treated with CPA, MPA and DES respectively. The remaining 182 patients went off study mainly because of progression (46%) or because they were lost to follow up (16%) (Table 8). Nineteen patients were ineligible and should not have been entered in the protocol while six patients were taken off treatment due to toxicity, three on MPA (pulmonary embolism, retinal atrophy, acute aortic insufficiency), two on DES (hepatic toxicity and phlebitis, thrombophlebitis) and one on CPA (Meniere's syndrome).

Table 8. Protocol 30761 - Reasons for going off study (stopping treatment)

REASON OFF TREATMENT	CPA	MPA	DES	TOTAL
STILL ON TREATMENT	42	34	37	113
PROGRESSION (Including deaths due to prostatic cancer)	26	35	24	85
LOST TO FOLLOW UP	16	8	6	30
INELIGIBILITY	4	9	6	19
TREATMENT REFUSED	6	5	3	14
DEATH (Not due to cancer)	4	2	4	10
EXCESSIVE TOXICITY	1	3	2	6
PROTOCOL VIOLATION	1	1	1	3
OTHER	3	4	8	15
T O T A L	103	101	91	295

Side Effects

Painful gynecomastia was more frequent on DES than on the other two arms being reported by 40% of the patients treated with DES against only 6% of the patients treated with CPA or MPA.

On the other hand gastro-intestinal side effects were almost negligible and did not require the treatment to be permanently stopped in any patients. Only 2% of the patients taking DES or CPA reported any gastro-intestinal side effects as opposed to 8% of the patients on MPA.

The total incidence of cardiovascular side effects reported in this study was 25%, namely 14, 23 and 38% on CPA, MPA and DES respectively (Table 9). The difference between CPA and DES is significant at P = 0.01 and indicates that patients treated with DES have a significantly higher chance of developing cardiovascular side effects than patients treated with CPA. If we now look separately at those patients without a history of cardiovascular disease at entry on study, the frequency of cardiovascular side effects reported during treatment in 8 ,26 and 32% for CPA, MPA and DES respectively and again the difference between CPA and DES is significant (P =0.02). In the patients with a history of cardiovascular disease, no statistically significant difference was observed between the treatments although the data again suggest that DES patients tend to develop more cardiovascular side effects than the patients treated with MPA or CPA.

Table 9. Protocol 30761 - Cardiovascular side effects

TREATMENT	NO HISTORY OF CARDIOVASC. DISEASE		HISTORY OF CARDIOVASC. DISEASE		TOTAL		TOTAL
	SIDE EFFECTS		SIDE EFFECTS		SIDE EFFECTS		
	NO	YES	NO	YES	NO	YES	
CPA	37	3(8%)	12	5(29%)	49	+ 8(14%)	57
MPA	28	10(26%)	21	5(19%)	49	++ 15(23%)	64
DES	25	12(32%)	11	10(48%)	36	+++ 22(38%)	58
T O T A L	90	25(22%)	44	20(31%)	134	45(25%)	179

\+ 4 cardiovascular deaths
++ 1 cardiovascular death
+++ 2 cardiovascular deaths

Of the cardiovascular side effects reported during treatment, 42% involved fluid retention: heart failure, hypertension, oedema and dyspnoea while ischemic heart disease (infarctions, R.B.B.B., ventricular ectopic beats) and venous thromboembolism (thrombophlebitis, deep venous thromboembolism, cerebrovascular accidents, pulmonary embolism) were each reported by 28% of the patients who developed cardiovascular side effects. Four cardiovascular deaths were reported on CPA as opposed to one on MPA and two on DES.

Tumour Response

Since this protocol was closed to patient entry only recently, a separate analysis of the response of the prostatic tumour and the bone metastases has not yet been carried out. Therefore, the criteria of response in this protocol are different compared to the criteria of protocol 30762, first because no distinction between local and distant disease is made and second because any increase in size of a lesion is sufficient to consider that lesion as progressive as opposed to a 50% increase in protocol 30762. The criteria of response adopted in this study are presented in Table 10. One hundred and sixty-five patients with follow up data at two months or for whom progression is known to have occurred prior to two months are included in the treatment comparison. The overall response rate (CR + PR) is 32%, namely 33, 18 and 44% on CPA, MPA and DES respectively (Table 11). The difference between MPA and DES is significant at P =0.005 in favour of DES. None of the other treatment differences are statistically significant at this time. However we would like to emphasize again that extramural review of scans and/or X-rays is planned for determing the response of the bone metastases while the response of the prostatic tumour will be reassessed according to the criteria of protocol 30762. Consequently, the response rates given in Table 11 must be considered as very preliminary. The amount of follow up data is not sufficient yet to enable a meaningful comparison of the three treatments with respect to the duration of survival.

CONCLUSIONS

Since this protocol was closed to patient entry only recently, a considerable amount of patient follow up data must still be received and the current results must be considered as being more preliminary than those reported in protocol 30762.

Painful gynecomastia was reported more frequently by the patients treated with DES while gastro-intestinal side effects were almost negligible. Patients treated with DES tended to develop more cardiovascular side effects than those patients treated with MPA or CPA, independently of whether or not a patient had a previous history of cardiovascular disease.

Table 10. Protocol 30761 - Criteria of overall response (local + distant)

COMPLETE REMISSION

1. Absence of any clinically detectable soft tissue tumour mass
2. Recalcification of all osteolytic lesions if present
3. No evidence of progression of osteoblastic lesions if any are present

PARTIAL REMISSION

1. A significant decrease in size in at least 50 % of all soft tissue lesions
   - measurable lesions : decrease of 50 % of the product of the 2 largest tumour diameters
   - non-measurable lesions : reduction of at least three-fourths of the estimated volume
2. Recalcification of some osteolytic lesions if present

- No increase in any other lesion and no new areas of malignant disease may appear
- No significant deterioration in weight (> 10 %), symptoms or performance status (one score level)
- Return of an elevated acid phosphatase to normal
- If hepatomegaly is a significant indicator, there must be a reduction in liver size and at least a 30 % improvement of all pretreatment abnormal liver function tests.

NO CHANGE

- No new lesions appear and no lesion increases in size
- No significant deterioration in weight, symptoms or performance status

PROGRESSION

- Any lesion increases in size or any new lesion appears, regardless of what the response of the other lesions may have been
- Significant deterioration in symptoms, decrease in weight or decrease in performance status
- An increase in acid or alkaline phosphatase alone is not to be considered as an indication of progression.

Table 11. Protocol 30761 - Overall response rates (local + distant)

TREATMENT	CR	PR	NC	PROGR.	TOTAL	% CR + PR
CPA	2	15	14	21	52	33
MPA	-	10	23	23	56	18
DES	7	18	18	14	57	44
TOTAL	9	43	55	58	165	32

OVERALL RESPONSE RATE : MPA VS DES, P = .005
CPA VS DES, P = .32
CPA VS MPA, P = .12

The overall response rates (CR + PR) of both the prostatic tumour and the bone metastases indicate that DES is significantly superior to MPA (P =0.005) while no other statistically significant differences have been observed.

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Editorial Note - P.H.S.

Although not directly comparable, these two trials have many features in common. They represent an attempt to compare the effectiveness of agents which are potential alternatives to conventional oestrogen therapy and which are all significantly more expensive. Also, patients entered into the studies show similar characteristics in relation to age, T, G, and M categories, prostatic size, and the incidence of pain. However, those entered to the study comparing Estracyt and Stilboestrol had a somewhat higher performance status - 65% vs 40% having a normal status.

The evaluation of the results of therapy is very difficult in patients with prostatic cancer since objective remission of the commonest metastatic lesions - those in bone - is slow and difficult to measure with accuracy.

These very preliminary results have however shown that a dose of DES of 1 mg tds carries a distinct cardiovascular risk, especially in those with a previous history of cardiovascular disease. This showed clearly in each study. However, it seems likely that patients receiving DES will prove to show the highest incidence of objective remission, a point which can be determined only at subsequent analysis where other critical factors, including time to progression and survival will be assessed.

The best treatment for metastatic prostatic cancer is still by no means clear and further studies of low dose Stilboestrol (1 mg daily), other agents, orchiectomy and chemotherapy, alone or in combination, will be required to clarify the situation.

THE VALUE OF THE ANCILLARY FACTOR SCORING SYSTEM

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INTRODUCTION

An accurate objective assessment of the response to treatment in patients with advanced prostatic cancer is not an easy task. The problems associated with the evaluation of treatment response have been considered by Eagan, Hahn and Myers (1) and Kvols, Eagan and Myers (2) who developed a scoring system which they used in an attempt to assess a patient's response to treatment. An overall ancillary score was calculated based on changes in the value of six different ancillary factors. This score was then used to assess a patient's response to treatment.

In order to determine the validity and suitability of this system for assessing response to treatment and for comparing two or more different treatments, the EORTC Genito-Urinary Tract Cancer Cooperative Group applied this scoring system to one of its ongoing trials. The purpose of this paper is to present the results of this investigation and discuss how such an index might be improved.

THE ANCILLARY FACTOR SCORING SYSTEM

The objective measurement of tumour response in prostatic cancer patients is difficult at best due to problems in accurately measuring the primary lesion and due to difficulties in interpreting successive bone scans. It is thus natural to attempt to assess a patient's response to treatment in terms of those aspects of the

disease which can be accurately and objectively measured and which are correlated with the objective tumour response.

Eagan, Hahn and Myers (1) and Kvols, Eagan and Myers (2) proposed a scoring system to be used in assessing response to treatment for patients with advanced metastatic disease. As shown in Table 1 this system is based on information provided by six ancillary factors: pain, performance status, weight, hemoglobin and the total alkaline and acid phosphatase levels. Scores are computed for each factor based on the change in the factor relative to its initial value. For a given factor, a score of zero at follow up represents a normal value for the factor and a score of one represents an improvement without returning to normal. No change in an abnormal factor is scored as a two while worsening of the factor is coded as a four. A total ancillary score is then calculated by summing the scores for each individual factor. A low total ancillary score suggests that the patient has improved during the course of the study while a high score indicates an overall worsening of the patient's condition. Kvols et al (2) found that among the seven patients who were considered to have responded using conventional evaluation criteria, all seven had total ancillary scores of less than ten.

The EORTC Genito-Urinary Tract Cancer Cooperative Group has applied a slightly modified version of this scoring system (Table 2) to data from its trial comparing Stilboestrol and Estracyt in previously untreated T3, T4 prostatic cancer patients (3). The score for weight had to be modified since only a patient's initial weight was available at entry on study and not his normal weight. In addition alkaline and acid phosphatase were considered separately and a change of 50% rather than 25% was required for the change to be considered significant.

CORRELATION OF THE ANCILLARY FACTOR SCORES WITH CLINICAL RESPONSE

Local Response

In order to determine whether or not a correlation exists between the ancillary scores and the response of the primary tumour as measured by rectal palpation, scores for each factor were calculated at the time of the local response. The guidelines of the World Health Organization (4) were used to assess a patient's response to treatment, except that an increase of 50% in the cross product of the two largest perpendicular diameters was required in order to demonstrate a progression. The following response categories were determined based on changes in the size of the prostatic lesion:

1. Complete response (CR): complete disappearance of the local tumour
2. Partial response (PR): decrease of at least 50% in the cross product of the two largest perpendicular diameters

Table 1. Ancillary scoring system (all changes relative to starting value) (2)

Ancillary factors	Scores and Changes 0	1	2	4
Pain	None	Improved	Stable	Worse
Performance status (ECOG)	Asympto-matic	Improved	Stable	Worse
Weight (%)	Normal	Increased >5	Stable (≤ 5)	Decreased >5
Hemoglobin (g/100ml)	Normal*	Increased >1	Stable (≤ 1)	Decreased >1
Total alkaline and acid phosphatase levels (%)	Normal	Decreased >25	Stable (≤ 25)	Increased >25

An overall score of ≤ 9 considered a response; overall score of 10-14 considered stable; and an overall score ≥ 15 considered progression.

*13g/100ml

Table 2. Ancillary factor scoring system studies by the EORTC GU Group

Ancillary Factors	Scores and Changes 0	1	2	4
Pain	None	Improved	Stable	Worse
Performance status	Asymptomatic	Improved	Stable	Worse
Weight	Stable or increase	-	-	Decrease $\geq 5\%$
Hemoglobin	Normal+	Increase $\geq 10\%$	Stable	Decrease $\geq 10\%$
Alkaline phosphatase	Normal*	Decrease $\geq 50\%$	Stable	Increase $\geq 50\%$
Acid phosphatase	Normal*	Decrease $\geq 50\%$	Stable	Increase $\geq 50\%$

+13g/100ml Equivalent, *Hospital limits

3. No change (NC): less than 50% increase or decrease in the cross product of the two largest perpendicular diameters
4. Progression (PROG): increase of at least 50% in the cross product of the two largest perpendicular diameters.

Patients were initially evaluated at two months and then again at six months and every six months thereafter as long as the patient remained on-study. If the response of the local lesion was CR, PR or PROG, then the ancillary scores for each factor were calculated at the moment of the response. If a patient's response to treatment was NC, then the ancillary scores were calculated at six months, or at two months if no data at six months were available.

One hundred and eighty-seven patients receiving Estracyt or DES and for whom the required data are available are included in this analysis. The complete and partial responders were grouped together for analysis purposes. Table 3 presents the local response and the corresponding total ancillary factor score calculated at the time of the response. Patients are divided into three categories according to the value of the total score: 0-3, 4-7 and ≥ 8. While this division into three groups is somewhat arbitrary, it was chosen for statistical reasons which will not be detailed here. While there is a significant correlation between a patient's response to treatment and his total score ($P = .0003$), a closer investigation of this table reveals that the total score is not very informative and cannot be used to accurately assess a patient's response to treatment. First it should be noted that 53% of the patients fall in the NC category with only a slight variation as the score increases. For patients with a total score of ≥ 8, approximately the same percentage responded as those who progressed. Thus no discrimination between responders and failures is possible in this group of patients. While in each of the categories 0-3 and 4-7 patients are more likely to respond than to progress, no discrimination between responders and patients with no change is possible. Thus from a practical point of view, knowledge of the total ancillary factor score does not allow one to accurately determine a patient's response to treatment.

An examination of the individual ancillary factor scores for each factor included in the total score reveals that only the ancillary score for the performance status is highly correlated ($P = .0006$) with the response of the local tumour. However once again one cannot accurately determine the response of the local lesion based on the knowledge of the ancillary factor score for performance status. From these analyses it is evident that knowledge of the total ancillary factor score or of any of its components cannot be used to assess a patient's response to treatment.

Table 3. Local response by total ancillary factor score

Score \ Response	CR - PR	NC	PROG	TOTAL
0 - 3	34 (48%)	32 (45%)	5 (7%)	71
4 - 7	27 (41%)	36 (55%)	3 (5%)	66
$\geq$ 8	9 (18%)	31 (62%)	10 (20%)	50
Total	70 (37%)	99 (53%)	18 (10%)	187

Test for correlation
Kendall's TAU = .23 P = .0003

Distant Response

As in the case of the local lesion, ancillary scores were calculated at the time of the response of the distant lesions for the 87 patients with bone metastases. An extramural review committee evaluated the scans and X-rays of 66 of the patients in order to determine their response to treatment. For the remaining 21 patients a review was not available and the evaluation of the local investigator was used. As there was only a small number of patients with a complete response of their distant lesions, complete and partial responders were grouped together for analysis purposes.

The distribution of the distant response to treatment according to the total ancillary factor score is presented in Table 4. Once again there is a significant correlation ($P < .0001$) between a patient's response and his total score; however the score's usefulness in assessing response remains limited. Fifty-seven percent of the patients were classified as NC with little variation according to the score.

Although only three patients with a total score of 0-3 progressed and only one patient with a score of ≥ 8 responded to treatment, Table 4 reveals that in patients with a score of 0-3, no discrimination is possible between CR-PR and NC. In addition there is a similar percentage of responders and failures in the group with a score of 4-7 and in the group ≥ 8 no discrimination between NC and PROG can be made. As before the usefulness of the total score remains limited. When analyzing each ancillary factor separately, it was found that there was a significant correlation between the response to treatment of the bone lesions and the ancillary scores for performance status ($P = .0001$), weight ($P = .0004$), alkaline phosphatase ($P = .004$) and

Table 4. Distant response (bone lesions) by total ancillary factor score

Score \ Response	CR - PR	NC	PROG	TOTAL
0 - 3	15 (41%)	19 (51%)	3 (8%)	37
4 - 7	5 (18%)	19 (68%)	4 (14%)	28
≥ 8	1 (5%)	12 (55%)	9 (41%)	22
Total	21 (24%)	50 (57%)	16 (18%)	87

Test for correlation
Kendall's TAU = .38 P = <.0001

pain (P = .005). These correlations are deceptive however as one cannot accurately determine a patient's response to treatment based on the scores for any of these variables.

ASSESSMENT OF THE ANCILLARY FACTOR SCORING SYSTEM

Although there is a significant correlation between the total ancillary factor score and a patient's response to treatment, the ancillary factor scoring system as developed by Kvols and Eagan is not recommended for use in clinical trials as an alternative method for assessing a patient's response to treatment for the following reasons:

1. This scoring system does not adequately take into consideration a patient's initial disease status. It makes no distinction between a patient who initially has an abnormal value for a factor which returns to normal at the time of response and a patient who initially has a normal value for the factor and which remains at a normal level during the course of the trial. Thus two patients with the same score might have had quite different amounts of tumour growth or shrinkage. In addition one patient may achieve a lower score than another patient simply because he was in better overall condition at the start of the study.

2. Secondly, this scoring system assumes that the six factors which comprise it are all of equal importance with respect to assessing a patient's response to treatment. It was found that only the score for the performance status was correlated with the response of the local lesion while for the distant metastases, four of the factors were found to be of importance. Yet all six factors are

given equal weight in computing the total score. In addition one might expect several of the factors to be correlated with each other so that information provided by some of the individual scores may be redundant. There is no justification for assigning equal weights to the six ancillary factors.

Since this scoring system does not adequately take into consideration a patient's disease characteristics at entry on study and since it has no statistical basis, it should not be used to assess a patient's response to treatment or to compare the relative efficacy of two or more different treatments.

DEVELOPING AN ANCILLARY FACTOR SCORING SYSTEM

In constructing an index to aid an investigator in assessing a patient's response to treatment, it is logical to restrict oneself to those factors which are of prognostic importance with respect to the endpoint under study. The prognostic importance of the baseline values of the following variables has been studied using Cox's proportional hazards regression model and linear logistic regression:

1. T category
2. M category
3. G category
4. Age
5. Performance status (E.C.O.G. scale)
6. Chronic diseases
7. Weight
8. Pain
9. Hemoglobin
10. Alkaline phosphatase
11. Acid phosphatase
12. Size of the primary lesion

Taking into account the correlation between these variables, Table 5 presents in the order of their relative importance those variables which are of prognostic importance with respect to the duration of survival and the response to treatment of the local and distant lesions. It is seen from this table that the most important ancillary factor for both survival and response to treatment is the patient's performance status at entry on study. Thus any scoring system would most likely give the greatest weight to changes in this variable. Although the size of the local lesion is positively correlated with the response rate, the significance of this variable remains unclear as only a very small change which may be due to measurement error is required for a response or progression in very small lesions.

Based on these observations one should correlate the response to treatment with changes in a patient's performance status,

Table 5. Prognostic factors in prostatic cancer

Duration of survival		Response	
All causes of death	Death due to malignant disease	Local lesion	Distant metastases
1. Performance status	1. Performance status	1. Size of local lesion	1. Performance status
2. Presence or absence of chronic diseases	2. G category	2. Performance status	2. Acid phosphatase
3. G category	3. Presence or absence of pain	3. G category	

G category, pain and acid phosphatase levels and construct an index using these variables or a subset of them. The practical problem of how to construct such an index or how to measure changes in the factors retained are important problems which will not be discussed here.

DISCUSSION

It may be questioned whether or not one should attempt to assess a patient's response to treatment by taking various ancillary factors into account or whether one should rely only on objective tumour measurements, however accurate or inaccurate they may be. Despite the fact that measurement of the primary lesion by rectal palpation is criticized as being subject to considerable measurement error, we have found a significant correlation between the response of the local tumour as measured by rectal palpation and the patient's overall duration of survival. Thus it is important to assess the response of the primary tumour even if only by rectal palpation. However newer techniques such as ultrasonography should permit a more accurate and objective evaluation of the prostate than is possible by palpation.

The EORTC Genito-Urinary Group has set up a central review of all patient's scans and X-rays in order to assess the response of the bone metastases. While the conclusions concerning the problems in interpreting successive scans and X-rays and their reliability are still pending, it is felt that the review has provided an important standardization in the assessment of the distant lesions.

No ancillary factor scoring system can at this time replace the classical techniques of tumour evaluation since no satisfactory scoring system currently exists. However the information provided by changes in factors such as a patient's performance status and degree of pain should not be neglected when assessing a patient's overall well being and quality of life as these factors provide supplementary information concerning a patient's progress under treatment.

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THE CORRELATION BETWEEN GRADING AND CLINICAL RESPONSE

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INTRODUCTION

The prognostic importance of the histological grading of prostatic cancer has received considerable attention during the past decade. Classification systems such as those developed by Gleason (1), Mostofi (2) and Gaeta et al (3) have shown a direct correlation between a patient's histological pattern and his prognosis, especially with respect to duration of survival.

The EORTC Genito-Urinary Tract Cooperative Group has adopted the histological grading system published by the International Union Against Cancer (UICC) in the TNM Classification of Malignant Tumours (4). A G grade is determined based on the worst degree of tumour differentiation which can be identified:

G0 : No evidence of anaplasia

G1 : High degree of differentiation

G2 : Medium degree of differentiation

G3 : Low degree of differentiation or undifferentiated

GX : Grade cannot be determined

The usefulness of this classification scheme as an indicator of a patient's prognosis was studied in a randomized trial comparing Estracyt to Stilboestrol in previously untreated prostatic cancer patients with advanced T3 or T4 disease (5). The purpose of this paper is to present some interim results of the prognostic importance of this grading system.

Table 1. Correlation Between Grading and Biopsy Procedure

	NEEDLE	TUR	TUR + NEEDLE	TOTAL
G1	15 (33%)	21 (23%)	8 (13%)	44 (22%)
G2	12 (27%)	49 (53%)	25 (40%)	86 (43%)
G3	18 (40%)	23 (25%)	29 (47%)	70 (35%)
TOTAL	45	93	62	200

Test for Correlation Between Grading and Biopsy Procedure

Kendall's TAU B = .13 P = .02

DISTRIBUTION OF G GRADE AT ENTRY ON STUDY

Out of the 248 patients entered in this study, data concerning the initial G grade are available for 220 patients. Table 1 presents the distribution of the G grade by biopsy procedure in the 200 G1, G2 or G3 patients for whom the grading at entry on study was determined by needle biopsy, TUR or TUR plus needle biopsy. As can be seen from the table, 22% of the patients were G1, 43% were G2, and 35% were G3. This table shows however that the G grade is correlated with the biopsy procedure and appears to depend on the amount of material available for the biopsy. Patients with a TUR plus needle biopsy have a worse average G grade than patients with either a TUR or needle biopsy alone ($P = .01$). In addition it is seen that the percentage of G1 patients decreases as the amount of material for the biopsy procedure increases, going from 33% for needle biopsy alone to only 13% for TUR plus needle biopsy.

The above analysis is however subject to bias as the choice of biopsy procedure may depend on the patient's clinical characteristics. The correlation between the biopsy procedure and the G grade might be due to the correlation of the G grade with the T classification, the M classification and a patient's performance status or perhaps with other variables not taken into account. However further analyses revealed that after adjustment for the T classification, the M classification, the performance status and the presence or absence of pain and urinary symptoms that the correlation between the biopsy procedure and the G grade persists and that the G grade thus appears to be influenced by the biopsy procedure employed. This analysis suggests that with either TUR or needle biopsy alone, a significant proportion of the patients may be understaged, thus leading to an incorrect prognosis for the patient.

Table 2. Correlation Between Grading and Response of the Local Prostatic Tumour.

	CR	PR	NC	PROG	TOTAL
G1	2	22	23	1	48
G2	5	29	33	7	74
G3	1	16	34	8	59
TOTAL	8	67	90	16	181

Test for Correlation

Kendall's TAU C = 0.165 $P < 0.01$

THE PROGNOSTIC IMPORTANCE OF THE G GRADE

The importance of the G grade in determining a patient's response to treatment was studied by first considering the G grade separately and then by considering its relative importance when other clinical variables were accounted for. Table 2 presents the correlation between the grading and the response of the primary lesion. The response criteria are based on those of the World Health Organisation (6). A 50% response rate was observed among G1 patients, 46% for G2 patients and 29% for G3 patients. A test for an overall correlation between the response to treatment and the G grade, taking the ordering of both variables into account, is significant at $P < .01$. If one considers the correlation between the grading and the response of the primary separately for each type of biopsy procedure, it was found that a significant correlation exists only for those patients who had both a TUR and a needle biopsy at entry on study. The misclassifications which may result when patients are biopsied by TUR or needle alone tend to conceal the true importance of the G grade as a prognostic factor in these patients. This analysis thus shows that the G grade is an important prognostic factor with respect to the response of the prostatic tumour.

The distribution of the response of the bone metastases according to the grading of the primary tumour is shown in Table 3. As can be seen from this table the correlation between these two variables is not statistically significant; however it is based on a relatively small number of patients and no adjustment for type of biopsy procedure has been made. Thus while the grading of the primary tumour appears to be of no direct prognostic importance with respect to whether or not the bone metastases respond to treatment, further follow up is required before a definite analysis can be carried out.

Table 3. Correlation Between Grading and Response of the Distant Metastases

	CR	PR	NC	PROG	TOTAL
G1	2	2	11	1	16
G2	1	10	8	6	25
G3	1	4	14	6	25
TOTAL	4	16	33	13	66

Test for Correlation

Kendall's TAU C = 0.128 P = 0.12

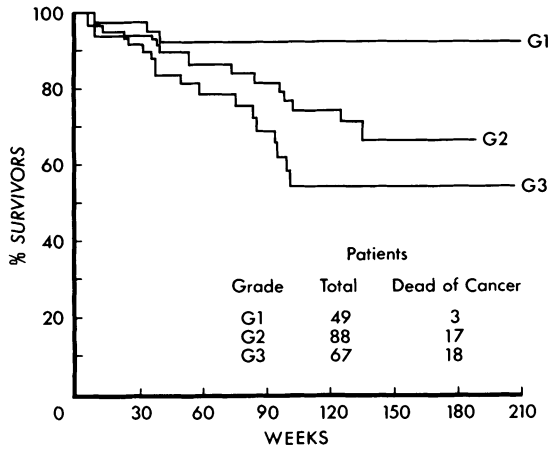

Fig 1. EORTC Protocol 30762. Duration of survival of T3, T4. Prostatic cancer patients according to G grade and deaths from prostatic cancer (April 1981).

Figure 1 presents the duration of survival by G grade where now we are considering only those deaths due to malignant disease. As seen from this figure the duration of survival is directly related to the G grade ($P < .01$, Logrank Trend Test) with the relative death rate for G2 and G3 patients being respectively twice and three times that for G1 patients. Similar results are obtained if we consider all causes of death, rather than just those due to malignant disease. When we consider the correlation between the grading and the duration of survival for each biopsy technique separately, the difference between the curves is significant at the 5% level after adjustment for the performance status only for those patients with a TUR and needle biopsy. Considerably less of a separation between the curves is noted for patients with a TUR or needle biopsy alone.

These results show that despite possible inaccuracies in assessing the G grade, which means that a certain percentage of patients will inevitably be understaged, the G grade is an important prognostic factor when considered by itself with respect to the response rate of the prostatic tumour and a patient's duration of survival. The importance of the grading would be even more dramatic if all patients were correctly staged without error since such misclassifications only mask to some degree the true prognostic importance of this variable.

No analysis would be complete however without considering the importance of the histological grading relative to other clinical variables which may influence a patient's prognosis. Any effect attributed to the grading may in practice be due to its correlation with other more important variables.

When considering the correlation between the grading and other clinical variables, it was found that the G grade was correlated with a patient's performance status ($P < .01$), the T classification ($P < .01$) the level of acid phosphatase ($P = .02$) and to a lesser extent, the M classification ($P = .06$) and the patient's degree of pain ($P = .08$). No correlation was detected between the G grade and the patient's age, weight, associated chronic diseases, the size of the primary lesion or the level of alkaline phosphatase. A lower degree of differentiation was associated with a worse performance status, a higher T classification, a higher level of acid phosphatase, a greater probability that distant metastases are present and a higher degree of pain.

Since the G grade is correlated with several variables of prognostic importance, it is essential to determine the relative importance of the G grade with respect to these variables. One must verify whether or not the G grade retains its importance as a prognostic factor once these variables are taken into account.

Linear logistic regression was used to determine the relative importance of the G grade with respect to the response of the local and distant lesions while Cox's proportional hazards regression model

Table 4. Prognostic Factors in Prostatic Cancer

Duration of Survival		Response	
All Causes Of Death	Death Due To Malignant Disease	Local Lesion	Distant Metastases
1. Performance Status	1. Performance Status	1. Size of Local Lesion	1. Performance Status
2. Presence or Absence of Chronic Diseases	2. G Grade	2. Performance Status	2. Acid Phosphatase
3. G Grade	3. Presence or Absence of Pain	3. G Grade	

was used for the same purpose with regard to the duration of survival. Table 4 presents the results of this analysis. For each endpoint, response rate or duration of survival, this table presents those variables which are of prognostic significance in the order of their relative importance. Thus if one considers death due to malignant disease, the performance status is the most important variable. However once one adjusts for the performance status, the G grade becomes the next most significant prognostic factor. Despite the correlation of the G grade with a patient's performance status, the additional information provided by the G grade over and above that given by the performance status improves one's ability to predict a patient's overall duration of survival.

This table shows that in spite of the correlation of the G grade with other prognostic factors, the G grade remains of prognostic importance with respect to the response rate of the primary tumor and a patient's duration of survival. Thus the significance of the G grade is not simply due to its correlation with other variables but it is an important prognostic factor in its own right even once these other factors have been considered. Thus assessment of the G grade plays a vital role in the patient's evaluation. Despite the inaccuracies inherent in its determination, the G grade is shown here to be more important than either the T or M classification in predicting a patient's local response or duration of survival.

The EORTC attempted in this protocol to correlate the local tumour response with the change in the G grade at eight weeks. However since there was no standardization of the successive biopsies,

it is not surprising that no significant correlation was detected. While a needle biopsy was routinely performed at eight weeks, only 22% of the patients had their G Grade at entry on study determined by a needle biopsy alone. For this reason no conclusions can be drawn concerning the usefulness of a follow-up biopsy in assessing a patient's response to treatment.

DISCUSSION

Due to its prognostic importance, it is mandatory that the G grade be accurately assessed in all patients with prostatic cancer. This requires a standardization of biopsy techniques and a central review of all slides. Only in this manner can a patient's prognosis adequately reflect the importance of the histological grading and the appropriate treatment be chosen. Without this knowledge we may be under staging and under treating our patients and neglecting what is one of the most useful indicators of a patient's overall prognosis.

Editorial Comment (C.B. and P.H.S.)

This paper clearly emphasizes the importance of the G grade in the prognosis of prostate cancer. We were however surprised to find no correlation between grade and type of biopsy or amount of tumour thereby made available. This might be due to: a) Selection of patients - those who have a TUR having a more advanced local tumour, or b) Alteration of the tissue sample by electroresection leading to the appearance of a more undifferentiated tumour.

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CRITERIA FOR EVALUATING RESPONSE TO TREATMENT IN PROSTATIC CANCER

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ABSTRACT

Better delineation of criteria for both objective and subjective responses to treatment of prostatic cancer will aid in evaluating new cytotoxic agents as well as combinations of endocrine manipulation and chemotherapy. The inclusion of the radionuclide bone scan in such criteria will help in the evaluation of the many patients with skeletal involvement only.

The need for criteria of response to treatment of prostatic cancer has arisen only in the past few years. Between the early 1940's and the early 1970's when endocrine manipulation (bilateral orchiectomy and/or exogenous estrogens) was the only treatment available for advanced prostatic cancer, only a rare patient could be identified as becoming tumor-free. When endocrine therapy was started, generally in patients suffering from some clinical disability, e.g., bone pain or urinary tract obstruction, a beneficial response of uncertain duration could be predicted in about 75% of men. Clinical responses were most often subjective in nature including alleviation of pain, improvement in voiding, weight gain and a feeling of well-being.

Another significant problem with which many cooperative groups and individual investigators have been grappling recently, is the difficulty in making objective assessments of a patient with advanced prostatic cancer (1). The primary tumor, if not removed by total prostatectomy, is notoriously difficult to measure. Notwithstanding recent attempts utilizing ultrasonography or computerized tomography, the margin of error in such measurements is great. Soft tissue lesions (enlarged lymph nodes, pulmonary

nodules and other masses) are infrequently seen even in advanced prostatic cancer; thus few patients in this category are available for treatment and assessment of their responses.

The use of the serum acid and alkaline phosphatase activities for assessment of response to therapy has had its pitfalls. Perhaps 20 to 25% of patients with Stage D prostatic cancer will never demonstrate an elevated serum acid phosphatase by any method. Changes in elevated serum acid phosphatase activity, whether increases or decreases, have been interpreted by varying individual criteria, e.g., serum acid phosphatase activity decreasing to normal and for various durations or simply a decrease in activity to a level greater than 50% of the pretreatment value.

The alkaline phosphatase activity is also unreliable since the source of this enzyme may be other than bone. Important to note is the classical paradoxical increase of an already elevated serum alkaline activity in response to hormonal therapy; here the increased activity represents a "healing reaction" of normal bone. Yet in the same patient, at a later date, an increased alkaline phosphatase activity may represent increasing osseous metastatic disease.

Until the advent of reproducible high-quality technetium bone scanning, the common osteoblastic metastases of prostatic cancer were followed as unreliable indicators of disease activity vis a vis response to treatment. Osteoblastic metastases often increase dramatically in patients who otherwise are "doing well" (2), and only a rare patient demonstrates complete disappearance of such metastases. The less common osteolytic metastases need to be observed for recalcification, a somewhat subjective interpretation.

In the United States, the National Prostatic Cancer Project (NPCP) has focused on these problems by setting down criteria in various categories of objective response: complete response (CR), partial response (PR), stable disease (S) and progression (P). These criteria have slowly evolved and continue to be modified (3). In addition, criteria for subjective responses have been formulated. The current NPCP criteria for objective and subjective responses are presented in Table 1-5. Recently the NPCP has demonstrated that survival is similar for patients considered stable and in partial regression compared to those in progression according to the response criteria as listed (4).

To further the input of radionuclide bone-scanning in evaluating treatment responses, the NPCP in 1980 held a workshop to study this aspect of diagnosis (5). Criteria of response utilizing the bone scanner are listed in Table 6. Employing these criteria solely, we recently evaluated 41 patients with stage D prostatic cancer receiving various treatment including chemotherapy and/or hormones and compared their responses to survival. The Figure identifies the

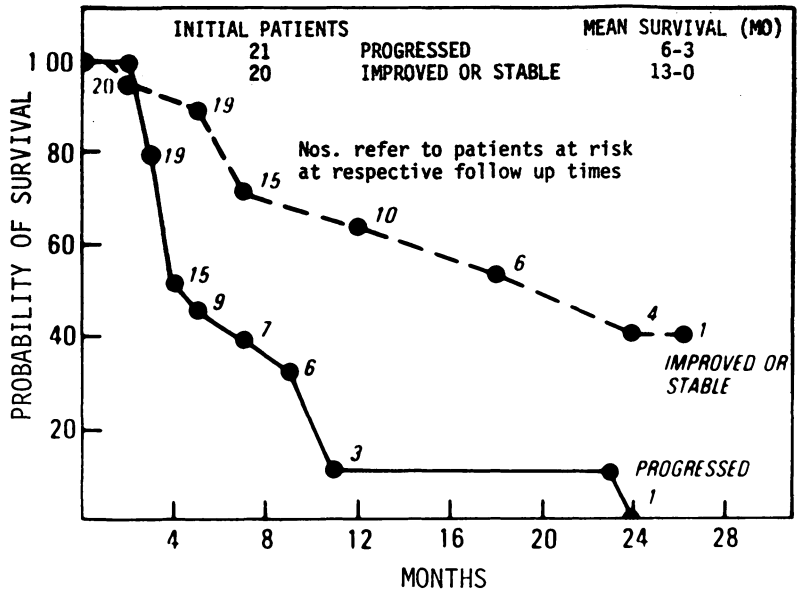

Fig. 1. Survival of 41 patients with stage D prostatic cancer evaluated according to response on radionuclide bone scan.

Table 1. Criteria for Complete Objective Regression (NPCP)

(All of the following must be present)

1. Tumour masses, if present, totally disappeared and no new lesions appeared. The primary tumor is included.

2. Elevated acid phosphatase, if present, returned to normal.

3. Osteolytic lesions, if present, recalcified.

4. Osteoblastic lesions, if present, disappeared.

5. If hepatomegaly is a significant indicator there must be a complete return in liver size to normal, i.e., no distention below both costal margins at the mid-clavicular lines and from the tip of the xiphoid process during quiet respiration without liver movement, and normalization of all pretreatment abnormalities of liver function including bilirubin mg/dl and SGOT.

6. No significant cancer related deterioration in weight ($>10\%$), symptoms, or performance status.

Table 2. Criteria for Partial Objective Response (NPCP)

(All of the following)

1. At least one tumor mass, if present, is reduced by > 50% in x-sectional area.
2. Elevated acid phosphatase, if present, returned to normal.
3. Osteolytic lesions, if present, underwent recalcification in one or more, but not necessarily all.
4. Osteoblastic lesions, if present, did not progress.
5. If hepatomegaly is a significant indicator, there must be at least a 30% reduction in liver size indicated by a change in measurements, and at least a 30% improvement of all pretreatment abnormalities of liver function, including bilirubin mg/dl and SGOT.
6. There may be no increase in any other lesion and no new areas of malignant disease may appear.
7. No significant cancer deterioration in weight (> 10%), symptoms, or performance status.

Table 3. Criteria for Objective Stable Disease (NPCP)

(All of the following)

1. No new lesions occurred and no measurable lesions increased more than 25% in x-sectional area.
2. Elevated acid phosphatase, if present, decreased.
3. Osteolytic lesions, if present, did not appear to worsen.
4. Osteoblastic lesions, if present, remained stable.
5. Hepatomegaly, if present, did not appear to worsen by more than a 30% increase in the measurements, and hepatic abnormalities did not worsen including bilirubin mg/dl and SGOT.
5. No significant cancer related deterioration in weight (> 10%), symptoms, or performance status.

Table 4. Criteria for Objective Progression (NPCP)

(Any of the following)

1. Significant cancer related deterioration in weight (>10%), symptoms or performance status.
2. Appearance of new areas of malignant disease.
3. Increase in any previously measurable lesions by greater than 25% in x-sectional area.
4. Increase in osseous metastases as shown by scan.

Note: An increase in acid or alkaline phosphatase alone is not to be considered an indication of progression. These should be used in conjunction with other criteria.

Table 5. Criteria for Subjective Response (NPCP)

(At least 2 of the 3 criteria must be present)

1. Weight gain of 3% in any 4 week period without evidence of edema.
2. Correction of anemia (hemoglobin > 10.5 grams%).
3. Relief of pain on a scale 1-4, with 4 being maximum relief.

Table 6. Objective Response Criteria Utilizing Bone-Scanning

Complete Response (CR):		No evidence of metastases
Partial Response (PR):		(a) Reduction in intensity of uptake
	or	(b) Reduction in extent of uptake
	or	(c) Disappearance of some metastases
	and	(d) No new lesions
Stable (S):		No significant change in uptake pattern.
Progression (P):		(a) Increase in intensity of uptake
	or	(b) Increase in extent of uptake
	or	(c) Appearance of new lesions

strikingly improved survival of patients considered as objectively improved or stable via bone scan criteria compared to those patients considered in progression. Clearly this is a preliminary observation and will need to be clarified and confirmed by other investigators. Problems to be worked out include standardization of scanning techniques and equipment, differences in radiopharmaceuticals, time intervals between scanning procedures and reproducible means of quantitation of bone scan changes.

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Editorial Note (P.H.S.) This paper and that by the same author on combinations of chemotherapy and hormones in prostatic cancer illustrates some of the work of the National Prostatic Cancer Project. It is clear that the NPCP is as concerned as the EORTC about the problems of evaluation of response in prostatic cancer.

The concept of stable disease as an objective response is attractive but even though it is clear that patients showing stable disease survive longer than those who continue to progress, it is

by no means certain that this finding can yet be attributed to the treatment given.

The criteria of response as listed in the two articles vary slightly and presumably patients with recurring anaemia or who develop ureteral obstruction will no longer be regarded as progressing. This is likely to increase the number of patients with "stable disease" but may reduce any difference in survival between the two groups. It is unlikely that cytotoxic or other therapy will be of real value unless the patients show large numbers of complete or partial objective responses and it may be preferable to limit the term "objective response" to these two categories.

CANCER OF THE KIDNEY

AETIOLOGY OF KIDNEY TUMOURS

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Surveys of possible aetiological factors were made by one of us in 1963 and in 1967 (1,2), reviewing 441 articles hoping to find some clues to the causes of tumours of the kidney.

It is apparent that, from the aetiological standpoint, renal cell carcinoma must be kept separate from nephroblastoma, transitional cell carcinoma, sarcoma and other types of renal neoplasia, although in rare instances the same cause was experimentally found to be responsible for the occurrence of renal tumours of different histotypes. For instance, benzpyrene was found to produce renal cell carcinoma, kidney sarcoma or squamous cell carcinoma of the renal pelvis, depending on the experimental animal or on the route of administration.

Apart from this, and other exceptions, it was apparent that adenoma and adenocarcinoma (renal cell carcinoma) were often caused by the same factors, whereas Wilms' tumours and the other neoplasias were related to different aetiological factors.

1. Nephroblastoma Nephroblastoma is not infrequently congenital and bilateral. It can be associated with various congenital malformations especially hemihypertrophy and aniridia. It has been suggested that the presence of aberrant embryonal germ cells or the abnormal growth of multipotent cells during foetal life, might be responsible for their formation. Heredity may represent a predisposing factor, whereas geographical and environmental conditions, including chemical carcinogens and hormones, do not appear to play a significant role in their aetiology. It is extremely difficult to produce Wilms' tumour experimentally, although a virus-induced nephroblastoma could be obtained in the fowl by the myeloblastosis

avian tumour virus (3).

2. Squamous Cell Carcinoma has been described in patients as a late complication of retrograde pyelography with a radioactive compound, thorotrast. Squamous cell carcinoma has also been produced experimentally by the intrarenal administration of chemical carcinogens. Renal stones, infection and other factors producing "chronic irritation" are likely to play a role in the development of both squamous cell carcinoma and mucus-secreting adenocarcinoma of the renal pelvis.

3. Transitional Cell Carcinoma of the renal pelvis can be produced by the same carcinogens, either exogenous or endogenous, that are held responsible for the induction of bladder tumours. Papillary tumours of the renal pelvis have been produced experimentally using 2-naphthylamine and have been detected in workers exposed to industrial carcinogens. Urothelial carcinoma of the renal pelvis has often been found in association with two types of chronic interstitial nephritis, namely Balkan nephropathy, and nephritis due to prolonged use of phenacetin-containing analgesics.

4. Renal Adenoma and Adenocarcinoma A more detailed discussion will be devoted to the spontaneous occurrence of renal adenocarcinoma in animals, as well as to the various aetiological factors that have been investigated in different animal species. Adenoma and adenocarcinoma will be considered together, as we believe that there is no clear distinction between the two conditions and that the traditional differentiation based only on the size of the tumours is arbitrary. In addition, both adenoma and carcinoma can be produced by the same carcinogen in various animal models and serial transplantation of renal adenoma may give rise to a frankly malignant carcinoma. In the following lines only the term "adenocarcinoma" will be employed. It includes adenoma and is considered synonymous with "renal cell carcinoma."

A. Spontaneous Renal Tumours in Animals

Spontaneous renal tumours are relatively rare, both in wild and domestic animals. Among the latter ones, poultry, horses and swine are affected most frequently. Although mixed tumours, similar to Wilms' nephroblastoma and renal urotheliomas have been occasionally found, only adenocarcinoma will be considered here. The incidence of renal adenocarcinoma in rats ranges between 0.0004 to 0.2% in various strains. Genetic as well as hormonal factors seem to play a role in a strain of Wistar rats in which 24% of males and 51% of females were affected (4). Similarly in mice the overall incidence is 0.02% (5), but a given strain was found to be affected in about one half of the animals (6). Spontaneous adenocarcinoma has been found in 0.5% of hamsters (7). The occurrence of renal adenocarcinoma in frogs will be discussed as an example of viral aetiology.

B. Association of Renal Tumours with Other Pathological Conditions

Horseshoe kidney and other congenital malformations are occasionally associated not only with nephroblastoma but also with renal cell carcinoma. According to Patoir (8), who collected 30 such cases in the medical literature up to 1959, the incidence of renal tumours of horseshoe kidneys is higher than in the average population. Unilateral or bilateral renal cancer has also been observed in association with polycystic disease of the kidneys (9, 10), multilocular cystic disease (11), diabetes mellitus (12) and a variety of other diseases (2). It has also been suggested that adenomas are more likely to arise in atrophic kidneys with focal tubular hyerplasia than in normal organs. It is difficult to be certain that such associations are due to a cause-effect relationship, rather than merely being coincidental. There are however two conditions in which renal tumours, often bilateral, are significantly more frequent than in the non-affected population: a) renal hamartomas in Bourneville's tuberous sclerosis and b) renal adenocarcinomas in von Hippel-Lindau's haemangioblastoma. For an extensive bibliography concerning these pathological associations, see refs. 1 and 2.

C. Possible Aetiological Factors for Renal Adenocarcinoma

Such factors include race, heredity, viruses, alterations in the host's immunological reactivity (renal transplantation), irradiation, hormones, endogenous and exogenous carcinogens, smoking habits, beverages, food and other environmental factors. A detailed discussion of these factors is beyond the scope of the present article. For detailed information and bibliography the reader is referred not only to our previous reviews (1,2) but also to more recent papers on closely related topics (13,14). Only brief comments and recent references will be given here.

C1. Race, epidemiology and other environmental factors

Epidemiological studies, with regard to renal cancer, are relatively scarce (15). It does not seem, however, that racial factors play a major role. In the United States no significant difference in the frequency of renal tumours was observed among Caucasian, Negro and Mexican ethnic groups, although the incidence in the Japanese population has been reported to be slightly higher. As far as geographic distribution is concerned, only minor fluctuations have been described. Comparison of mortality rates from renal malignancies in selected countries permitted a distinction between three different groups (16). a) countries showing low rates (Ireland, Italy, Japan,, Spain, Venzuela) b) countries showing high rates (Denmark, Norway, Scotland, New Zealand) c) countries with an intermediate incidence (Belgium, Holland, France, England, Wales, Australia, the U.S.A.). It can be inferred that the highest frequency of carcinoma of the kidney is found in the industralized countries of

western Europe, especially in the northern areas (Scandinavia and Scotland) and, to a lesser degree, in the U.S.A.

Urbanization and high social class appear to be associated with a comparatively high incidence of renal cancer. This does not appear to be related to animal protein but rather to fat intake. A positive correlation with obesity has been suggested. No correlation with coffee consumption has been found. The role of smoking is still controversial, although some reports show a greater incidence of renal tumours not only in cigarette but especially in cigar and pipe smokers. This may depend, at least in part, on the fact that tobacco smoking leads to increased absorption and inhalation of "renal carcinogens", such as methylnitrosamine and cadmium, as well as to enhanced urinary excretion of 3-hydroxyanthranilic acid and other abnormal tryptophan metabolites. Occupational hazards should also be considered and workers involved with coke ovens appear to be especially at risk (17).

C2 Heredity This is not usually considered of primary importance in this context, although genetic factors have been shown to play a definite role, not only in rats and mice, but also in Rhesus monkeys. In the latter species, a family has been described in which four animals were affected by renal adenocarcinoma (18).

In humans there are also several observations of renal adenocarcinoma in members of the same family. Brinton in 1960 (19) described a family in which at least four persons died from kidney carcinoma in two different generations. Ten people in three generations were found to be affected in a more recent report (20). The role of genetic factors is stressed by the higher incidence in subjects with blood group A, as well as by the reports of cases of hereditary renal cell carcinoma associated with chromosome translocations (21) and with colour blindness (22). Reddy (23), in addition to his own observation of bilateral renal cell carcinoma in a father and his two sons, reported 114 cases of familiar renal cancer that he had been able to find in the English literature up to 1981. The problem of heredity in renal cancer should be investigated with greater attention in the future, especially in cases of bilateral tumours.

C3 Irradiation Whole body irradiation induced renal adenocarcinoma in 30% of intact mice and in 53% of animals with single kidneys (24). Fast neutrons lead to similar results. A case of renal adenocarcinoma following retrograde pyelography with thorotrast, a colloidal suspension of radioactive throrium dioxide, has also been described (25), in addition to the more numerous cases of squamous cell carcinoma, which can be ascribed to the same aetiological factor. Radioactive compounds, such as Polonium-210 and Strontium 90, have also been incriminated in renal carcinogenesis.

C4 Viruses The viral aetiology of a spontaneous renal carcinoma (Lucké's tumour) occurring in the North American leopard frog (Rana pipiens) has been demonstrated beyond doubt. The lesions are well differentiated adenocarcinomas which, under favourable conditions, metastasize in 80% of cases. When adult frogs are kept in the laboratory for eight months, the incidence may be as high as 25%, whereas the field incidence is between 3 and 9%. An updated review was presented in 1980 by Beckley (26). Already in 1938, Lucké (27) had demonstrated that this tumour is strictly species-specific. If its cells or their extracts are injected into green frogs (Rana clamitans), bull frogs (Rana catesbiana), or even into a different sub-species of Rana pipiens, none of the frogs of foreign species develop renal cancer. The aetiological agent has been identified as a herpes simplex virus (28). Interestingly enough, in a recent investigation by Cocchiara, Tarro et al (29) herpes simplex virus (HSV) specific antigens were identified in a human adenocarcinoma of the kidney. As the presence of HSV tumour- associated antigens has been described in a variety of human tumours, it remains uncertain whether or not this really represents the demonstration of their viral aetiology. Renal tumours can also be produced by the polyoma virus, the simian virus (SV 40) and by the adenovirus-7 in hamsters and in other rodents. They are usually different from adenocarcinoma, a sarcomatous pattern being the most frequent histotype.

C5 Hormones It has been known for many years that renal adenomas and adenocarcinomas can be provoked by prolonged administration of both natural and synthetic oestrogens in the male golden Syrian hamster. A recent report suggests that renal tumours can also be consistently induced in the European hamster (30). It appears that the male hamster is the only animal species in which renal tumours are caused by oestrogens. If administration of hormones is stopped even after long periods of time, regression of the tumours will take place. These tumours are similar to adenocarcinoma of the human kidney, including the high lipid content of the tumour cells. They are often multiple and bilateral. Their growth is initially expansive and eventually becomes infiltrative. True metastases are rare. If the oestrogenic treatment is extended for at least 250 days, kidney tumours develop in about 97% of treated males. No tumours appear in organs other than the kidneys. Oestrogen-induced renal adenocarcinoma may develop, not only in intact male hamsters, but also in castrated males. In females, oestrogens can induce renal tumours only before the onset, or after the cessation, of sexual activity.

The tumours can be serially transplanted into other male hamsters provided the recipient animals are also treated with oestrogens. Such hormone-dependence, however, can be lost after several passages in animals, so that, after many transfers, takes can be obtained even if the recipient animals are not given oestrogens.

This animal model lends itself to attempts at treatment with a variety of hormone manipulations. Thus, it has been shown that deoxycorticosterone inhibits the growth of renal carcinoma in oestrogen-treated male hamsters (31). By transplantation of an oestrogen-independent tumour into animals treated with various hormones or submitted to endocrine ablation procedures (32), it has appeared that a) cortisone produces marked tumour inhibition, b) a progestational agent, if used without the concurrent administration of cortisone has comparatively little effect upon the tumour growth rate, c) adrenalectomy produces growth reduction, d) orchidectomy is followed by inhibition of tumour development and by prevention of further growth in established transplants, e) the effect of orchidectomy can be abolished by the administration of testosterone or oestrogens, f) bromocryptine inhibits primary renal tumours induced by DES. The above-mentioned experiments have been followed by many attempts to treat human renal adeonocarcinoma with hormones. Medroxyprogesterone acetate, testosterone and cortisone or related steroids have been used for this purpose. Human renal adenocarcinoma has long been considered to be responsive to hormonal treatment and even to be a hormone-dependent tumour. This view was recently supported by the finding of hormone receptors in normal kidney tissue and in renal cancer (33). This has however been challenged in the last few years, due to conflicting clinical and experimental results (34). This topic will form the object of a more thorough discussion elsewhere in this book.

There seems to be little doubt that human renal adenocarcinomas are under some sort of hormonal influence. There is a significant sex ratio, the tumour being more frequent in males than in females. In the series of Pignalosa and Fernandez (35) 69.9% of renal tumours were found in males. This difference tends to increase after menopause, reaching a male to female ratio of 4:1. The dependence of "hypernephromas" on endocrine disturbances has often been postulated. It was claimed, for instance, that renal adenocarcinoma is more frequent in patients with hypercorticism or other hormonal imbalance states than in the average population. It has also been hypothesized that spontaneous regression of renal tumours may be under the influence of endocrine changes (36). The relationship between hormones and renal cancer remains a controversial issue. Oestrogen-induced renal carcinoma in the male hamster is an important experimental model but this animal is unique in showing this particular behaviour. The gap still remains to be filled between laboratory animals and human beings and, in our view, it is doubtful whether conclusions drawn from investigations performed in the hamster can be safely applied to renal tumours in man.

C6 Chemicals Endogenous carcinogens may be involved not only in the production of urothelial tumours but also in that of renal parenchymal cancer. A case of an adenocarcinoma associated with increased urinary excretion of Tryptophan metabolites was described

in 1963 (37) but, to our knowledge, this finding has not been confirmed in subsequent years. This remains, however, a very interesting avenue for future research. A larger number of observations has been obtained with regard to exogenous chemical carcinogens. A table with a long list of substances having been tested for renal carcinogenic activity was presented in our review of 1967 (2). Further data from the current literature were reviewed by Sufrin in 1980 (14).

It has been shown that several exogenous chemical carcinogens are able to produce renal tumours in various laboratory animals. There are also some interesting clinical observations that this may also be true in the human being. Among the substances that are carcinogenic for the kidney, nitroso-compounds, aromatic amines, hydrazines, alkylating agents, anticancer chemotherapeutic agents, metals and natural compounds deserve special attention. A few miscellaneous agents will also be considered.

a. N-nitroso compounds

Nitrosamines are currently employed in rubber vulcanization and textile fibre industries.

Dimethylnitrosamine (DNM) has for many years been known to be a potent oncogenic agent. The chronic administration of DMN to the rat gives rise to degenerative changes and to neoplasms in the liver and lungs. In acute experiments with high doses of DMN, the liver undergoes marked regression without secondary tumour formation. Instead, renal adenoma or adenocarcinoma appear in about one half of the surviving animals. Even a single dose of DMN will produce renal tumours in 63% of female rats, in spite of the fact that DMN is rapidly excreted and metabolized. In other animal species, DMN feeding induces renal adenocarcinoma in 72% of Swiss mice, but is ineffective in European hamsters. The renal tumours induced by DMN and related compounds are of two main histological types, i) a differentiated neoplasm, resembling clear cell carcinoma and ii) an anaplastic infiltrating tumour.

Diethylnitrosamine, if given by a single intravenous bolus to Sprague-Dawley rats induces bilateral and multifocal renal tumours in 30% of males and 80% of females. Tumours tend to be present in other organs too. The same compound is also effective in inducing renal adenocarcinoma in either sex in the golden Syrian hamster.

Nitrosomethylurea gives rise to anaplastic or sarcomatous renal tumours if administered to adult rats, whereas adenocarcinoma can be obtained in 25% of Wistar rats treated at birth or within the first days of life. Similar results were obtained in mice.

Nitrosethylurea Following treatment with this compound rats

develop nephroblastoma, but mice develop adenocarcinoma. This is another interesting confirmation that the same oncogenic agent can produce various tumour histotypes if employed under different experimental conditions and in different animal species.

b. Aromatic amines

4 fluoro-4-aminodiphenyl This amine induces renal adenocarcinoma in 80% of treated male Wistar rats. They are often bilateral and multifocal and are usually associated with tumours of other organs. Similar tumours that can be serially transplanted are induced in rats by a closely related compound, N-(4-fluoro-4-biphenyl) acetamide.

2-Acetylaminofluorene This compound is a well known carcinogen which is responsible for the induction of transitional cell carcinoma from the urothelium of bladder and renal pelvis. It can also induce renal adenocarcinoma in 10% of treated rats.

c. Hydrazines

Renal adenocarcinomas were obtained in 80% of Sprague-Dawley rats treated with formic acid-nitrofuryl-thiazolyl hydrazide (FNT). The other hydrazines tested so far appear to be less effective.

d. Alkylating agents

An alkylating agent, the flame retardant tris (2,3-dibromopropyl) phosphate (TBP), if given in high doses, is capable of inducing renal adenocarcinoma in 39% of rats and in 21% of mice. As recently pointed out by Reznick et al (38) the use of flame retardants has greatly increased in recent years, following the introduction of U.S. federal regulations concerned with fabric flammability, particularly those requiring treatment of all children's sleepwear. In addition, TBP is used in carpets, plastics, house furnishings and in building materials. The oncogenic effects of TBP in rodents occur not only after oral administration, but also if it is painted on the skin of experimental animals. This has created great concern in the United States for fear that a carcinogenic substance might enter the bodies of children by being absorbed through the skin or by being ingested by children sucking their clothing. TPB was therefore banned from commerce starting April 1977. Interestingly enough, large quantities of children's pyjamas treated with flame retardants, are now being exported from the U.S.A. to Europe. This fact has even led to an official investigation in March 1981 by a member of the European Parliament. Flame retardants are certainly toxic. They cause testicular atrophy and chronic interstitial nephritis in rabbits. If traces of TPB (1 p.p.m.) are added to the water containing goldfish, all the fish die within five days. There is however no proof until now that TBP and related compounds are responsible for the induction of renal cancer in man.

e. Anticancer agents

Streptozotocin, administered by a single intravenous dose, induces diabetes mellitus immediately and causes delayed renal adenocarcinoma in 50% of treated male Holzinan rats. Only 7% of Wistar rats are affected, suggesting an important strain difference.

Daunorubicin an anthracycline anti-tumour antibiotic closely related to doxorubicin (adriamycin), if administered to Sprague-Dawley rats, has been reported to produce chronic glomerulonephritis in nearly all animals, as well as renal adenocarcinoma in 21% of treated rats (39).

f. Metals

Lead In 1962 Kilham et al (40) reported that a high prevalence of renal tumours had been observed in wild rats living in a suburban industrial area, in the vicinity of burning refuse dumps containing substantial amounts of lead. The lead content of various tissues was abnormally high and typical lead inclusions were discovered in the renal tubular cells of these animals. Furthermore, it was demonstrated that prolonged administration of lead acetate or phosphate results in the development of kidney adenocarcinomas in the rat. Hyperplastic and cystic lesions occur after short periods of treatment. Such tumours can be obtained only in rats and mice, probably because of their tolerance of high doses which would be lethal to other animals.

Attempts to ascertain if chronic lead intoxication is a factor in the development of renal tumours in man have yielded negative results so far. Despite marked reduction of saturnism in the typographic industry, chronic lead poisoning is still possible. Work involving soldering, painting, battery changing and insecticide spraying are particularly hazardous from this standpoint. Heavy air pollution in most cities, especially since lead tetraethyl is used as an additive to petrol, is a factor to be kept in mind. This may be a possible explanation for the higher incidence of renal cancer in urban than in rural areas. However, follow up studies of workers exposed to lead vapour inhalation have failed to demonstrate any greater incidence of renal tumours among those workers than in the general population.

Cadmium A relatively high incidence of renal cancer has been described in workers exposed to cadmium poisoning (15). This metal is also being suggested as a possible oncogenic factor for prostatic adenocarcinoma. It is of interest that the incidence of both cancers is especially high in Sweden where chronic cadmium poisoning represents a very severe problem. Exposure to this substance may occur in the manufacture of almost all industrial products from cameras to aircraft brakes, from semi-artificial agricultural

fertilizers to dyes and washing machines. It has been reported that Sweden actually uses from 80 to 100 tons of cadmium yearly and that the manufacturing process often gives rise to highly toxic clouds. It has also been calculated that its concentration in the ground rises yearly by a factor of 0.5%. It has more than doubled within the last 40 years. A similar trend has also been observed in other countries. This should lead to a more careful search for a correlation between exposure to cadmium and renal oncogenesis so that timely preventive measures can be taken, if this correlation can be confirmed.

g. Natural products

Cycas Circinalis is a palm-like plant which is used as a source of starch among the Chamorro, the indigenous population of Guam, Mariana Islands. There is evidence to indicate that the seed of this plant can induce a high yield of kidney and liver tumours when incorporated in the diet of rats. The renal tumours correspond even ultrastructurally to human adenocarcinomas (41). The responsible substance is a glycoside named cycasin. Its hydrolysis liberates the active aglycone, methyl-azoxy-methanol (MAM). Intraperitoneal MAM induces renal adenocarcinoma or, less frequently, a renal sarcoma in 78% of rats. The tumours are bilateral in 57% of cases. The incidence of MAM on cycosin-induced tumours is much lower in mice and hamsters.*

Aflatoxin produced by the fungus Aspergillus flavus which not rarely contaminates peanut meal has also been shown to be able to produce renal adenocarcinoma in rats.

h. Miscellaneous compounds

A list of substances experimentally found to be capable of inducing renal cancer would not be complete without ochratoxin A, safiole, niridazole, urethane and elaiomycin. The potent and almost ubiquitous polycyclic aromatic hydrocarbon carcinogens, benzpyrene, 20-methylcholanthrene and dibenzanthracene are rather ineffective as renal oncogens since less than 1% of treated animals develop kidney adenocarcinomas.

Finally a recent WHO report, quoted in the Bulletin of the Italian Ministry of Health (41) suggests that chloroform is also a relatively potent agent responsible for experimental induction

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* There is not yet any clear-cut evidence that the population of Guam presents a strikingly high incidence of renal cancer. However the frequently held view that normal, unadulterated foodstuff is always free from carcinogenic hazards is no longer tenable.

of renal adenocarcinoma in rats and mice. In the rat, renal cancer was obtained in 8% and 24% of animals treated with daily doses of 90 and 180 mg/kg respectively. Higher doses induced liver carcinoma. In the mouse renal adenocarcinoma developed in 10-25% of males treated with 60 mg/kg/day. Chloroform has therefore been considered as a potential carcinogenic agent in man and the U.I.C.C. has suggested that all pharmaceutical products containing chloroform should be withdrawn from free commercial distribution. This recommendation has been put in practice in Canada, the U.S.A., Japan, Norway, Poland, Sweden and Switzerland. The European Community has however not restricted the use of tooth paste containing 4% chloroform, postponing final decisions until January 1981. In the United Kingdom a maximum chloroform concentration of 0.5% is still allowed in pharmaceutical products pending more definitive information about its actual carcinogenic risk in man. Again, as noted with regard to flame retardants, no controls or restrictions are requested in European countries belonging to the Common Market with regard to products imported from abroad.

Conclusions

In conclusion, a few interesting animal models have emerged from the study of spontaneous and experimental animal tumours. They are appealing, in spite of the obvious reservations and limits, for the investigation of potential forms of treatment (43). They have however given us relatively little insight into the unsolved problem of aetiology of renal cancer in man. Important information on the early events in renal carcinogenesis has resulted. It is of interest that damage to the tubular cells appears to be the first recognizable event and that the appearance of cystic lesions often precedes that of adenoma and adenocarcinoma in a few experimental renal tumours. Species or strain specificity and sex also appear to play a significant role. It is likely that in man heredity, coexistence of other diseases, smoking and, in rare instances, irradiation represent possible aetiological factors. It also appears that hormonal, chemical and other environmental factors can play a role. The importance of herpes virus in renal adenocarcinoma needs to be elucidated. Many suspects are at hand, but a major villain still remains to be identified. It is hoped that further research will be continued, so that data obtained from experimental work can lead to a better understanding of aetiology and hopefully to clues for prevention of human renal cancer.

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RENAL CANCER: IN VITRO MODELS AND ANIMAL TUMORS

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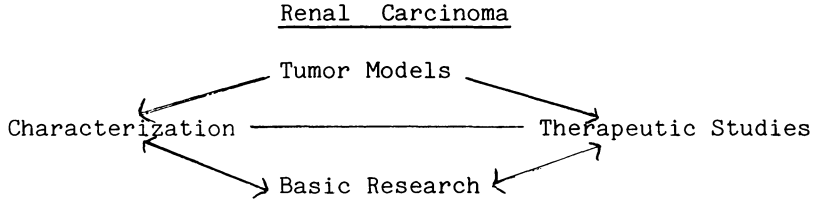
Model systems for human tumors are necessary tools of basic and clinically related research that can not be carried out in humans. It is desirable that these models reflect the properties of the human tumor as closely as possible. Before assuming that results obtained with a tumor model are relevant to the management of patients, a model system has to be characterized, and the similarity of the model with the human situation has to be firmly established. In the case of human renal carcinoma (RC) research with tumor models could be oriented in the directions indicated in this diagram:



Prior to considering treatment studies, characterization is an obligatory step. There should be correlation with the basic research that can be conducted with established models.

ANIMAL MODELS

Review of the literature reveals a large number of available animal tumor models and surprisingly little work that has been done with relation to human RC.

Spontaneous tumors occur in rats, mice and in the Syrian golden

hamster (1,2,3,4). Kidney tumors with great morphological similarity to RC can be induced in Syrian golden hamsters by prolonged estrogen treatment (5). This tumor has been shown also to depend on progesterone, androgens and glucocorticoids in a complex manner (6).

Virally induced tumors occur in the frog rana pipiens (7) and have also been described in hamsters, mice, rats and chicken.

Chemically induced kidney tumors have frequently been found in chemical carcinogenesis studies in various animals. They usually occur together with other malignant tumors which determine the prognosis of the laboratory animals. The renal tumors are a by-product found at autopsy. Two rat tumor lines induced by mono- and diaminobiphenyl compounds (MK1 and MK3) have been well characterized and have proved to be useful for chemotherapy studies (8,9). This subject has recently been reviewed by Sufrin (10).

Human tumor transplants in animals. Heterotransplantation of human RC tissue has occasionally been possible. Katsuoka described five transplantable lines in 'nude' mice. He attempted transplantation of 24 different tumors, a success rate of about 20% (11). The tumor line NC 65 has, together with a more recent line, been isolated in our own laboratory (12). The results obtained with the NC 65 line which has also resulted in two cell culture lines will be described in greater detail. Recently Day et al. (13) mentioned the use of two new RC lines in nude mice on which chemotherapy experiments were performed. The transplantation of the RC cell lines CAKI 1 and 1072 F into the cheek-pouch of the hamster, an immunologically privileged site, has been accomplished and used for the study of cholesterol metabolism by Clayman (14).

Selected Results

Animal tumors. The Syrian golden hamster RC has been subject to a large number of publications which were recently reviewed by Sufrin (10). This tumor, which has great morphological similarity to human RC can be induced in male hamsters by the application of natural or synthetic estrogens in 9-12 months. The same techniques fail to induce RC in intact female animals. However, if the females are pretreated by androgens immediately after birth, if the estrogen treatment is initiated in pre-puberal animals or after castration, RC will also develop in females exposed to estrogen. The agent that protects intact female animals from getting the tumor has been identified as being progesterone. Simultaneous application of progesterone with estrogen in intact males or castrated females prevents tumor induction. Also testosterone, deoxycorticosterone, bromoergocryptine 20-methylcholanthrene and nafoxidine prevent tumor induction if concurrently administered with an estrogenic hormone. Cortisone enhances tumor induction, prolactin has no effect. The induced RC

requires estrogen for its maintenance. The tumors spread and infiltrate locally, rarely metastasize but eventually kill the animals.

Estrogen induced hamster RC shows strong morphological and biochemical similarities with human RC. The possibility of progesterone dependence of human RC has been deduced from this model. Unfortunately, the initial enthusiasm for gestagenic treatment of RC has been shown to be unjustified. Only an occasional patient responds to this treatment and it must be concluded that endocrinologically the similarities between estrogen induced hamster RC and human RC are limited. Review of the literature has not revealed any application of chemotherapy to this model system.

A large volume of work has been carried out to characterize the two chemically induced rat RC lines MK1 and MK3 biochemically, morphologically and genetically. Furthermore biochemical parameters such as enzymes of glucose, pyrimidine and purine metabolism have been studied in these slow growing, well differentiated adenocarcinomas and have been compared to the same parameters in normal human and rat kidney and to human RC.

The pertinent findings have been recently reviewed by Weber (9). The studies show convincing similarities between the MK1 and MK3 transplantable lines and human RC. The findings obtained led Weber and his research group to the conclusion that "the reprogramming of gene expression manifested in the enzymatic imbalance in human RC in a large extent was similar to that observed in the chemically induced rat kidney carcinomas". The strongest linkage between renal neoplasia and enzymatic changes was found for the increased activities of pyruvate kinase,uracil phosphoribosyl transferase, IMP dehydrogenase and AMP deaminase. A number of enzyme ratios were found to be increased and some enzymes of glucose metabolism were decreased in activity. The strategy of this research lies in identifying "Key enzyme systems" which are essential for DNA replication and which can not be replaced by salvage pathways. Such systems can then be used as targets for chemotherapy which can be specifically designed. This highly sophisticated approach could make clinical research for chemotherapy more efficient and cut down on the need for clinical experimentation if it proves successful.

In 1973 Murphy and Hrushesky (15) described a tumor line which was derived from a spontaneous, transplantable RC of a BALB/c Cr mouse. The tumor metastasizes and grows fastest when transplanted under the renal capsule. Presence of the tumor leads to an increase of the hematocrit by secretion of "erythrocyte stimulating factor". The tumor is fast growing and kills all animals. Its growth is enhanced by exogenous testosterone and DES but it is unaffected by gestagens. This model has recently been used by Sufrin in chemotherapy studies and serves presently as a model for immunotherapy in the hands of Pontes (16). CCNU, BCNU and adriamycin were active

agents; cytosine arabinoside, bleomycin and cyclophosphamide showed little activity.

Soloway and Myers (17) studied the effect of hormones in another mouse tumor line (BALB/c/cf/cd) named MKT-Cdl. They found suppression of growth by estrogen and testosterone.

White and Oson (18) characterized and used for chemotherapy experiments a spontaneous RC tumor line growing in Wistar Lewis rats. The tumor resembles human RC morphologically, it metastasizes and kills the animals. Preliminary chemotherapy studies were carried out.

Human RC in "Nude mice". In a recent review of this subject (19) only 11 human RC lines were found in the literature. It seems that with the techniques used at this time a take rate of 20% in human RC is the maximum that can be expected. Only one of these lines has been characterized to some extent and it will therefore serve as an example.

The transplantable tumor line NC65 was initiated in 1976 from a moderately differentiated RC and from a hilar lymph node metastasis (20). The tumor has also given origin to two permanent cell culture lines which bear the same name. The animal tumor will readily grow in culture but culture lines cannot be transplanted successfully back into the mice. Morphologically the tumor can easily be recognized as RC. It consists of clear and granular cells growing in trabecular fashion. The speed of growth and the morphological appearance have remained remarkably stable over the years. The doubling time varies between four and six days. The tumor has been characterized biochemically and genetically (21,22). It was proven by chromosone analysis that the tumor derives its stroma from the mouse. The model shows very strong similarity with human RC and is presently used for chemotherapy studies.

CELL-CULTURE

A large number of normal animal kidney cell lines are available and have been recently reviewed (19). Also, at least 10 cell lines have been isolated from human RC and have at least been partially characterized (19). Very little has been done to establish their possible usefulness as models for RC. Some of these lines are transplantable into 'nude' mice.

Organ cultures and short term cell cultures of RC can be established with a good chance of success; however very little work has been done in this field. Atkins (23) has shown in organ cultures that human RC tissue remains functionally active and produces prostaglandin E_2 leading to bone resorption in co-cultivated mouse calvaria.

RC cells may be clonogenic in soft agar (24). Also micro-transplantation under the renal capsule is applicable to transplantable lines and perhaps to fresh tissue (25). These techniques may prove to be useful for therapy studies; preliminary results will be reviewed.

Proper attempts to characterize these models have been carried out only in very few instances. Many potential markers are available in human RC and need to be exploited. The development of new markers is one of the important aspects of this research.

Selected Results

At least 12 cell lines have been isolated from human RC. Few attempts to characterize cell lines have been made. As already mentioned, a cell line also resulted from the tumor which gave origin to the transplantable line NC 65.

This cell line was initiated from tissue of the primary tumor; attempts to grow cells from hilar metastases were unsuccessful. Within the cell population marked morphological differences were seen and in the 25th passage cloning was undertaken which resulted in one line consisting of round cells called NC 65 R and another consisting of spindle cells called NC 65 SP. Another line, NC 65 V, could be initiated from the nude mouse line which was originally grown from the hilar metastases. This line consisted only of granular cells and the cell culture line NC 65 V only of round cells. All three lines were characterized genetically. They were shown to possess a human karyotype, at least three common marker chromosomes being identified. These studies suggest that the NC 65 R and V cells may be identical with granular cells and that the NC 65 SP cells are derived from the clear cells of the tumor. Confirmation by re-transplantation has not yet been obtainable.

Long term cell lines are ideal models for the study of in vitro killing conditions by cytostatic drugs. Lines have been used to set up such studies which can then be used for freshly grown cells from individual tumors.

Short term cultures from human RC can be established with a good chance of success. Such cultures have been shown to carry on the abnormal pattern of lipid metabolism which is typical for human RC (26). Short term culture has been widely used in immunological studies (19). Some renal carcinomas have been shown to be clonogenic in soft agar (24) and this property is now widely exploited for in vitro chemotherapy studies.

CLINICAL APPLICATIONS

As mentioned above animal and in vitro models have been successfully used to identify endocrine properties of RC. Marker substances such as prostaglandins, parathormone and other peptide hormones have been identified. Human cell lines and transplantable tumors should be especially suitable material for the search for marker substances. Such research is presently conducted in some laboratories at a low scale and should be encouraged.

The endocrine and immunological properties of human RC are still unclear. Well documented evidence suggests that such properties can be used at least in some instances for controlling growth in patients with metastatic disease. Animal and culture models should be suitable to investigate this important field in a more conclusive way with the goal of identifying tumor and host factors which can be manipulated for controlling growth in patients.

Chemotherapy testing has become a vast field of research. Some laboratories feel that the suitability of available techniques should be further documented, whilst others have immediately applied experimental results to patient management to try to correlate these results retrospectively to patient response. In the U.S. soft agar cloning assays have already become commercially available.

Four testing systems are presently used experimentally:

1. Soft agar clonogenic assay (23,26).
2. ^{3}H uridine incorporation assay (27).
3. Measurement of subcutaneous nude mouse transplants.
4. Microtransplantation under the renal capsule of nude mice (24).

Space does not permit a detailed review of the reported results. In summarizing the available information it can be stated that up to now no effective single agent has been identified. Sarosdy et al. (28) feel that individual tumors will require individual treatment and that RC is less sensitive to commonly used drugs than other solid tumors. This observation made with the soft agar assay is confirmed by Day et al. (13). Extensive drug resistance has also been reported by Lieber (29).

Belitsky et al (30) linked Adriamycin to an antirenal carcinoma antibody and were able to target the drug into the renal carcinoma of BALB/c mice. They were able to inhibit tumor growth more effectively than by using the non-conjugated drug. This technique could be a major break-through if it proves to be applicable to human RC.

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THE EVALUATION OF CHEMOTHERAPY IN A MURINE RENAL ADENOCARCINOMA

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A spontaneously arising renal adenocarcinoma in a male Wistar-Lewis rat has been used over the past three years as a testing system for various chemotherapeutic agents, used alone or in combination. We have also utilized this model in an effort to evaluate the ability of cytoreductive surgery to render chemotherapy more effective. Finally, we have looked at the sensitivity of this tumor to hyperthermia delivered alone or in combination with cyclophosphamide.

DEVELOPMENT OF TUMOR MODEL

We have previously described in detail the method by which a nodule and metastatic model were produced (1) and will only outline them here in brief.

Nodule Model

0.06 to 0.08 grams of the spontaneously arising renal adenocarcinoma is taken from a single donor and placed subcutaneously in the flank of a syngeneic rat anesthetized with ether. The incision is closed with a single clip. Three weeks later 90% of animals will have palpable tumors.

Metastatic Model

Male Wistar-Lewis rats, anesthetized with ether, underwent splenectomy after which 0.06 to 0.08 grams of tumor, taken from a single donor animal, was placed intra-peritoneally. The peritoneum and skin were closed with clips and treatment began five weeks later

at which time 90% of animals had metastatic intraperitoneal tumor growing.

SINGLE AGENT STUDIES

Nodule Model

In order to test the effectiveness of single agents against this tumor system, the tumor was implanted in the flank of the rat and allowed to grow for a week. The animals were then treated with intraperitoneal (IP) injections of various agents on weeks one, two and three. On week four the animals were sacrificed, the tumor removed and the wet tumor weight recorded. The effectiveness of the chemotherapy was measured by the reduction in the number of animals bearing tumor at this time compared with their own controls and in the reduction of the tumor weight as compared to its own controls. The results can be divided into the agents that were or were not effective in the dosages and time sequence utilized. Ineffective agents included 5-fluorouracil, cis-platinum, bleomycin, hydroxyurea, streptozotocin, neocarzinostatin, chlorzotocin, carminomycin and methyl-GAG. Maytansine did not reduce the number of tumors present, but did significantly reduce the weight of the tumor present in comparison to its own control. The agents which appeared effective either by reducing tumor weight or by reducing the amount of tumor surviving at the time of sacrifice were cytoxan, adriamycin, vinblastine and vindesine. By far the most effective agents, both reaching statistical significance, were cytoxan and vindesine.

Metastatic Model

The obvious limitations of a nodule model is that it does not metastasise and therefore does not in any way reproduce the situation in humans. For this reason, a metastatic model was utilized to further evaluate the efficacy of the agents which had proven effectiveness in the nodule model, these agents being used either alone or in combination. In the drug regimens utilized, the tumor was allowed to grow for five weeks post implantation and then treated for three weeks with intraperitoneal injections. The animals were sacrificed the following week, which in most cases was the ninth week following implantation. The effectiveness of the single agent or combination therapy was judged by assessing the number of animals that had tumor in comparison to their own control or by the wet weight of the tumor present. This was gauged by the removal of all visible tumor, obtaining a wet weight and comparing this to the wet weight of the removed tumor in that animal's own control. Agents employed were: cytoxan alone, vindesine alone, cytoxan and cis-platinum, cytoxan and vinblastine, cytoxan and adriamycin, and cytoxan, adriamycin and vinblastine. The problem with all the combinations was that 80 - 100% of the animals had persistent tumor present at the time of sacrifice and those combinations which

produced any marked decrease in tumor weight had an unacceptably high animal mortality.

Following this vindesine, cytoxan and vindesine, cytoxan, vindesine and cis-platinum were tried in various combinations. By far the most effective combination tested was vindesine 0.5 mg/kg on weeks five and seven, with cis-platinum 5 mg/kg and cytoxan 40 mg/kg on week six and eight, the animals being sacrificed on week nine. Of the forty rats so treated, 51% had no evidence of tumor at the time of sacrifice and in those in whom tumor was present, the weight ranged from 6.5 to 15 grams as compared to the control in which the weight of tumor ranged between 36 and 143 grams. The average reduction in tumor weight compared to its own control was 90% - the reduction in the incidence of tumor and in the tumor weight reaching significance.

The extrapolation of any results from animals to human is always fraught with danger and in renal cancer the ideal animal tumor model system should (a) arise spontaneously, (b) be histologically a pure adenocarcinoma, (c) have a predictable growth rate, (d) have the capacity to metastasize, (e) be hormonally independent and (f) respond to chemotherapeutic agents as does the disease in humans. This present model does have some of these characteristics. It arose spontaneously, is histologically a pure adenocarcinoma, and has a predictable growth rate. It does not metastasize from the flank but produces a metastatic disease model if placed intraperitoneally. The tumor was tested for its hormonal responsiveness and was found initially to be hormonally independent. Interestingly, in later generations its growth in females appears retarded although the tumor histologically has not changed. Regarding the tumor's response to chemotherapeutic agents, it has, like its human counterpart, remained resistent to most chemotherapeutic agents - cytoxan being the only exception. This tumor certainly appears more sensitive to this alkylating agent than its human counterpart.

THE EFFECT OF CYTOREDUCTIVE SURGERY ON THE TUMOR'S RESPONSE TO CHEMOTHERAPY

In order to see if debulking would improve the effectiveness of vindesine (VDR), 22 male Wistar-Lewis rats were innoculated with tumor to produce both a flank and a metastatic tumor model. Seven weeks later the rats were divided into three groups, six designated as controls, eight receiving vindesine 0.5 mg/kg IP at week seven and week nine, and eight having cytoreductive surgery. In this last group the flank tumor was removed prior to a similar dose of vindesine IP at week seven and nine. At week ten all animals were sacrificed, the tumor removed and the wet weight recorded in grams. Values representing mean and standard error are shown in Table 1. The reduction in flank, IP and total tumor weight between the control and VDR animals was in cases significant ($p<0.05$, <0.25 and <0.001

Table 1. Tumor Weight at 10 Weeks

	Flank Tumor Wt.	IP Tumor Wt.	Total Tumor Wt.
Control	44.83 ± 7.84	46.5 ± 12.45	91.33 ± 10.15
VDR	23.95 ± 6.52	14.21 ± 5.2	35.16 ± 5.87
VDR & Debulking		39.64 ± 8.67	39.64 ± 8.67

respectively). In contrast, in the debulked group, the IP tumor weight was similar to that of controls and significantly larger than the IP tumor weight in the VDR group, ($p < 0.025$). The IP tumor weight in the debulked group was the same as the total weight (flank + IP tumor) in the vindesine group. Therefore in this, albeit small, study debulking did not appear to statistically increase the effectiveness of the chemotherapeutic agent, in this case vindesine, against the remaining intraperitoneal tumor. Certainly from this experiment it could be suggested that surgery and/or anesthesia had nullified the effectiveness of vindesine against IP tumor growth. Further studies have been done and in none of these have we found that debulking in any way improved the effectiveness of vindesine.

SUSCEPTIBILITY OF TUMOR TO HYPERTHERMIA

These experiments were carried out on the flank nodule. The tumor was allowed to grow to a diameter of $1\frac{1}{2}$ to 3 cm and was then subjected to localized hyperthermia to a temperature of 43° for twenty minutes at weekly intervals. The hyperthermia was delivered via a focus ultrasound machine which was constructed by a Professor Lele at the Massachusetts Institute of Technology. The heat was confined strictly to the tumor nodule (2). Tumor size was measured weekly by three perpendicular diameters with Vernier calipers. Tumor sensitivity was assessed by differences in the tumor size between the treated and control animals. In the ten animals treated, there was a 41% reduction in tumor volume after one week; 43.3% reduction after two weeks and 71.3% reduction after three weeks of hyperthermia. The control animals uniformly showed progressive increase in their tumor size.

The experiment was then repeated, but on this occasion with four groups of animals, one group treated with hyperthermia, one group treated with cyclophosphamide, one group treated with hyperthermia and cyclophosphamide and a control group. Though these studies are still in a pilot stage, the combination of hyperthermia 40° for twenty minutes and cyclophosphamide (20 mg/kg IP weekly) showed a greater decrease in tumor size than when the hyperthermia

or chemotherapy were used alone. Cytoxan alone produced a 40% decrease in tumor size as compared to the controls, hyperthermia alone a 47% reduction and cytoxan and hyperthermia 85% reduction.

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Editorial Note (M.P.)

Dr. deVere White and his colleagues deserve to be congratulated on this very careful study. There is certainly a great need for studies of this type, in the hope of finding a reliable animal model for therapeutic research on renal cancer.

Unfortunately, as the authors rightly point out, there is a large gap to be filled between in vitro or animal studies and the clinical situation in man and attempts to extrapolate results from animals to humans are fraught with danger. This murine tumor is responsive to both cyclophosphamide and vindesine. Unfortunately, there is no evidence that this is also true for human renal cancer. Probably cyclophosphamide has not been sufficiently evaluated in man, but the few available results in small series do not indicate a significant activity. Vindesine is currently being evaluated by the EORTC Urological Group. It is too early to reach any conclusions but the preliminary results do not appear to be encouraging apart from an occasional response. The neurotoxicity is not negligible. The correspondence between response in this animal model and that obtained in humans seems to be imperfect.

EPIDEMIOLOGY OF RENAL TUMORS

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INTRODUCTION

Many different tumors may arise within the kidney. Although the commonest are tumors of epithelial origin including adenoma (benign) and adenocarcinoma (also called hypernephroma or Grawitz' tumor) together with tumors of the renal pelvis (transitional cell, squamous or undifferentiated) one must not forget nephroblastoma, also called Wilms' tumor, and its variants. Less commonly benign and malignant mesenchymal tumors may be found. The benign include leiomyoma, leiomyolipoma, angiomyolipoma, hemangioma, juxta-glomerular tumor, lymphangioma, fibroma and the malignant, leiomyosarcoma, rhabdomyosarcoma, liposarcoma, angiosarcoma, fibroxanthosarcoma, oesteogenic sarcoma and hemoblastoses.

EPIDEMIOLOGY

A. Benign mesenchymal tumors are rare. Most are detected incidentally at autopsy and usually being too small to produce clinical symptoms during life. In some cases, they give hematuria or hypertension; they are more frequent in women than in men.

Malignant mesenchymal tumors are also rare. The leiomyosarcoma is that most frequently formed but less than 100 cases have been reported in the literature and with the exception of well-known association of angiomyolipomas with Bourneville's tuberous sclerosis, no data are available on the epidemiology of these tumors (1).

B. Nephroblastoma or Wilms' tumor is usually discovered during infancy or childhood. It is a malignant embryonic tumor originating from metanephrogenic blastema. Although it represents 6% of all

malignant neoplasms in children below the age of 15 years, it is a rare tumor and its incidence is approximately one case per 150,000 children per year. The incidence is the same in boys and in girls.

Ten per cent are discovered during the first year of life; 50% before the third year and 80% before the fifth year. A few cases have been reported in adults (approximately 3%). Nearly 5% of the cases are initially bilateral.

The constancy of incidence in every nation and race indicates that the tumor is not dependent on any kind of carcinogenic influence but that it results from a stable endogenous process. Its association with various malformations or other tumors and familial incidence indicate the importance of genetic factors in the occurence of this type of tumor.

C. Tumors of the renal pelvis are about three times less frequent than adenocarcinomas of the kidney. They are histologically identical to tumors of the ureter or of the bladder and their epidemiologic and etiologic factors are also the same (2). They have been extensively discussed.

D. Adenocarcinoma of the kidney

Erroneously called "hypernephroma" as it was formerly considered to be derived from vestiges of ectopic adrenal tissue, renal cancer is now recognized to be derived from the epithelial cells of the proximal convoluted tubules.

In many epidemiological reports, renal cell carcinoma is grouped together with pelvic tumors, nephroblastoma and the more rare forms under the general definition of renal cancer, which makes a correct evaluation of the incidence of the disease rather difficult.

In the United States (3,4), cancer of the kidney is slightly more common among white males - the age standardized incidence rate being 8.6/100,000 as compared with 7.6/100,000 in the black male.

The incidence of white and black males is twice as high as in white and black females (4 and 3.8/100,000 respectively).

Table 1 gives the age-specific and age-standardized incidence rates (per 100,000 person-years) of renal cancer in the United States (4).

Figure 1 clearly shows that the incidence of cancer of the kidney in the United States (1969-71) is related to age in both sexes and races. Extremely rare before 40 years, it reaches a plateau at about 75-80 years; the incidence is then about 50 cases per 100,000 person-years in white males.

Table 1. Age-specific and age-standardized incidence (per 100,000 person-years) of renal cancer in the United States of America.

AGE	MALE		FEMALE	
	WHITE	BLACK	WHITE	BLACK
15	0.9	1.1	1.1	1.0
15-29	0.2	0.7	0.2	0.7
30-39	1.6	0.9	1.0	1.2
40-49	7.2	8.6	3.2	3.0
50-59	16.7	17.2	8.5	9.4
60-69	33.6	25.3	14.0	12.5
70-79	46.0	40.7	19.7	14.3
80-84	52.5	26.7	24.4	27.7
85+	53.5	17.3	22.3	5.5
All ages*	8.6	7.6	4.0	3.8

* Standardized to the 1970 U.S. standard population

In blacks, the incidence falls after this age but it is probable that this decrease is due to failure of diagnosis in many and to the fact that fewer black males survive beyond this age.

In Belgium, 451 cases of renal carcinoma were reported in 1975 but only 59 cases of pelvic (and ureteral) cancer (5). The incidence rate for renal cancer is 6.1/100,000 in males and 4.4/100,000 in females. If we exclude the tumours of the renal pelvis and ureter, the incidence rate is 5.6/100,000 in men and 4/100,000 in women. When compared with those in the United States the rates are slightly higher for women and lower for men. The distribution of the cases in the different regions (mainly industrial and urban or rural) does not show any significant differences suggesting that industrial carcinogens play no obvious role in the development of renal cancer.

Table 2 illustrates the age-standardized incidence rate (cases per 100,000 person-years) in several countries (6).

The incidence rates are higher in North America and South America. The difference is similar to, but much less marked than that seen in similar geographical areas in prostatic cancer. The sex ratio varies from 1.3 to 2.4, but in every country, the incidence is higher among men.

Table 2. Age-standardized incidence of renal cell cancer by country (cases/100,000 person-years).

COUNTRY	MALE	FEMALE	SEX RATIO
DENMARK	7.2	5.1	1.4
USA (ALAMEDA COUNTY, CALIFORNIA)			
White	7.1	3.6	2.0
Black	6.0	2.5	2.4
FINLAND	6.3	3.9	1.6
BELGIUM	6.1	4.4	1.4
ENGLAND AND WALES	4.4	2.0	2.2
YUGOSLAVIA	3.5	1.8	1.9
ISRAEL			
Jews born in Israel	3.7	2.8	1.4
Jews born in Europe	7.2	4.3	1.7
JAPAN	1.5	1.2	1.3
HAWAII (Japanese)	4.0	1.5	2.6

The difference observed between people of different origins but living in the same country (e.g. Jews in Israel) or people of the same origin but living in different countries (e.g. Japanese in Japan and in Hawaii), suggest that genetic and environmental factors probably play a part in the occurence of this disease.

Finally, time trends of incidence and mortality rates show a doubling among men from the mid-1930's to the mid-1950's. Thereafter, there has been little change, and it is very probable that

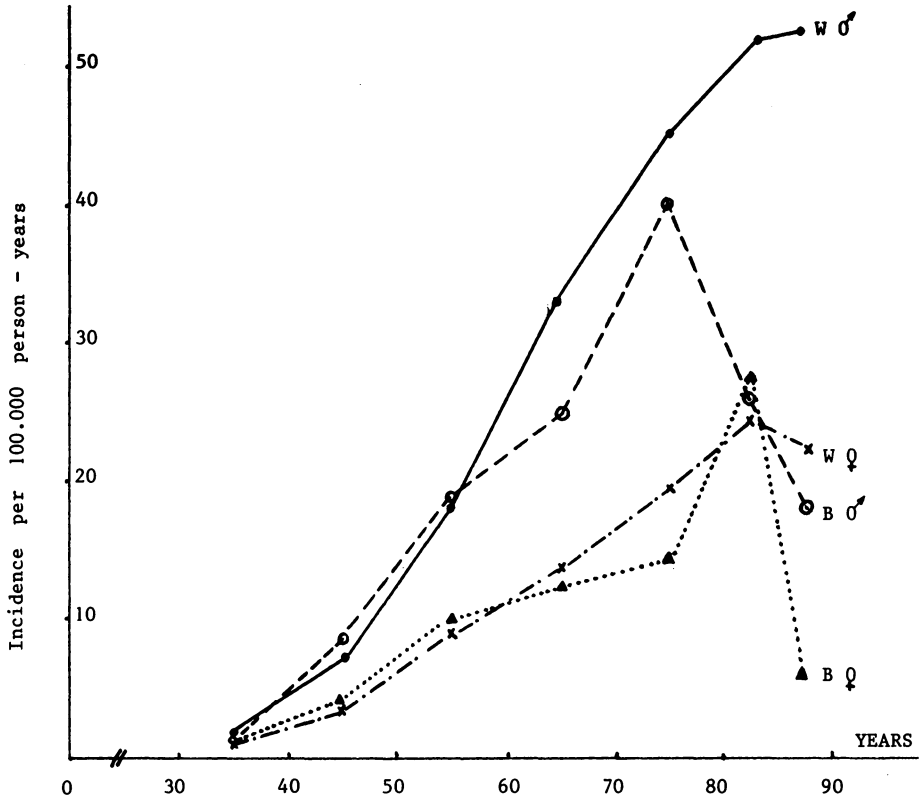

Fig. 1. Incidence of cancer of the kidney in the United States of America (1969-71) by age, sex and race. (B = black; W = white).

this increase is fictitious and related to improvements in the diagnostic methods.

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UNRECOGNIZED RENAL CELL CARCINOMA

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In a series of 16,294 autopsies, 350 cases of renal cell carcinoma were found. Of these tumours 235, i.e. two-thirds, were unrecognized during the patients' lifetime. Metastatic spread was revealed in 56 patients with unrecognized renal cell carcinoma (24%) and was the main cause of death in 49 patients (21%).

The number of metastasizing tumours increased significantly with the size of the primary tumour. Local aggressiveness of the primary tumour was also more common in large tumours but was much more closely correlated to metastatic spread than to size. Invasion of the renal vein by tumour was significantly more common in metastasizing tumours than in non-metastasizing lesions.

The present study confirms that an analysis of local aggressiveness of the primary renal tumour is of prognostic value and might be useful in defining the group of patients who may benefit from adjuvant treatment such as radiation therapy, chemotherapy and immunotherapy.

Editorial Note (P.H.S.)

If further autopsy studies in other parts of the world prove that renal cell cancer is three times as common as the clinical incidence suggests, one can justifiably argue both for non treatment in the elderly patient, as suggested by Denis at this meeting (especially if the diagnosis is made by chance in a patient free from symptoms) and also for an effective screening programme as suggested by Blandy in this volume, since the tumour is the cause of death in 21% of the affected patients.

THE USE OF ELECTRON-MICROSCOPY IN THE CONTROVERSY OF RENAL ADENOMA VERSUS CARCINOMA

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The debate as to whether or not renal adenomas are benign or malignant tumors is of great academic and epidemiological importance. Within Bennington and Beckwith's Tumor Fascicle on Kidney, Renal Pelvis, and Ureter (1), it is stated (and these authors concur) that the so-called renal adenomas are more frequent than renal adenocarcinomas. They believe, as we do, that renal adenoma is a small carcinoma and that whatever the etiological agents, it is through this evolutionary stage that renal adenocarcinomas develop. Unfortunately, in terms of time sequence, the surgical pathologists see these tumors as slow-growing. One may expect that with increased longevity in our growing geriatric age group, the incidence of clinically apparent renal adenocarcinomas will increase and the earlier diagnosis of these small cortical glandular tumors will become more important. Bennington and Beckwith (1) have gathered an impressive array of facts that demonstrate very clearly the similarity between the so-called renal adenomas and the renal adenocarcinomas, including the following:-

1. Both renal adenomas and adenocarcinomas arise from cells of the proximal convoluted tubule. This has been demonstrated both immunologically and ultrastructurally by several groups of workers (2,3).
2. There are no light histology, histochemistry, or electron microscopic features that distinguish an adenoma from a clear cut similar cell type carcinoma of the kidney either in the human or in experimental animals.
3. Adenomas occur more frequently in a kidney containing a renal adenocarcinoma (4).
4. Adenoma and adenocarcinoma occur in the same age group and Bennington and Beckwith state that they are rarely found before the age of 30. They also show a marked predisposition for the human male (2-4:1).

5. Both renal adenomas and adenocarcinomas occur with the same increased frequency among tobacco users. Bennington and Laubscher (5) have done extensive epidemiological research concerning this issue.
6. There is a direct relationship between tumor size and frequency of metastases considering all the renal cortical glandular tumors (6).

Bennington and Kradjian (7) also present convincing evidence that there are no correlative/morphological criteria that can distinguish renal adenomas from the small cortical glandular tumors that manifest their aggressiveness by perforating their capsule. They have shown that a small renal adenoma ($<$ 1.5 cm) demonstrates perforation of the tumor capsule at several points. This is one of the important criteria for the diagnosis of cancer, i.e. the ability to break out of tissue architecture. They believe that the so-called adenoma is actually a small renal adenocarcinoma which, in many instances, has not yet metastasized. They also point out that the small renal cortical glandular tumors that are $<$3.0 cm in greatest diameter are almost invariably an incidental finding at autopsy so that their classification is of no clinical consequence to the patient. However, when such a tumor is found at surgery, the diagnostic terminology becomes more important. If one gives a diagnosis of renal adenoma, it signifies a benign tumor, but it occasionally metastasises. On the other hand, a diagnosis of renal adenocarcinoma unlikely to metastasise, portends lifelong overtones of cancer for the patient and his family. When they are found in surgery, these authors, like Bennington and Beckwith (2) feel that it is best to diagnose such small cortical glandular neoplasms of the kidney as renal adenocarcinomas. It is imperative for all concerned that the tumor diameter size also be reported to make the urologisit or the oncologist aware that their patient's tumor may not have reached the minimal invasive size. Bennington and Beckwith (1) point out that enough sections should be taken to determine whether this "renal adenoma" is truly invasive into the surrounding parenchyma or through its tumor capsule. Such tumors offer an excellent prognosis and a comment may also be reported in the diagnosis but with the stipulation that tumors of this size may occasionally metastasize. Unfortunately, one cannot predict histologically which will behave in this way but the mention of the possibility that metastases might occur allows the urologist to plan careful follow up for the patient.

Briefly, we would like to discuss the ultrastructural pathology of human renal tumors. For several years, there have been extensive review articles and original research on the ultrastructural pathology of renal neoplasms (8). The clear and granular cell tumors are the predominant cell types in most renal carcinomas. They are found either in the tubular or papillary form. We, like several other investigators, feel that renal carcinomas do arise from the renal tubular epithelium and prefer the term "renal cell carcinoma" to be appended to the surgical pathology reports. Many observations of light histology patterns support the renal tubular epithelial origin

of renal cell carcinomas. These patterns may mimic normal tubular structures or they may exhibit transitions from normal tubules to those arranged in a papillary or cord-like fashion. There is even further support for this concept of origin. Electron microscopic studies demonstrate many similarities between renal carcinoma cells and the normal epithelial cells of the proximal convoluted tubule (8).

Before discussing the ultrastructural pathology of the granular cell tumors that are found both in renal cell carcinomas and in adenomas, a brief resumé of the ultrastructure of the normal renal tubules seems warranted. The appearance of the different parts of the tubules in man does not differ significantly from that found in animals. The main distinguishing features of the proximal convoluted tubules of the kidney are:-

1. The presence of a brush-border containing many tightly packed microvilli which contain filaments.
2. Invaginations of the apical plasmic membrane provided with membrane coatings.
3. The occurrence of vesicles or vacuoles with a characteristic structure in the apical cytoplasm.
4. An abundance of mitochondria.
5. Deep invagination of the basal plasma membrane creating slender cytoplasmic compartments.

Cells of the distal tubules do not demonstrate any of these apical cytoplasmic specialties. However, they contain abundant mitochondria and show deep basal plasma membrane invaginations. In the other parts of the tubular system, the cells are less specialized and show a similar structure of the plasma membrane as well as of the cytoplasm.

When the granular cell tumors of either the adenoma or carcinoma are examined, they are found to contain mitochondria. Occasional granules interposed between these organelles and interpreted as glycogen are also seen in the clear cell carcinomas. These granules are PAS-positive. In the cytoplasm of many of these tumor cells, there is marked infolding of the nuclear membrane with deep invaginations of the cytoplasm into these structures. The cytoplasm of many of these tumors contains intracytoplasmic well differentiated, brush-border like structures similar to those previously mentioned. In several instances where serial sectioning of the same block is performed, these intracytoplasmic brush-border lumens do not communicate with the exterior of the cell. At this institution, this attempt at brush-border formation seems to be more prevalent within the granular cell tumors than in the clear cell carcinomas of the kidney. These cells, i.e. the granular cells found both within the granular cell adenomas and granular cell carcinomas of the kidney are identical in morphology. We and Bennington (personal communication) have likened these granular cells to those of the proximal convoluted tubules of the normal kidney because of the similarities that would be found:-

1. Microvilli and brush-border elements.
2. Membrane associated vesicles which might be involved in pinocytosis.
3. Membrane coatings.
4. Basal infoldings of the plasma membrane.
5. Intercellular junctions and most important of all
6. Abundant mitochondria.

As now seems to be the prevalent feeling, there is a continuum between adenomas of the granular cell type and carcinomas of the kidney. As a result, more definitive epidemiological and ultrastructural analyses can be done in terms of time sequence studies of various agents that might be considered important in renal cell carcinomas.

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RENAL ONCOCYTOMA

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An oncocytoma is a neoplasm composed entirely of oncocytes. Oncocytes are cells rich in mitochrondria but sparse in other organelles. Oncocytoma has been described in many organs. Interest in oncocytoma of the kidney was recently revived by Klein and Valensi (1). They proposed that renal oncocytoma be considered a proximal tubule adenoma with oncocytic features. The importance of renal oncocytoma is as follows.

These tumors are solid and frequently large, i.e., $\geq$ 5 cm. Klein and Valensi proposed that they are essentially benign tumors. Their differentiation from renal carcinoma is then of more than academic interest. If oncocytoma is presently being confused with carcinoma, needless nephrectomies are being performed. Finally, are survival statistics for various modes of therapy being favourably influenced by the unwitting inclusion of oncocytoma?

We may pose several questions concerning oncocytoma. Is this a sufficiently well described entity to allow for consistent diagnosis by an informed pathologist? What percentage of clinically detected solid renal masses represents oncocytoma? Can the diagnosis be established with confidence without surgery? Klein and Valensi propose that their data suggest a recent increase in the incidence of this disease. Is this correct? Klein and Valenis also propose that the clinical history, specifically the lack of hematuria, might be a useful diagnostic clue. Is this correct? Under what circumstances, if any, can the diagnosis of oncocytoma be established by frozen section? Finally, is this invariably a benign disease?

The following abbreviated case report may illustrate the

challenge and opportunity presented by this disease. The full case report is awaiting publication (2). The patient was a 42 year old white married nulliparous previously healthy female. She complained of colicky left flank pain and was admitted to a local hospital with the presumptive diagnosis of a left ureteral calculus. The admitting physical examination and laboratory data were within normal limits except for microhematuria. An excretory urogram revealed a large middle pole mass of the left kidney and a smaller upper pole mass of the right kidney. Sonography revealed both masses to be solid. An aortogram and bilateral selective renal arteriograms were performed. An evaluation for metastases was negative. The patient was referred to us with a diagnosis of bilateral renal cell carcinoma.

Our evaluation of the patient failed to reveal any evidence of von Hippel-Lindau disease or tuberous sclerosis. Ultrasonographically controlled "skinny" needle aspiration cytology of the masses was negative for malignant cells from the right and technically unsatisfactory from the left kidney.

Since in our opinion the most likely diagnosis was malignancy with, however, the remote possibility of an unusual benign tumor we chose to approach the problem by performing a radical nephrectomy of the kidney with the greatest tumor burden, i.e., the left. We modified the procedure, however, by leaving the vessels intact until we had mobilized the entire specimen. We then secured the vessels, removed the specimen and immediately perfused the kidney via the renal artery with cold Collin's solution. The mass was shelled out with a combination of blunt and sharp dissection. The entire mass which weighed almost 400 grams and was 10.5 cm in diameter was given to our pathologist (Dr A Nicastri). Frozen section was interpreted as a benign tumor. The kidney was reconstructed and then autotransplanted into the left iliac fossa by our transplant surgeon, Dr Khalid Butt. The regional lymph nodes looked and felt normal and this together with the frozen section report of benign disease prompted us not to perform a lymphadenectomy. The patient was discharged on the eleventh post-operative day. The final pathological diagnosis was oncocytoma.

The patient was re-admitted 35 days after her initial discharge. We performed a simple enucleation of the right kidney mass and a second previously undetected mass. Again the frozen and permanent section diagnosis was oncocytoma.

We believe this is the first report of bilateral renal oncocytoma. Forty eight months have now elapsed since her surgery and the patient has no evidence of malignant disease.

An attempt will now be made to answer the questions posed in the initial part of this article. Based on a review of the

literature, I am of the opinion that a well informed competent pathologist can be expected to reliably establish the diagnosis especially when the entire tumor is available for study. Reports in the literature have suggested that the percentage of clinically detected solid renal masses which subsequently prove to be oncocytoma ranges between 5-15%. It is our opinion that no publication has documented a reliable method of establishing the diagnosis prior to surgery. Publications subsequent to that of Klein and Valensi (1) have failed to confirm their suggestion that the incidence of this tumor may be increasing. Klein and Valensi also suggested that these tumors might have a characteristic clinical history. Most significantly they suggested a lack of hematuria. This case report, however, and many other reports in the literature document that the clinical history does not differ significantly from that of renal cancer. A frozen section based on a small volume of tissue as obtained from, for example, a needle biopsy, is unlikely to be of value because needle biopsy is highly selective. The current definition of an oncocytoma requires that the tumor be entirely composed of oncocytes. If, as in this case, the entire tumor can be examined closely by the pathologist and frozen sections taken, then frozen sections may be reliable.

Until the recent report from the Mayo Clinic by Lieber et al (3) no oncocytoma had been reported to have produced metastases. They reported 90 cases divided in two groups - 62 cases with what they categorise as Grade I oncocytoma and 28 cases with Grade 2 disease. No case in the Grade I group developed metastases, but six of the Grade 2 group did. Their report suggests that a spectrum of clinical behaviour exists for these tumors. Confirmation of the malignant potential of Grade 2 oncocytomas as noted by the Mayo Clinic group is currently lacking.

The use of radical nephrectomy and autotransplantation in the management of renal masses of uncertain nature has been previously discussed (4) and is of considerable value in certain cases.

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CIRCULATION TIME AND RENAL BLOOD FLOW IN KIDNEY CARCINOMA

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Renal carcinomas are often highly vascular tumours. From angiographic as well as from direct pathologic examinations it is well known that some tumours show arteriovenous shunts (1,2). A rapid opacification of the renal vein is sometimes seen indicating shunt flow. However, as a rather large amount of contrast medium is needed to visualize the renal vein, the incidence of arteriovenous shunts might be much more common than is shown by angiography. Information on renal blood flow and arteriovenous shunts is of basic interest in conjunction with the development of future alternative therapeutic methods in inoperable cases of renal carcinoma. Thus, dose calculations for direct arterial infusion of chemotherapeutic agents or embolization with microseeds loaded with cytostatic or radioactive material might be dependent on the knowledge of tumour blood flow.

MATERIAL AND METHODS

Renal circulation was studied in 15 patients with renal carcinoma by a dye-dilution technique at the time of angiography. No premedication was given. Both the renal artery and the renal vein were catheterized and a bolus injection of indocyanine green was given into the artery while blood was continuously sucked from the renal vein through a spectrophotometer. The resulting concentration curves facilitate the calculation of renal blood flow, appearance time of dye in renal vein and disclose, by curve irregularities, shunt flow which in many cases can be calculated and separated from total renal blood flow too (3,4). The vasularity of the tumours and the size of the kidneys were determined from the angiograms.

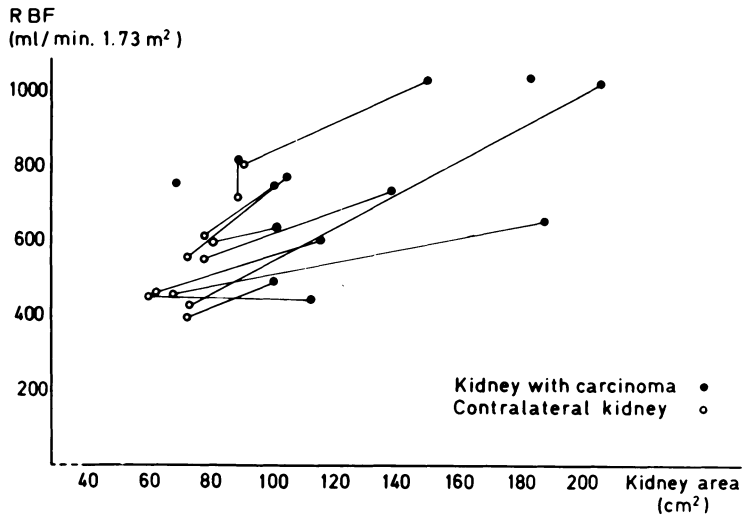

Fig 1. Renal blood flow (RBF) in tumour kidneys compared to contralateral kidneys and plotted against the size of the organs as measured from the angiograms. In all but one case the tumour kidney had the highest blood flow.

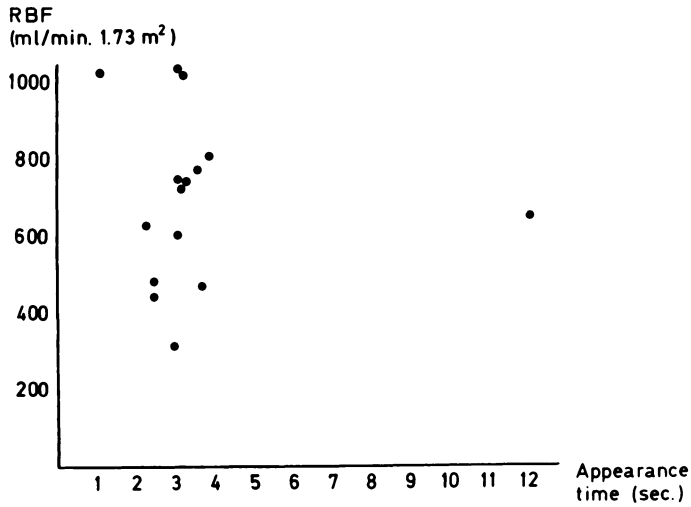

Fig 2. RBF in tumour kidneys plotted against appearance time of dye in the renal vein. No correlation was seen between RBF and appearance time, which was extremely short only in one case and was prolonged in one case in spite of a rather high RBF.

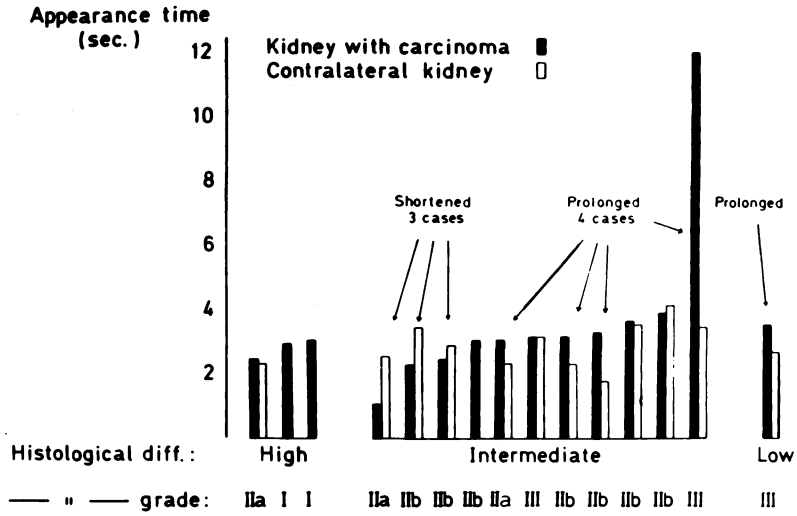

Fig 3. Appearance time of dye in tumour kidney compared to the contralateral one. Three cases had a shorter appearance time on the tumour side and five had a prolongation, while it was approximately the same in the others. (In three patients the contralateral kidney could not be studied for technical reasons.)

RESULTS

The total renal blood flow was as a rule increased on the tumour side. Large tumours had the greatest blood flow. In three cases the blood flow was more than 1000 ml. per minute (fig 1). One of these tumour kidneys had a rapid appearance of dye in the renal vein but no correlation was seen between appearance time of dye and increase in renal blood flow (fig 2). In all but one kidney obvious curve irregularities showed the occurence of abnormal shunt flow. Compared to the appearance time of the contralateral kidney the tumour kidney had a shorter time in three cases and a longer in five. In the other cases the differences were small (fig 3). There was no correlation between the appearance time of dye or contrast medium and histologic differentiation or grade; nor were these parameters correlated to the renal blood flow.

CONCLUSION

Angiography gives mainly morphologic information on renal carcinomas and tumour vessels and is only able to reveal a few cases of arteriovenous shunting. All renal carcinomas, except for those totally necrotic, can be shown to have pathological arteriovenous tumour shunts. Massive increase in total renal blood flow may or

may not occur in tumour kidneys in conjunction with rapid opacification of the renal vein at angiography. Large communications between central vessels predispose to rapid opacification at angiography. Large peripheral arteriovenous shunts in the tumour can however give a great increase in blood flow even if centrally located communications are absent. This is the reason why even great shunt flow in carcinoma may be undetectable by arteriography. The capacity of the feeding arteries to the tumour in relation to the volume of the sinusoidal vessels inside the tumour determine whether or not there will be an early opacification of the renal vein in these cases. A high vascular density of the tumour as judged from the angiograms is not synonymous with a high renal blood flow. The degree of vascular density is more dependent on the vascular volume of the tumour. The largest tumours had the greatest increase in blood flow. This is why there seems to be some correlation between renal blood flow and prognosis. However, there was no correlation between the appearance time of dye or contrast medium and histologic differentiation or grade; nor were these parameters correlated with the renal blood flow.

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HISTOCHEMICAL DETECTION OF STEROID BINDING SITES IN HUMAN RENAL TISSUE AND RENAL CELL CARCINOMA: A PRELIMINARY REPORT OF WORK IN PROGRESS

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The degree of interest in the hormonal milieu as a factor influencing the natural history of renal cell carcinoma is, perhaps, out of proportion to what is warranted by the cumulative experience. Controversy regarding this topic might best be relegated to the medical equivalent of Shakespeare's "Much Ado About Nothing". As a minor offshoot of our binding site studies involving prostatic cancer (described in another section of this book: see 'Histochemical determination of androgen binding in prostatic adenocarcinoma: clinical correlation') we succumbed to this apparently irresistable phenomenon. The following is a preliminary report on work in progress.

Using the histochemical technique, eighteen non-cancerous kidneys were analyzed for estrogen, progesterone and testosterone binding sites. This work has been previously published (1). Binding of all three hormones was detected in eight of eighteen specimens, 5/18 exhibited various combinations of binding and 5/18 were negative. The predominant site of binding was the epithelial cells within the glomerular capillary loops. Binding was also frequently seen in Bowman's capsule. Almost half of the positive specimens showed additional binding in the epithelial cells of the proximal and/or distal convoluted tubules. The results of competitive and non-specific binding studies have been published (1).

Thirty-six specimens of either primary site or metastatic renal cell carcinoma were assayed histochemically but only for estrogen binding. The results, previously unpublished, were as follows: 2/36 necrotic - not assayable; 9/36 negative;

4/36 - low level binding (circa 10% of cells positive); 4/36 - 25% of cells positive; 3/36 - 50% of cells positive; 14/36 - 75% of cells positive. Most cells that were positive exhibited predominantly nuclear binding. Assuming that no nuclear estrogen receptor assay is usually performed with biochemical methods, only 4/36 specimens exhibited significant enough numbers of positive cells to have potentially detectable cytoplasmic binding by the usual biochemical methods.

Preliminary clinical correlation was available in fifteen of these thirty-six cases. In twelve cases the specimens were of the primary site, i.e. kidney, and in three of metastatic deposits. Of the group of twelve primary cases, eight were men and four were women. The average age was sixty-four years. Table 1 shows the level of estrogen binding as a function of stage of disease. Table 2 relates the subcellular location of estrogen binding to binding intensity. Table 3 studies the same relationship but for the specimens of metastatic disease.

Our work is still at too preliminary a stage to draw any meaningful conclusions. The potential role played by steroid hormones in the kidney has been discussed (1).

Table 1. Level of Estrogen Binding Relative to Disease Stage

	Low	Moderate	High	Total
Localized to Kidney	-	-	6	6
Beyond Kidney	-	2	4	6
Total	-	2	10	12

Table 2. Renal Cell Carcinoma - Binding Site Study (Primary Tumours)

Binding		Nuclear	Cytoplasm	Mixed	Total
Low	<10%	-	-	-	-
Moderate	10-49%	1	-	1	2
High	>50%	8	-	2	10
Total		9	-	3	12

Table 3. Renal Cell Carcinoma - Binding Site Study (Metastatic Lesions)

Binding		Nuclear	Cytoplasm	Mixed	Total
Low	<10%	-	-	-	-
Moderate	10-49%	1	-	-	1
High	>50%	-	1	1	2
Totals		1	1	1	3

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Editorial Note (M.P.)

Although the authors state that their results are still preliminary, their 36 cases of renal cancer, that have been assayed for estrogen receptors by histochemical technique, lend themselves to some considerations.

It is hard to establish any correlation between this and the conventional biochemical techniques as the latter, as indicated by the authors, are unable to identify the presence of nuclear receptors, but merely reflect the presence of receptors in the cytoplasm. In the present series, fluorescence in the cytoplasm was found, along with nuclear fluorescence, only in three out of 36 patients. This would appear to confirm the findings of those workers who were unable to detect biochemically significant amounts of estrogen receptors in the cytosol of renal adenocarcinoma in humans.

The clinical significance of the relatively high number of cases showing nuclear binding sites by histochemical techniques needs to be clarified if the findings are confirmed by further study. It is also of interest that estrogen receptors in the normal kidney seem to be located more in the glomerular tuft and in Bowman's capsule than in the convoluted tubules from which renal adenocarcinomas originate. This work does not help to unveil the mystery of the role of hormones in the natural history and in the treatment of renal cell cancer.

HORMONE THERAPY AND RECEPTOR STUDIES IN HUMAN RENAL CELL CARCINOMA

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INTRODUCTION

There is very clear experimental evidence of the hormone-dependence of renal cancer in the male hamster (1-5) but little evidence of the effectiveness of hormone therapy in humans. Opinions are controversial and Hrushesky & Murphy (6) pointed out that the cumulative reported response rate to either progesterone or androgen therapy in advanced renal cell carcinoma was 17% in the literature up to 1971 and less than 2% afterwards. In recent years, antiestrogens have been tried and the response rate reported in the largest published series (7) is 4% objective remission and 28% subjective improvement. Different criteria of evaluation may be responsible for the widely differing results reported in the older literature.

In recent years steroid receptors have been widely studied in renal cancer and normal kidney parenchyma. With the exception of Concolino et al (8,9), who faithfully trust in the hormone-dependence of human renal cancer, high affinity binding sites to estrogens (ER) and to progesterone (PR) are found at a very low concentration in a small proportion of kidney tumor cytosols (10,11). In particular, estrogen treatment does not induce PR synthesis in human renal cancer (12) and both ER and PR binding is higher in the normal kidney than in the tumor (13). Recently a significant concentration of binding sites to androgens (AR) and glucocorticoids (GR) has been demonstrated in both the tumor and in normal tissue (13-17), while renal cancer appears not to contain binding sites to mineral corticoids (MR) (17).

PERSONAL EXPERIENCE

In a preliminary investigation of the hormone-dependence of human renal cell carcinoma, we studied the ER content in both the

tumor mass and the surrounding healthy parenchyma of 31 kidneys consecutively removed at the Istituto Tumori for cancer (18). ER were non negative in 12/31 neoplastic tissue cytosols (38%) and in 13/26 normal tissue samples (50%). Of these patients, 10 also had metastatic disease and were given high dose Medroxyprogesterone Acetate (MPA) as post operative treatment (19). Stabilization of the disease was achieved in two of five patients whose tumors were not ER negative. These preliminary results persuaded us to undertake a prospective cooperative study in Lombardy starting in July 1979; this is sponsored by the Consiglio Nazionale delle Ricerche (CNR), Rome.

The design of the study is outlined in Fig. 1. Every patient with renal cancer amenable to surgery undergoes a radical transperitoneal nephrectomy. No pre-operative embolization is allowed. Tissue samples are immediately taken from the tumor mass and the surrounding healthy parenchyma and stored at - 70°C. Receptor studies are carried out in the laboratory of the Istituto Tumori. ER, PR and AR are currently studied by the dextran-coated charcoal (DCC) technique as previously described (18). A further purification of the cytosol by precipitation with $(NH4)_2SO_4$ 35-50% was introduced in order to remove the steroid binding plasma proteins, as the kidney is a very vascular organ.

Up to March 31st 1981, 79 patients have entered this study (Table 1). Randomization of the 62 category MO patients is quite satisfactory (Table 2). ER and PR have been studied in 59 samples of neoplastic tissue and in 32 normal parenchyma. AR were studied in a smaller number of tissue samples (Table 3). ER and PR were detectable in 10% of renal cancer studied and in 40% of healthy tissues. AR were present in nearly the same proportions of healthy and neoplastic tissues studied and the concentration in the neoplastic tissue was twice that which was found in the normal parenchyma.

To date, relapses have occurred in 4 of the 46 evaluable category MO patients (8%). All relapses occurred in patients with locally extensive disease, 3 of whom had been randomized to receive MPA post-operatively (Table 4). However, as far as the receptor content in the tumor tissue is concerned (Table 5), relapses occurred only in ER- PR- and AR- patients and no response to hormone therapy could be expected in receptor negative cases. Twelve category M1 patients have been evaluated (Table 6). No objective response was observed, but four patients (33%) had stabilization of the disease for four to more than eight months. No correlation between clinical response and tumor receptor studies could be found. These results are very preliminary, and a larger number of cases must be studied to obtain significant information.

Resectable category M0 patients

RADICAL NEPHRECTOMY (ER,PR,AR)

STRAT
- hospital
- sex
- T.N.V.

RANDOM
- control group
- MPA 500 mg 3/w; x 12 mths.

RECURR
- MPA → testosterone
- Testosterone 100 mg 3/w

Resectable category M1 patients

NEPHRECTOMY (receptor studies) → MPA 500 mg/d. (for at least 2 mths)

EVAL
- Regression → MPA (500 mg 3/w)
- No change → MPA id.
- Progression → Testosterone

Fig. 1. Prospective Cooperative Study to Evaluate the Hormone-Dependence of Human Renal Cell Carcinoma (Lombardy Group 1979, sponsored by CNR, Rome)

Table 1. Patient entry from July 1st 1979 to March 31st 1981

Participating Center	No. of Cases	M0	M1	Registered only
Univ. Urol. School, Milano	10	9	1	-
Dept. of Urology, Lecco H.	7	6	-	1
Dept. of Urology, Brescia H.	27	23	3	1
Dept. of Urology, Bergamo H.	10	9	1	-
Dept. of Urology, Magenta H.	9	7	2	-
I.N.T. Milano	16	8	8	-
Total	79	62	15	2

Table 2. Distribution of 62 category M0 patients

	Stratification	No. of Cases	Randomisation MPA	Control
M	T1-2, N0, V0	21	10	11
	T3-4, N1-2, V1-2	21	10	11
F	T1-2, N0, V0	10	5	5
	T3-4, N1-2, V1-2	10	7	3
	Total	62	32	30

Table 3. ER, PR & AR Concentration and Binding Affinity in the Normal and Neoplastic Renal Tissue Samples Studied

Receptors	Tissue	Cases Studied	Positive cases		F mol/mg prot.	Ka 10^{-9} M^{-1}
			No.	(%)		
E.R.	Normal	32	13	(40)	2.75 ± 0.40	5.11 ± 0.95
	Neoplastic	59	6	(10)	4.34 ± 1.10	5.12 ± 1.02
P.R.	Normal	32	14	(43)	4.37 ± 0.77	3.41 ± 0.59
	Neoplastic	59	6	(10)	3.04 ± 0.92	1.03 ± 0.36
A.R.	Normal	20	6	(30)	5.33 ± 0.96	2.86 ± 1.24
	Neoplastic	47	11	(23)	9.21 ± 1.69	1.65 ± 0.19

Table 4. Recurrences in 46 evaluable category MO patients according to the extent of the disease

Treatment/Extent		No. of Cases	Relapses	
			No.	(%)
MPA	T1-2, NO, VO	13	-	-
	T3-4, N1-2, V1-2	12	3	(25)
Control	T1-2, NO, VO	10	-	-
	T3-4, N1-2, V1-2	11	1	(9)
Total		46	4	(8)

Table 5. Relapses in 46 evaluable category MO patients and receptor studies in their neoplastic tissue

Receptor studies in the neoplastic tissue	No. of Cases	MAP		Control	
		No.	Relapses	No.	Relapses
ER- PR- AR-	26	13	3	13	1
ER- PR- ARx*	7	5	-	2	-
ER- PR- AR+	5	4	-	1	-
ER- PR+ AR-	1	1	-	-	-
ER- PR+ ARx*	1	-	-	1	-
ER- PR+ AR+	1	-	-	1	-
ER+ PR- AR-	2	1	-	1	-
ER+ PR- AR+	2	-	-	2	-
ER+ PR+ AR+	1	1	-	-	-
Total	46	25	3 (12%)	21	1 (5%)

* ARx : AR not studied

Table 6. Receptor studies in neoplastic tissue and the results of high dose MPA treatment in 12 category M1 patients

Pt.	Sex	Receptors			Distant metastases	Response to MPA	Duration (months)	Survival (months)
		E	P	A				
1	M	-	-	-	adrenal (removed)	no change	8 +	8 +
2	M	-	-	-	paratesticular (removed)	"	5 +	5 +
3	M	+	+	+	bone	progression	-	3
4	M	-	-	-	bone and lung	"	-	6
5	M	+	-	+	lung	"	-	4
6	M	-	-	-	lung	"	-	15 + *
7	F	-	+	x	bone	no change	7	11
8	F	-	-	x	lung	"	4	6 +
9	F	-	-	-	lung	progression	-	11 + *
10	F	-	-	x	lung	"	-	13 + *
11	F	-	-	-	lung	"	-	3
12	F	-	-	-	bone	"	-	10

* 3 patients achieved stabilization by subsequent androgen therapy.

CONCLUSIONS

There is some evidence that a weak hormone dependence may exist in human renal cell carcinoma. Further studies are needed to investigate the mechanism of action of steroid hormones in this tumor, with particular reference to the possibility of inducing PR synthesis by tamoxifen administration (20) and to the significance of the high GR content in renal cancer.

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DIAGNOSTIC INVESTIGATIONS IN RENAL CANCER. ADVANCES IN DIAGNOSIS INCLUDING RADIOLOGY, LYMPHOGRAPHY, PERCUTANEOUS PUNCTURE ULTRASONOGRAPHY AND COMPUTED TOMOGRAPHY

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INTRODUCTION

The classical clinical triad of flank mass, pain and hematuria is seen in a small minority of patients. More than half the diagnosed renal masses, including tumors, are incidental findings during a urological investigation carried out for some other purpose or the result of a stubborn search for the aetiology of the origin of a variety of paraneoplastic symptoms or signs. In this respect renal cancer earns the nickname of the internist's tumor.

HISTORY AND PHYSICAL EXAMINATION

Hematuria is the initial symptom in about half the patients and is total in nature. Flank pain, usually dull or of a colicky nature when clots are passed, is another symptom leading to the diagnosis of renal cancer. Extra-renal signs, such as low grade fever, increased sedimentation rate, an expression of metastatic disease or one of the many para-neoplastic syndromes point in a slow but constant percentage of patients to the diagnosis (1). Physical examination allows the detection of a mass in a minority of patients. Edema of the lower extremities and weight loss are signs of advanced disease. As a rule we accept that a renal cyst of benign nature, a common disease in elderly people, should be considered as an incidental event and extreme caution is advised before attributing any of the aforementioned symptoms to this benign condition. Cukier et al (2) reviewed 184 cases of renal cancer and found hematuria (50%), flank pain (10%) and alteration of the physical status (10%) as the initial symptoms. In a retrospective study of 50 cases of renal cancer (from 1975-1980) and

50 cases of renal cysts (from 1977-1980) we found as the initial symptom of the 50 patients with renal cancer: 28 with hematuria (56%), pain of a dull nature in eight (16%), renal colic in another eight (16%) and an altered physical condition in three (6%). Only three of the 50 cases with renal cancer were referred with the diagnosis of flank mass and only two of the 50 cases with renal cyst presented symptoms that were attributable to the cyst. The sedimentation rate was elevated in all but three patients with renal cancer. A list of manifestations described in patients with renal cancer is presented in Table 1.

MEDICAL IMAGING STUDIES

Intravenous Urography and Nephrotomography

These studies are essential to an evaluation of the basic problem and their quality usually determines the chain of events leading to final diagnosis. To repeat an improperly executed intravenous urography (IVU) is good clinical practice. Irregularity of the excreting system by tumor invasion points to the diagnosis of transitional cell cancer. The real adenocarcinoma is revealed by the euphemistically called "space occupying lesion" with distortion of the collecting system and an enlarged displaced or non functioning kidney. In general one can state that regular, smooth and well defined masses are usually benign whilst irregularity, calcifications, amputations and variation in deformity are suggestive of neoformations. A typical renal cyst as an incidental finding can be demonstrated as a radiolucent mass, sharply defined against the normal parenchyma. In elderly persons we do not hesitate to accept this as a final diagnosis and avoid further investigations. Additional confirmation can be obtained in this examination by abdominal compression and mobility of the affected kidney (3). Normal variants which may mimic calyceal distortion such as exaggerated

Table 1: Clinical Manifestations in Renal Cancer

G.U.	Systemic Toxicity	Endocrine
Hematuria	Anemia	Erythrocytosis
Pain	Weight Loss	Hypercalcemia
Mass	Neuromyopathy	Hypertension
Varicocele	Fever	Ectopic HCG Production
AV Shunting	Amyloidosis	
	Hepatic Dysfunction	
	Increased Sedimentation Rate	

renal lobes or renal sinus fat deposit in the aged are typical and should be recognised. A renal scan may solve the problem in an individual case. Retrograde pyelography provides sharp definition by filling the collecting system but there is really no need for this investigation except for the collection of biopsy material in cases supected of having urothelial tumors. The variability of the collecting system and the variations in vascularity and excretion pattern appeal to the lyricism of the radiologist or to the logic of the computer (4) but the IVU is only the first step in a diagnostic pathway (Fig. 1) and a well educated guess with a lack of accuracy in a small but constant percentage of patients with renal masses. The prime example to illustrate the fallacy of tumor diagnosis on IVU only has been demonstrated by a review of 19 misdiagnosed cases in the National Wilms Tumor Study (5). An expert panel despite knowledge of the intent of the review, could not correct all the errors.

Ultrasonography and Computed Tomography

Ultrasonography (US) and Computed Tomography (CT) brought a new kind of diagnostic imaging, the cross-sectional display of organs and body. The physical basis of both techniques and the clinical priniples, limitations and applications in urological practice have been described elsewhere (6-8).

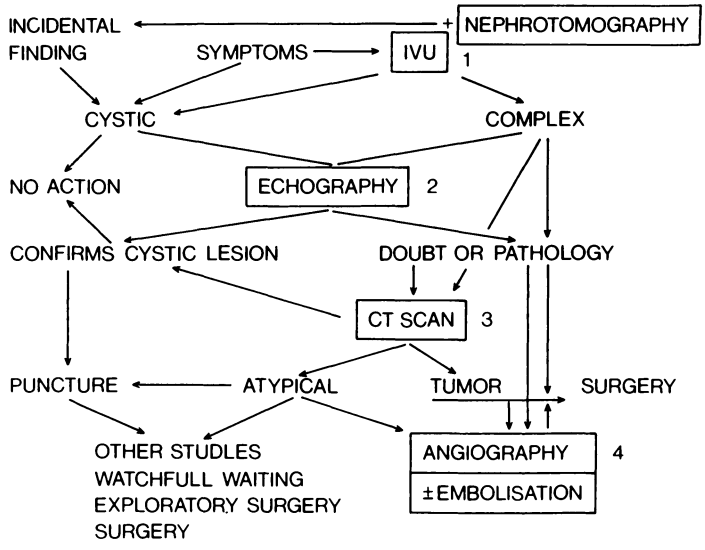

Fig. 1. Diagnostic Algorythm of Renal Mass

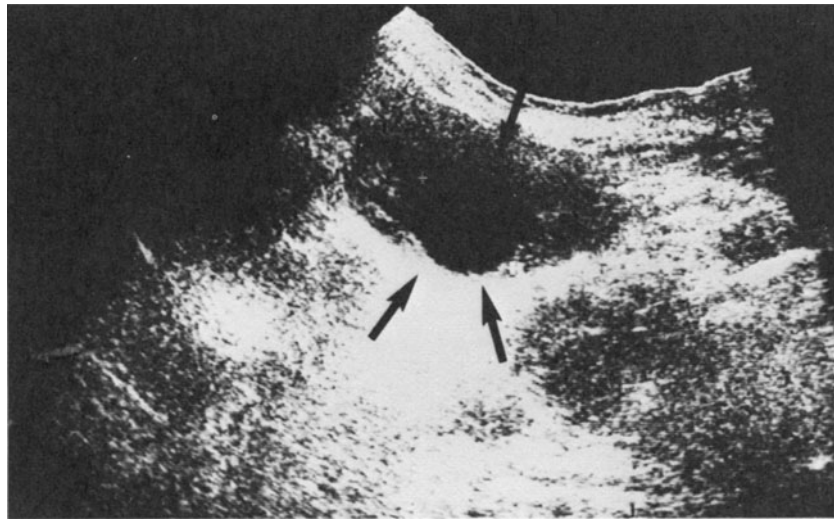

Fig. 2. Renal Cyst. Criteria on Ultrasonography include echo free lesions, sharp interfaces and enhanced transmission of sound through cystic space.

US became a natural second choice in the evaluation of renal masses because of its unusual accuracy in defining cystic structures, the commonest finding in renal masses. The complete absence of side effects, the low cost and the possibility of serial examinations justified the acquisition of the considerable skill required to learn to handle and interpret this technology. The US criteria for a cyst are well defined echo free lesions with sharp interfaces between fluid and normal tissue (Fig. 2). When dealing with an incidental finding we stop the investigation if this simple and clearcut definition is met. In doubtful cases and in those with echogenic masses further diagnostic tests are required. In a series of 266 renal masses the diagnostic accuracy for US was 100% for incidental cysts, 92% for renal cancer and 80% for symptomatic cysts (9). In our series one renal cancer was interpreted as a cyst in 1976. Advances in the equipment and greater experience allow better interpretation of the echogenic mass in angiomyolipoma (10), pelvic lipomatosis, infections and local obstructive uropathy (7). In the evaluation of extrarenal lesions however CT is superior to US (11,12).

In our algorythm to define renal mass we utilize US as a revolving door. Cystic lesions are left alone or punctured. Doubtful or complex solid lesions go directly to surgery if renal cancer looks obvious or go to CT or preoperative arteriography for further evaluation.

Compared to US the CT is more costly and requires the use of radiation and contrast media. However this technique is easier in operation and image interpretation and less limited by bowel artefacts and not at all by bone, fat or gas. In addition it is acknowledged that CT allows easy visualization of complete body slices with information on the suspected lesion and its adjacent structures. Staging of renal cancer and serial follow-up with accurate patient positioning seems to be superior by CT evaluation (13,14). Though we usually refer our doubtful or solid images on US to CT it is established that CT allows for accurate diagnosis of renal cysts. Here the mass lesion is well rounded with a uniform density close to that of water. This density does not increase after the administration of contrast material unlike that of the adjacent parenchyma. The cyst walls are not detectable outside the kidney structure. False negatives do occur when the cyst diameter is smaller than the slice thickness. The presence of a dense rim is suggestive of tumor especially when it is thick and irregular. Again a solid neoplastic mass is determined by its irregular form. Here the decreased area of density lies between the cyst pattern and normal parenchyma.(Fig. 3). Variation in these density readings usually indicate necrosis or hemorrhage.

It should be noted that small areas of calcification are easily detected on CT. The demonstration of extrarenal extension is optimal with CT including venous invasion, lymph node involvement and

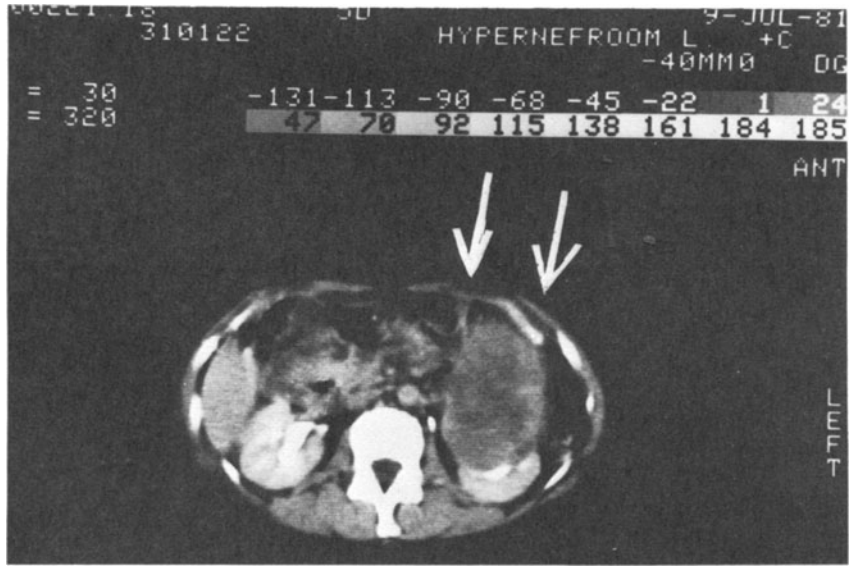

Fig. 3. Renal tumor is suggested by the irregular form and intermediate density on CT scan.

liver metastases. It is true that all these features are detectable by US subject to the skill and patience of the operator. We think however that US is more useful in detecting the presence of fluid collections such as abscesses, hematoma and urinoma around the kidney. In clinical practice CT proves invaluable since radiation treatment planning can be performed on the newest CT scanners (15). A tissue specificity with the exception of the typical angiomyolipomas is not possible and the etiology of extensive retroperitoneal tumors may be attributed to lymphoma, kidney, pancreas, retroperitoneal tissue, adrenals and metastatic disease. This lack of tissue specificity leaves us with the problems of indeterminate renal masses especially inflammatory disease that may mimic the characteristics of renal cancer on US or CT. It is at this point that the clinical evaluation of the individual patient requires further investigation or treatment by puncture and / or angiography.

Cyst Puncture and Aspiration Test Complex (CPATC)

Puncture of renal lesions is primarily reserved for the confirmation of cystic lesions by the aspiration of clear fluid without positive histochemical and cytologic examination. A cyst fluid culture is usually requested as a precaution. The histochemical analysis determines fat, protein, amylase and lactic dehydrogenese content. Cytologic examination establishes the presence of malignant or inflammatory cells (16). Contrast medium is instilled until the cyst is completely filled to reproduce the renal mass. The procedure is extremely simple and can be done under fluoroscopic, US or CT control (Fig. 4). The excellent accuracy and practical lack of complications leads automatically to a fine needle puncture in complicated cysts of an inflammatory or hemorrhagic nature. If the needle is in the lesion and no fluid is aspirated (a dry tap) a solid lesion is present. Aspiration biopsies by a thin needle technique are attempted in inflammatory lesions and hematomas (16). The hazard of puncturing a renal cancer is small indeed and only one case of needle tract seeding has been reported (17). We try to avoid the puncture of an obvious cancer but fine needle puncture aiming for cytological material is sometimes preferred to angiography in the elderly patient. False negative results occur but false positive results have not yet been seen. Negative cytological results after fine needle biopsy allow a wait and see attitude which we prefer to explorative surgery, a type of surgery which is costly to the patient and of poor diagnostic contribution. Cyst aspiration is without therapeutic benefit since most cysts recur after simple aspiration which is only to be expected. Therefore large cysts causing renal parenchymal atrophy or cysts in a perapelvic position should be excised.

Lymphangiography

The evaluation of suspected lymph nodes is based on lymph-

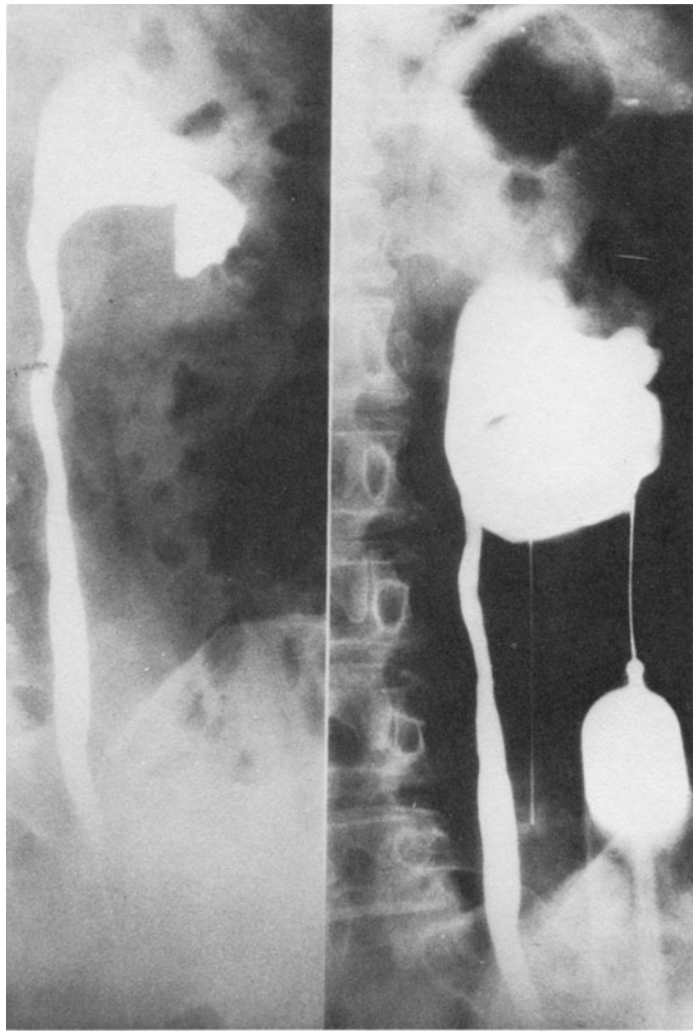

Fig. 4. Cyst puncture includes cytology, culture and chemical analysis of the fluid and radiological investigations.

angiography, CT scan, occasionally US and as an extremely rare event on physical examination. The problem of course with CT and US is the minimal mass required for discovery. The interest in lymphangiography is waning due to the number of false positive and false negative results and few centers perform this examination in patients with renal cancer (18). Many centers carry out a more or less extended lymphadenectomy after removal of the renal tumor. The therapeutic value of lymphadenectomy is not however clearly established (19). We continue to practice limited regional lymphadenectomy since we feel that the presence of tumor indicates dissemin-

ated disease which should be treated in a more systemic way. This kind of staging procedure adds no morbidity to the surgery and provides reliable data for final staging and treatment.

DISCUSSION

Ultrasonography and CT were instituted in our hospital in 1975 and 1976 respectively. They share cross-sectional images, non invasive characteristics and many similar applications in clinical organ imaging. Together, they changed the whole diagnostic pathway to the solution of the space occupying lesions in the kidney. Accurate clinical information and a perfectly performed intravenous urography with nephrotomography constitutes the first and basic step in this algorythm. We prefer US as our next step in the investigation due to its total lack of side effects and its relative low cost compared to CT. The diagnostic accuracy is near 100% for the uncomplicated cystic lesion, a prevalent renal mass in elderly people. In the presence of symptoms, in case of doubt or in solid lesions CT brings complementary and sometimes invaluable information.

CT is superior in the demonstration of extrarenal growth, the relation and assessment of other retroperitoneal structures and the confirmation of the cystic, solid or mixed state of the mass.

The enhanced possibility of percutaneous puncture of all and especially cystic structures allows for confirmation of the diagnosis. This technique should be utilized in more cases with contradictory findings. In a recent round table on diagnostic problems in renal tumors it became evident that most centers experienced special problems where US, CT or both failed to suggest the correct diagnosis or worse were misleading. Typical examples were presented (2). Enhanced experience and improved technology will diminish these problems. However a constant reassessment with clinical controls is necessary for proper evaluation. Newer and refined technology has changed our possibilities for proper evaluation six times in the last five years for US and three times for CT. At this moment we utilize the Aloka SSD 60 C and the Philips Sonodiagnost B 7000 for US evaluation of the kidney and, for CT, the ACTA Scan 200 F.S. The participation of many centers is required to evaluate future trends in cross-sectional imaging to define the exact clinical applications to give the greatest advantage to the individual patient and the least expenditure to an increasingly cost conscious society.

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ARTERIOGRAPHY, VENOGRAPHY AND RADIO-ISOTOPES IN THE DIAGNOSIS OF CANCER OF THE KIDNEY

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ARTERIOGRAPHY

In 1981, renal angiography is no longer necessary in order to make a diagnosis and to institute appropriate treatment for renal cancer. However it remains of great value in the diagnosis of extension of the tumour and for the knowledge of the arterial and venous disposition which the surgeon will encounter. From a technical point of view, this information is important.

TECHNIQUE

As with all invasive tests, arteriography involves certain risks. However in the hands of experienced operators, these are minimal. Our arteriographic team has done over 7,000 diverse angiographies and has experienced approximately one complication per 1,000 cases.

After puncturing the femoral artery according to the classical Seldinger technique, the aorta is catheterized after which:

(a) Aortography (Fig 1) allows one to visualize the entire abdominal arterial vessels to isolate the tumour, its pedicle or pedicles and to show an extension, particularly to the mesenteric territory, the adrenals, the contralateral kidney or the liver. It also allows the discovery of other vascular lesions.

(b) Selective opacification of the renal arteries (Fig 2a) should always be carried out, injecting a large quantity of

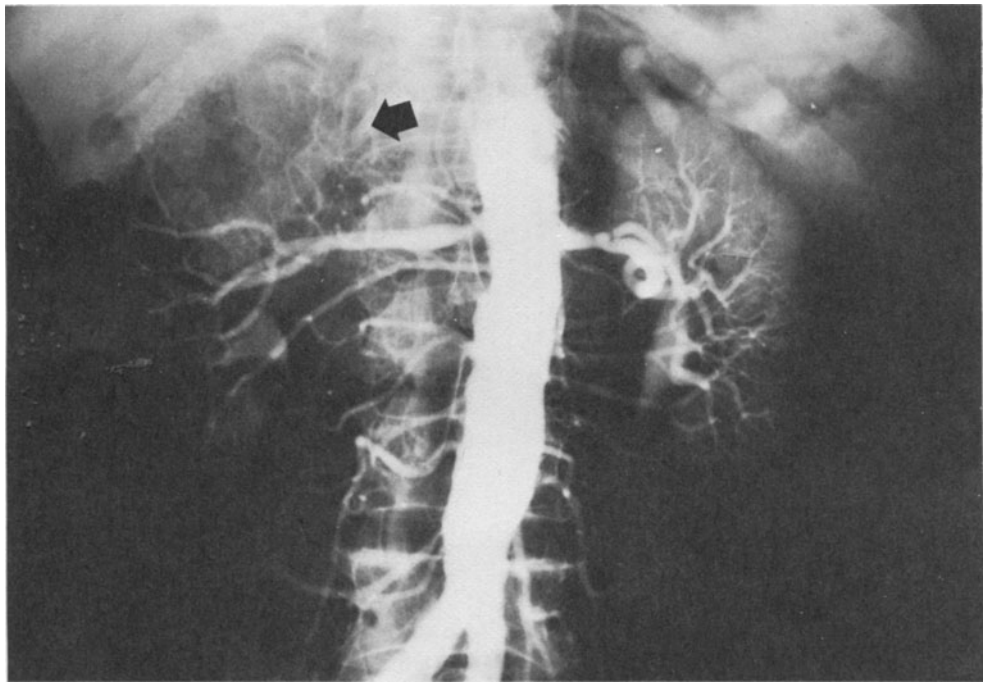

Fig. 1. Translumbar aortography - shows two right renal arteries with stenoses of the main right renal artery and of the left renal artery in a patient with a tumour of the upper pole of the right kidney (arrow).

contrast medium in order to obtain a venous return that can be analyzed (Fig 2b).

It also allows the utilisation of drugs to modify arterial function, if, after standard posterior, anterior and lateral views, the diagnosis remains uncertain. Selective exploration of the celiac trunk is desirable in order to search for a neighbouring extension of the primary tumour or of metastases (especially hepatic lesions).

RESULTS

If a diagnosis appears evident after angiography, the X-rays should be analyzed methodically starting with the trunk of the renal artery to the trunk of renal vein: vessels penetrating the tumour and their extension, tumorography, the peritumoural venous circulation and the large venous axes.

Angiographically, there are two different forms of renal

cancer, the hypervascular tumour, where all the signs of malignancy are formed and appear evident from the test injection on, and the hypovascular cancer in which there are discrete anomalies which only a methodical search can discover.

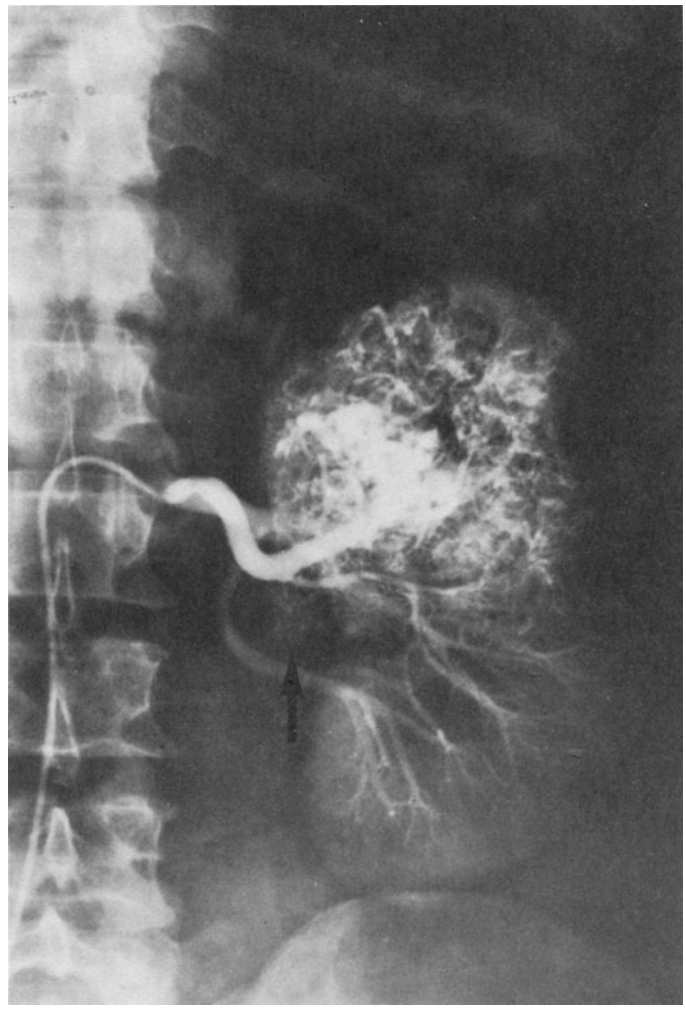

Fig. 2a. Left selective renal arteriogram - large hypervascular tumour at the upper pole of the left kidney with some new blood vessel formation in the renal hilum (arrow).

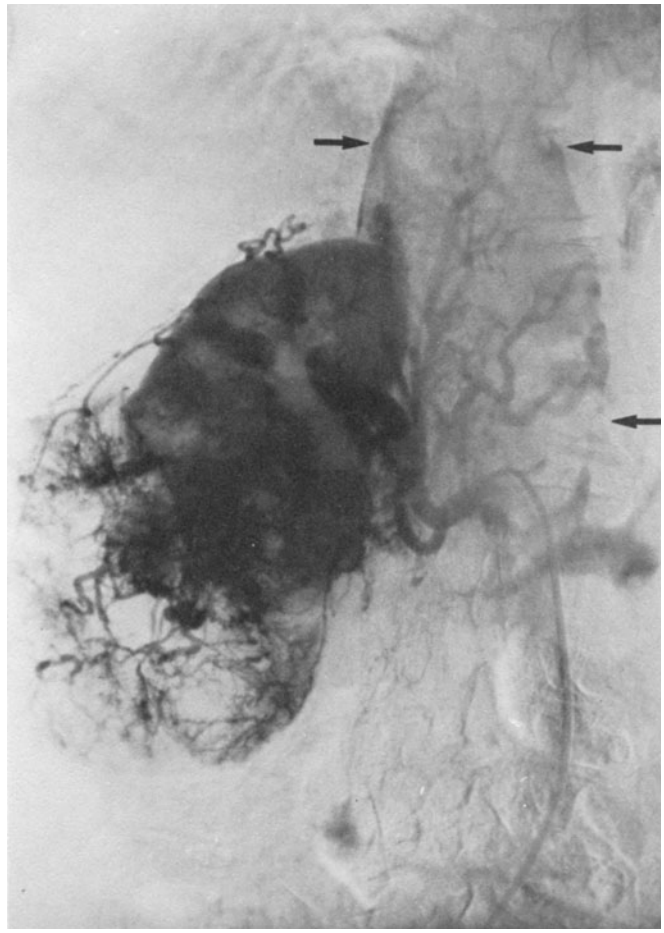

Fig. 2b. Right selective renal arteriogram - large tumour of the lower pole of the right kidney with obvious extension in the inferior vena cava. The neoplastic growth extending into the vena cava is partially vascularised by branches of the right renal artery. This permits the identification of the position of the inferior vena cava which is displaced medially. Only a slight amount of contrast medium is visible in the left renal vein.

(a) The Hypervascular Syndrome This combines all (or almost all) signs of renal cell carcinoma:

- augmentation of the size of the renal artery and its bifurcations
- displacement of the peritumoural arteries (less than in the case of a cyst)

- amputation of arteries or arterial branches
- neovascularization or anarchic distribution, the vessels being irregular in their direction, their caliber, and their divisions and sometimes forming sanguinous lakes
- very obvious tumorography, most often heterogenous with vascular zones corresponding to necrosis, sometimes well delimited and separated from non invaded renal parenchyma
- an abundant peritumoral venous circulation arising because of an obstruction of normal venous drainage: neoplastic outgrowth extending more or less towards the vena cava, which can be confirmed by the analysis of venous return during selective arteriography.

The diagnosis of adenocarcinoma of the kidney creates few problems of differential diagnosis in hypervascularized tumours. A hamartoma (angio-myolipoma) can have a similar angiographic appearance and biopsy alone will confirm the diagnosis if tomodensitometry is not conclusive.

(b) Hypovascular Cancer The diagnosis is more difficult and in doubtful cases necessitates special radiological manoeuvres, often the injection of vasoconstrictors into the renal artery or its branches (angiotensin, norepinephrine). In these patients:

- vascular spreading seems to be incomplete and some branches appear to penetrate the tumour
- neovascularization is seen mainly by arterial amputation and irregularities of the vascular pathway
- tumorography is absent but the parenchymal space occupying lesion "is regular". This aspect is different than that of a cyst.
- venous invasion is less frequent than in hypervascularized tumours.

The diagnosis of malignancy is more difficult to establish in these cases and will also be more difficult to relate to a specific pathological lesion. One can discuss various diagnoses including clear cell carcinomas with necroses, undifferentiated adenocarcinoma or basophilic cell tumour, renal metastasis of various tumours (gastro-intestinal, ENT, pulmonary) or of a hemolymphopathy, a mesenchymal tumour and of course cancer of the excretory pathway. Usually, however, these transitional cell lesions are recognized before arteriography is undertaken. If arteriography is carried out, it can show neovascularization which does not appear unless functional vascular modifiers are injected. Arteriography is of secondary importance in the diagnosis of excreto-urinary cancer, but it can help in resolving problems of non secreting kidneys.

Nephroblastoma is rare in adults and is usually seen in an extensive hypovascular lesion. The arteries are irregular and are spread out by the tumour formation. Neighbouring extension is

frequent, as well as metastasis to the contralateral kidney. However, these properties do not always confirm the diagnosis of nephroblastoma in adults.

CONCLUSION

Arteriography is no longer an indispensable test to establish the diagnosis of cancer of the kidney. This diagnosis can easily be made using non invasive methods such as intravenous urography and echography and eventually confirmed by CT scan which will show up extracapsular extension.

The value of arteriography is no longer to confirm a diagnosis but to add further information to a diagnosis already known or uncertain. Angiography allows one to appreciate with precision the size of the tumour in the frontal plane and its extension to other viscera. It also maps out the arterial and venous area that the operator will encounter during surgery.

PHLEBOGRAPHY

It is helpful to know the venous network in order to know the extent of spread of the renal cancer, to specify a prognosis, and, to establish a rational operative approach.

Usually, the nephrogram phase of renal arteriography gives a good visualisation of the venous bed. If the renal vein or veins are not well seen after arteriography or if an important collateral venous circulation is discovered, cavography (Fig 3a) or eventually venography (Fig 3b) are indicated.

For certain difficult diagnoses, selective phlebography of the trunk of the renal veins is indicated, especially for hypovascular tumours, for example, even if the venous bed can be seen during arteriography.

TECHNIQUE

In order to avoid dislodging a neoplastic thrombus, angiography of the inferior vena cava always precedes selective opacification of the renal vein but may be carried out by canulation via the superior vena cava and right auricle (Fig 4).

(a) The Inferior Vena Cava Cavography is usually performed after puncture of a femoral vein. The permeability of the iliac axis is verified by a test injection of the contrast medium. A catheter is placed in the inferior vena cava after having verified the permeability of this large venous trunk while the patient is performing a Valsalva manoeuvre: afterwards, the renal vein is selectively catheterized.

(b) The Renal Vein The technique of pre-operative arterial embolisation has the same effect on phlebography as does intra-arterial injection of angiotensin. As a result of the absence of arterial flow, venous reflux from the inferior vena cava into the renal vein is established. Therefore one can obtain good visualisation of the venous pedicle and its tributaries. Opacification of the intra-renal venous network is obtained using pharmaco-angiography (intra-arterial injection of angiotensin) or by using a catheter with a balloon which occludes the trunk of the renal vein.

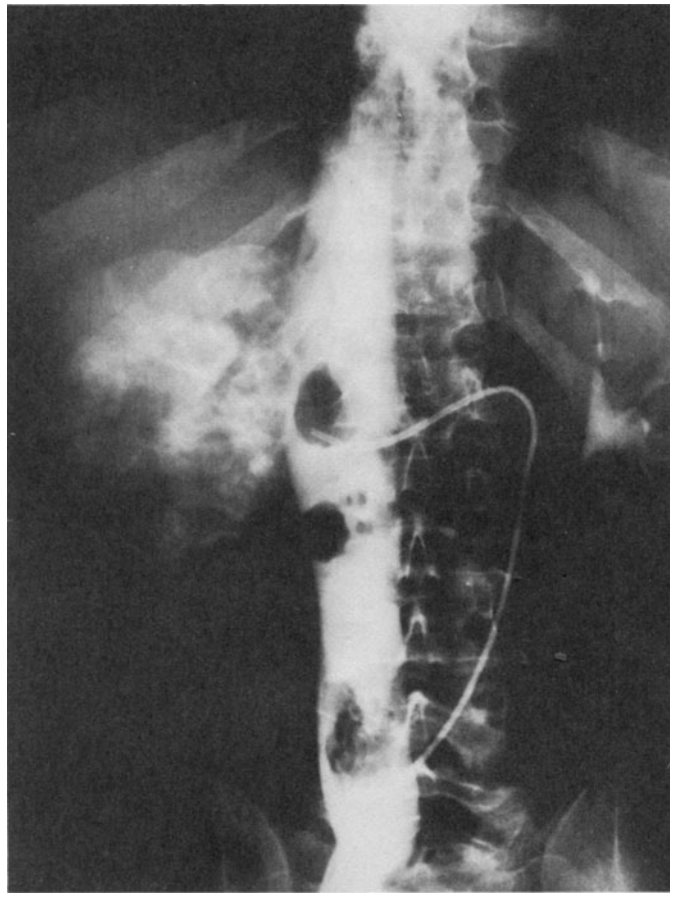

Fig. 3a. Inferior vena cavography after puncture of the right femoral vein. Multiple filling defects in the vena cava arising from a right renal tumour. This example underlines the importance of cavography before selective renal vein catheterisation.

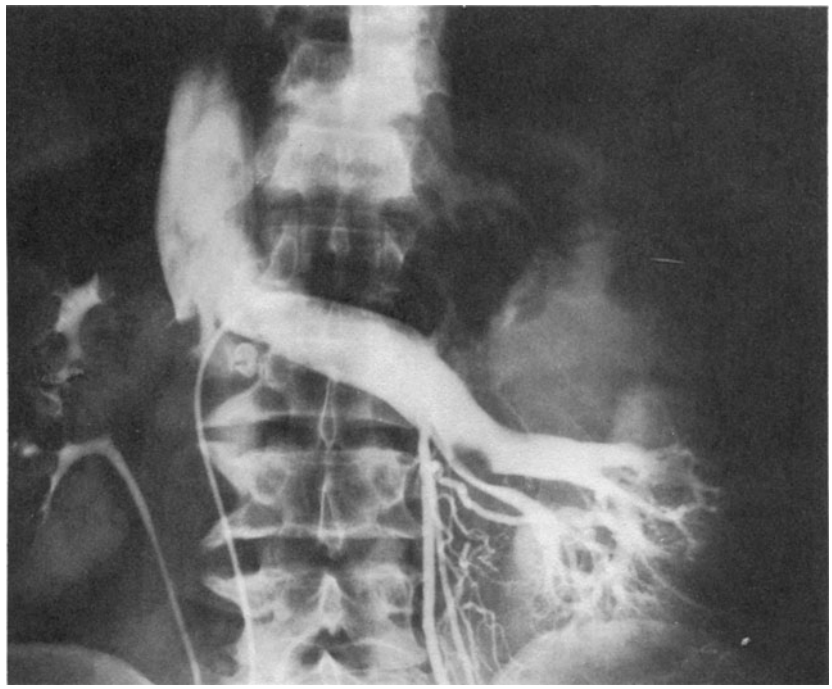

Fig. 3b. Selective catheterisation of the left renal vein - shows a filling defect in a branch of the renal vein, corresponding to the region of hilar vascularity shown in the arteriogram in figure 2a.

RESULTS

Invasion of the vena cava presents as a constant well defined sometimes polycyclic space occupying lesion. A differential diagnosis, but easy enough to establish with lateral view cavography, is the compression of this venous axis by metastatic adenopathies with give the false impression of space occupying lesions.

Within the renal veins, the radiological signs of a tumour includes irregular narrowings, interruptions of the venous axes, lateral imprinting of the venous wall and sometimes images of different density which are very difficult to diagnose. Certain renal vein space occupying lesions can be difficult to understand and correspond to the drainage of a hypervascularized tumour. It is important to compare arteriography and selective phlebography: an accelerated flow of venous drainage usually corresponds to hypervascularisation at the level of the renal parenchyma, due to actual intra-tumoral arterio-venous communications. This observation sometimes allows one to confirm an otherwise uncertain diagnosis of cancer of the kidney.

CONCLUSION

Plebography is no more essential than arteriography as an aid for the surgeon who is about to carry out an ablation for cancer of the kidney. However, phlebography is useful in that it allows one to appreciate the vascular extension of a cancer and to establish a rational operative approach.

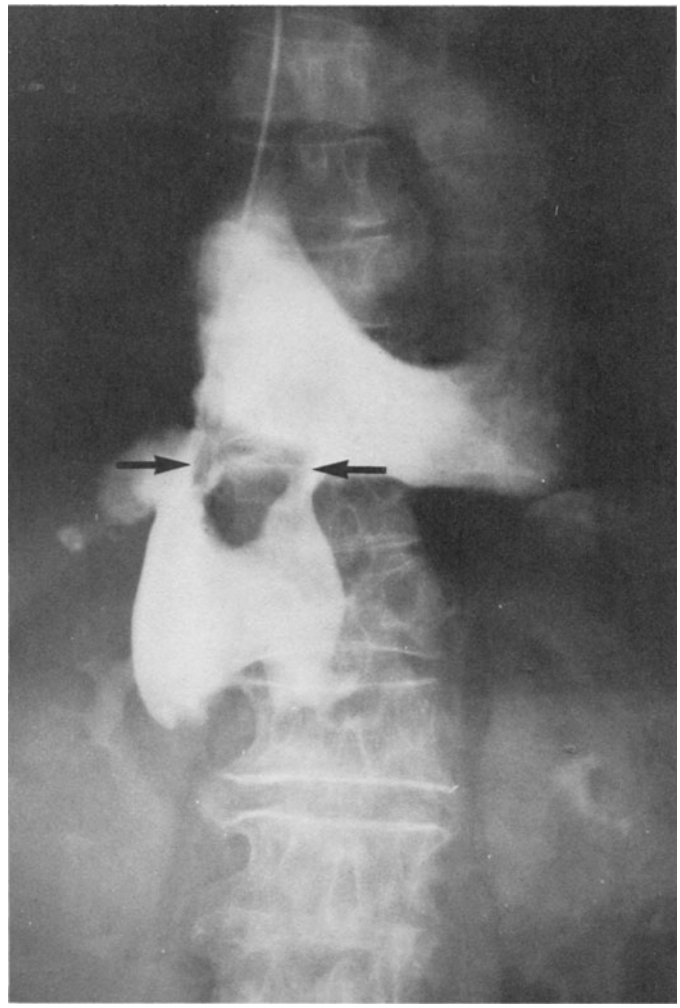

Fig. 4. Opacification of the inferior vena after catheterisation via the superior vena cava and right auricle. The extension into the inferior vena cava extended up to the right auricle and is revealed by the contrast.

RADIO-ISOTOPES

Diagnosis of the Primary Tumour

Renal investigation with radiopharmaceuticals provides a reliable, easy and non invasive method for appreciating renal morphology and estimating renal function. Because of revolutionary developments in echographic techniques (specially real time scanning) and in computed X-ray tomography, the renal scan has lost some of its interest for demonstrating anatomical defects although it may be helpful as the first approach. It is however increasingly used for the determination of divided renal function.

Static renal imaging technique. Until the early 1970's, the agent used to demonstrate the renal parenchyma was usually an organic mercury compound: Neohydrin labelled with Hg 203 soon replaced by Hg 197 labelled organomercurials. The molecules are filtered through the glomeruli and tightly bound in the tubules. They show relatively slow excretion - approximately 1% per 24 hours, so that long lasting images could be obtained. However their use was limited by the unfavorable dosimetry of Hg 203 and the suboptimal energy of Hg 197.

As a result the mercurial compounds were replaced by Tc 99 m labelled renal agents, iron ascorbate - Tc 99 m, Gluconate or glucoheptonate Tc 99 m, and DTPA - Tc 99 m (diethylene triamine pentaacetic acid) which is almost completely excreted by glomerular filtration and is used as a standard of glomerular filtration rate. DMSA - Tc 99 m (dimercapto succinic acid), like chlormerodrin, shows high cortical and low medullary concentration and low urinary excretion, allowing visualisation of the renal cortex without interference by the collecting system. When the preparation is injected intravenously, approximately 50% of the administered dose is localized in the kidneys. Quantification of the uptake of DMSA into each kidney is determined and the ratio of counts in each kidney calculated so that we can estimate individual renal function.

Results. When the kidneys are normal, the uptake is homogeneous - sometimes we can see the collecting system as an area of reduced activity. When there is a renal tumor, the normal renal cells concentrate the scanning agent, leaving the tumor as an irregular area of decreased activity. Yet an image of a parenchymal defect is not specific for such a defect may also be an abscess, hematoma or a renal cyst (Figs. 5 and 6).

Diagnosis of Metastases

Pulmonary and bone metastases are quite common in renal cancers. Bone scanning is a necessary technique to ascertain the spread

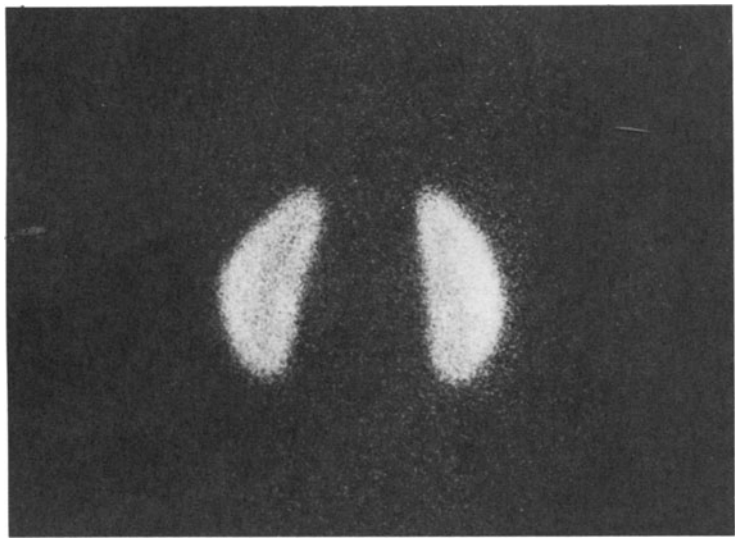

Fig. 5. Normal D.M.S.A. renal scan.

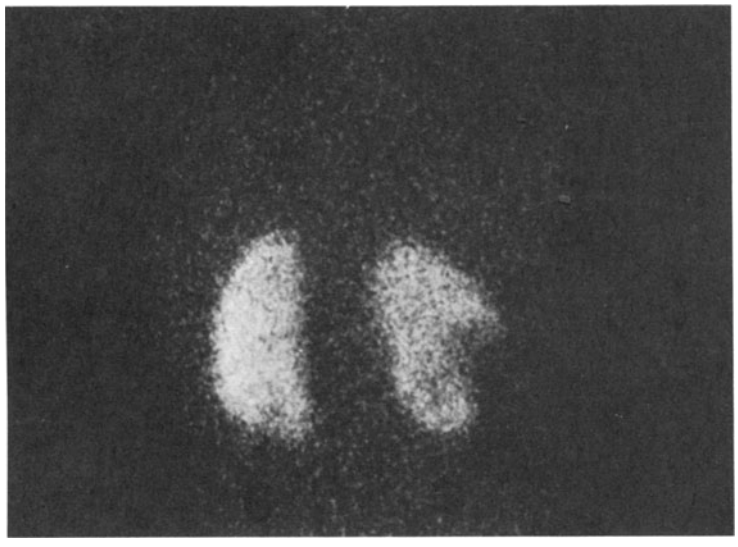

Fig. 6. D.M.S.A. renal scan showing filling defect of the infero-lateral part of the right kidney.

of bone-seeking cancers. A bone scan proves or excludes the diagnosis of bone metastases during the initial check-up, detects new localisations during follow-up and allows one to evaluate the efficiency of the treatment.

Technique. Bone tissue is constantly being remodelled. Normally there is an equilibrium between bone crystal and blood so that the calcium concentration in the skeleton is constant. The tumor takes the place of the calcium in bone structure, disturbing this equilibrium. The bone reacts by trying to lay down mineral substances all around the lesions. The calcium balance may be either deficient or in excess.

For bone destruction to be demonstrable by X-rays, the proportion of calcium in metastatic bone must be different from that in normal tissue by about 30%. Thus the lesions are discovered late. By using bone-seeking radio-isotopes, areas of osteolytic activity are visualized as well as areas of osteoblastic activity. These "hot spots" are seen early.

The ideal isotope would be calcium 47 or calcium 45 but the physical properties are not suitable. Ca 45 has too long a half-life (165 days) and Ca 47 has too high γ energy (1,3 Mev). Strontium (Sr 85 or Sr 87 m) follows calcium accurately in bone metabolism but is not commonly used.

The discovery of compounds of phosphate labelled with Tc 99 m allows the widespread use of bone scans. Polyphosphate, pyro-phosphate and mainly methyldiphosphonate (MPD) are used. The patients are given 15 mCi of MDP - Tc 99 m intravenously. Imaging is carried out two or three hours after injection.

A view of the skeleton is obtained with a camera equiped with a whole body system after which local views are recorded on film. Besides the anatomical study, the computer gives a pathological bone - to - normal bone ratio and a lesion - to - total activity ratio. This quantification allows us to follow the development of metastases and to appreciate the efficiency of the treatment.

Results. Metastases are shown by "hot spots." These areas of increased radioactivity are seen from three to nine months sooner than X-ray abnormalities. For each patient the ratios are calculated every three months. Three groups of patients are found: those whose pathological bone - to - normal bone ratio decreases, those whose ratio stays unchanged and those whose ratio increases. In this last group the treatment is not effective.

SUMMARY

Quantified bone scanning, with the computation of activity

ratios between pathological bone area and normal bone area, can be done every three months, and leads to an appreciation of the development or regression of bone metastases under treatment.

IS ARTERIOGRAPHY ANY LONGER NECESSARY IN THE ASSESSMENT OF RENAL CANCER?

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The process of diagnosis of renal tumors is usually triggered by the finding of a space occupying lesion at IVP. In the past the definitive diagnosis of the lesions depended most of the time on surgical exploration or needle aspiration.

Later the introduction of arteriography represented a major step forward in the diagnostic field of renal tumors. In the last few years, however, the development of ultrasonography and computerized tomography (CT scan) have dramatically changed the diagnostic approach to kidney tumors and rendered arteriography unnecessary in the majority of cases.

The diagnostic approach in kidney tumors must assure:

1. highest confidence level
2. least invasive diagnostic studies
3. minimal risk to patient
4. lowest price in time and money.

On every one of these criteria the association of ultrasonography and CT scan can score better than arteriography as we shall try to demonstrate.

In space occupying lesions of the kidney ultrasonography is a good screening study to differentiate between cysts and solid tumors. The confidence level is excellent in asymptomatic masses (the risk of finding a cancer is those cases is about 5%). Under such

conditions an unequivocal ultrasonic diagnosis of a cyst may be accepted as definitive. In our experience of 220 cases there was virtually no risk of missing a solid tumor.

If ultrasonography suggests a solid tumor and in patients with symptomatic lesions the confidence level of ultrasonography is no longer acceptable. These lesions should be explored further. Should such exploration be by arteriography, by CT scan or both? That is the question - or more precisely that was the question, for now we have the answer. CT scan is the best routine exploration for kidney tumors, arteriography being reserved as an additional study in a few particular cases since -

(i) CT scan can diagnose renal masses more accurately than arteriography.

At La Pitié Hospital in Paris 137 kidney tumors were explored by CT scan and 116 by arteriography (most of them had both investigations). Diagnosis was ultimately confirmed by surgical exploration and/or a two year follow up in cases not treated surgically.

This study showed that CT scan gave the exact diagnosis in 95% of cases and arteriography in only 79% of cases (Table 1). Details of the seven patients with erroneous or uncertain diagnosis on CT scan is given in Table 2. Details of the 21 patients with an erroneous or uncertain diagnosis at arteriography is given in Table 3.

Table 1. Diagnosis of Renal Tumors.

	CT Scan	Arteriography
No. of cases	137	116
Diagnosis accurate	130 (95%)	92 (79%)
Diagnosis erroneous or uncertain	7 (5%)	24 (21%)

Table 2. Patients in Whom Diagnosis by CT Scan Was Uncertain or Erroneous (7 Cases).

Diagnosis uncertain	5
Failure to differentiate benign and malignant tumors	2
False negative diagnosis of cancer	0

Table 3. Patients in Whom Diagnosis by Arteriography Was Uncertain or Erroneous (24 cases).

Diagnosis at Arteriography		Diagnosis at Operation	
Cysts	4	Cancers	2
		Bertin *	1
		Normal	1
Cancers	4	Cysts	2
		Angiomyolipoma	1
		Normal	1
Normal	3	Transitional Cell Tumor	2
		Cancer	1
Benign Solid Tumor	1	Cancer	1

12 Lesions of Uncertain Diagnosis

* Hypertrophy of Bertin's column.

(ii) CT scan can assess abdominal disease extension in renal cancer much better than arteriography. CT scan can study most of the parameters usually considered in the assessment of disease extension while arteriography can only explore the possibility of renal vein invasion or thrombosis (Table 4).

Table 4. Assessment of Possibility of Extension of Disease in Malignant Renal Tumors.

	CT Scan	Arteriography
Perinephric fat	+	
Renal vein	+	+ (10/18)
Vena cava	+	
Lymph nodes	+	
Extension to adjacent organs	+	
Abdominal wall	+	
Liver metastases	+	

In our experience CT scan gave a correct appreciation of disease extension in the abdomen as confirmed at operation in 85% of cases of renal cell carcinoma. Arteriography gave data only on renal vein involvement and even in this situation the information was less accurate than that given by CT scan. In our series arteriography diagnosed renal vein extension in 10 of the 18 surgically documented cases showing renal vein involvement. Without CT scan, cavography must be used together with arteriography to achieve a good level of confidence as far as renal venous involvement is concerned.

(iii) CT scan is a non-invasive study and is more comfortable and much safer than arteriography. Despite the excellent safety record of arteriography, complications do occur in 1-2% of cases reported in the world literature. In our 116 cases we had two complications (1.8%), one of septicaemia and one of arterial thrombosis at the injection site that necessitated surgery.

(iv) CT scan is also much cheaper than arteriography (Table 5). Furthermore, CT scan, by eliminating the need for hospitalization and reducing the rate of complications, leads to even lower costs in both money and time.

CONCLUSION

It is for these reasons that, at La Pitié Hospital in Paris, CT scan is the technique used routinely in the diagnosis of renal cancer, arteriography being reserved as an additional procedure for the doubtful case (Fig. 1). We must point out that arteriography is undertaken only in 5% of cases for diagnostic purposes. In a further 10-15% of cases it is done mainly for therapeutic reasons (Table 6).

Table 5. Costs of Ultrasound, CT Scan and Renal Arteriography (French Francs).

Ultrasound	CT Scan	Arteriography
200 FF	600 FF	2500 FF + 2 days in Hospital

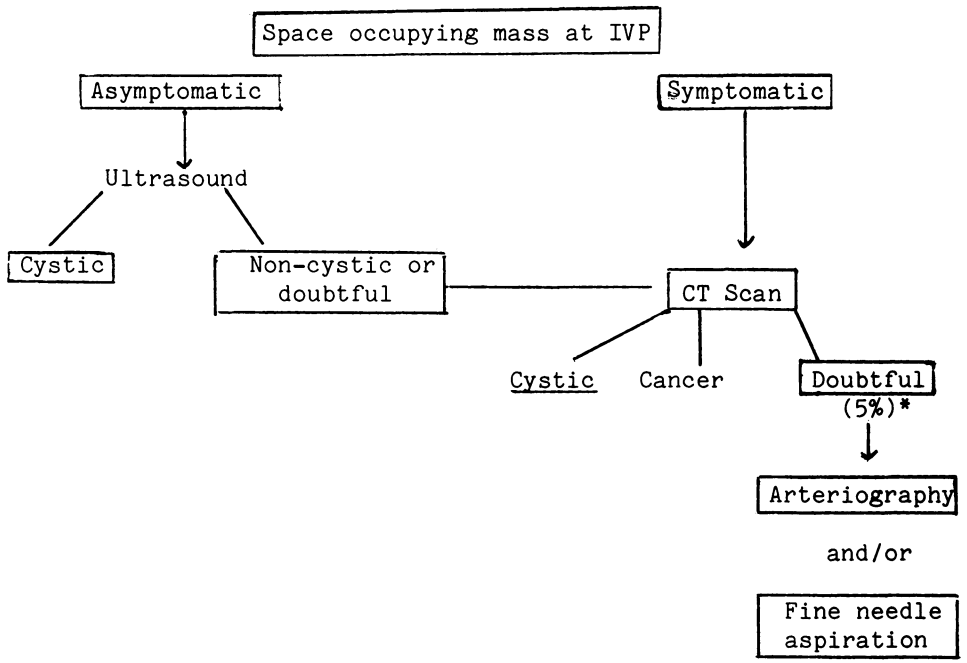

Fig. 1. A Systematic Diagnostic Approach to the Assessment of Renal Mass Lesions.

Table 6. Indications for Arteriography

1.	Cases unconfirmed by CT Scan	5%
2.	Cases where conservative surgery is considered	2 - 3%
3.	Tumors in which pre-operative embolisation is indicated	10 - 15%

CT scan has a high and very acceptable confidence level in the diagnosis of renal tumors and in the assessment of disease extension in the abdomen. In these respects it is more accurate than arteriography. It is also more comfortable, safer and cheaper. As a result is is now the technique of choice for the diagnosis and assessment of renal cell cancer, being effective when used alone in 95% of cases. Additional renal angiography is now required in only 5% of patients.

Editorial Note (P.H.S.)

Both ultrasound and computerized tomography are non-invasive, less demanding for the patient and cheaper than arteriography. Accepting that embolization is of value only for the larger renal tumors and that ultrasound and computerized tomography can reliably differentiate between cystic and solid lesions, the conclusions drawn by the authors are both logical and desirable. In my view this approach should be widely adopted.

ASPIRATION BIOPSY AND CYTOLOGY OF RENAL CANCER

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INTRODUCTION

In cases of renal carcinoma, preoperative cytologic verification of the diagnosis and assessment of the malignancy grade by aspiration biopsy is part of our diagnostic routine. Though echotomography and computerized tomography have reduced the need of diagnostic verification, there still remain a number of patients with a small tumour where needle puncture is useful to distinguish between a cyst and a tumour, sometimes a cystic tumour, and to distinguish adenocarcinoma from renal pelvic cancer. In addition aspiration biopsy is an easy and reliable method for preoperative malignancy grading.

METHOD

In principle the technique is the same as in aspiration biopsy of the prostate, introduced by Franzén, Giertz and Zajicek (1).

The puncture is done under television-monitored fluoroscopy and the kidney located by intravenous urography. In recent years ultrasound scanning has been used more often than fluoroscopy. With the patient in the prone position and local anaesthesia in the skin a trocar with a diameter of 1.5 mm is introduced from behind. When the tip of the trocar lies against the kidney the mandril is removed and a long thin needle introduced. The needle is attached to a syringe. Cellular material is aspirated from various parts of the tumour. The smears are either air-dried and stained by the May-Grunwald-Giemsa method or fixed in an ether-alcohol mixture and stained by Papanicolau's technique.

To evaluate the conformity between aspiration biopsy and histo-pathologic grading von Schreeb, Franzén and Ljungquist (2) performed puncture of the removed kidney within five minutes of dividing the blood vessels in 47 patients. In some cases pre-operative percutaneous puncture was also carried out. The smears were similar whether the puncture was done percutaneously or on the operative specimen.

The malignancy grading was determined by a cytologist and the histo-pathological evaluation by a separate person, a pathologist. If the smears contained cells of varying degrees of differentiation the most dedifferentiated cells were taken as indicating the malignancy grade. In the histo-pathological evaluation the highest malignancy grade also determined the classification.

Table 1 shows a comparison between cytologic and histologic malignancy grading in the 47 cases. In 36 cases there was agreement between the cytologic and the histologic evaluation, and in 11 cases there was a disparity, usually of one grade, but in one case of two grades. Sometimes tumours with no marked cellular dedifferentiation but with infiltrative growth are considered highly malignant. This may be one explanation of the disparity in some cases. Another explanation is the individual variation of judgement and there is no escape from the fact that a subjective judgement is part of both the cytologic and histo-pathologic evaluation.

Like cancer of the prostate, renal carcinoma often has different cell patterns in different areas. The risk of having a non-representative aspiration biopsy is however minimized by taking material from different parts of the tumour.

Table 1. Correlation Between Cytological and Histological Grading in 47 Patients

Cytologic Grade	Histologic Grade		
	High	Moderate	Poor
High	4	3	1
Moderate	-	13	6
Poor	-	1	19

Table 2. Relation between survival, degree of differentiation, size of tumour, and macroscopic and microscopic delimitation in 172 cases of renal cell cancer (from Arner, Blanck and von Schreeb, 1965).

	5 year survival
Histological Differentiation	
Well differentiated	82%
Moderately differentiated	50%
Poorly differentiated	15%
Size (Diameter)	
< 7 cm	69%
7 - 15 cm	40%
>15 cm	29%
Macroscopic delimitation	
Clear demarcation line	62%
No clear demarcation	39%
Microscopic delimitation	
Delimited tumours	61%
Unclear delimitation	31%

In a series of 187 cases of renal carcinoma, subjected to nephrectomy, Arner, Blanck and von Schreeb (3) studied the correlation between survival and various morphologic parameters such as the degree of cellular differentation, tumour size and macroscopic and microscopic delimitation of the tumour, in the 172 patients surviving more than one month after operation. All these factors are correlated with survival (Table 2). In the patients with poorly differentiated carcinoma there was a higher than expected incidence of large and poorly demarcated tumours.

Another question that has to be answered is whether puncture of the tumour implies risk of seeding tumour cells and producing metastases. In a previous series of renal cancer patients, puncture of the tumour and injection of contrast medium was performed. The needle used for injection had a larger calibre than the needle used for aspiration biopsy. Von Schreeb, Arner, Skovsted and Wikstad (4) compared the 5-year survival of 77 patients without obvious metastases and subjected to renal puncture with the survival of 73 control patients who had no puncture. When tumour size and degree of malignancy were taken into account there was no significant difference in 5-year survival between the two groups. Thus there is no reason to believe that aspiration biopsy involves risk of tumour spread.

Apart from verification of diagnosis and malignancy grading aspiration biopsy may sometimes be helpful to indicate the site of origin of a secondary tumour with a characteristic cell pattern.

SUMMARY

Fine needle aspiration biopsy was found to be a useful method of preoperative cytologic verification of the diagnosis and malignancy grading in patients with kidney tumours. The puncture is done with the aid either of ultrasound scanning or of television-monitored fluoroscopy. The relevance of cytologic grading was investigated by performing aspiration biopsy of tumours immediately after nephrectomy in 47 patients with renal cell carcinoma. In 36 cases there was conformity between the cytologic and the histopathologic grading. In the remaining 11 cases there was a disparity of one grade or, in one case, of two grades. There was no evidence of serious complications or tumour spread following the aspiration biopsy.

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DYNAMIC COMPUTED TOMOGRAPHY IN RENAL TUMORS

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Dynamic Computed Tomography is a method that allows the study of the circulation in both kidneys. We use a General Electric 8800 scanner with an experimental dynamic system. After injecting 50 cc of Uromiron 380 rapidly, we begin the cuts 10 seconds later. The CT scan allows us to obtain six cuts, each one in a scan time of 4.8 seconds and an interscan time of 1.2 seconds.

It is possible to obtain an incrementation scan by moving the patient in an axial direction and to obtain a succession of cuts at one centimetre intervals, or by doing the six cuts at the same anatomic level one can register a density histogram within 35 seconds.

We have applied this technique to 10 patients with renal tumors. It is possible to obtain images of the tumor's vascularization and to define a vascular pattern in the tomographic cuts. It is equally possible to characterize zones of necrosis, a technique which will be useful to control the evaluation of the embolization of tumors.

With the concentration values of contrast in an axial plan, we can construct a density histogram, to study the velocity of the intratumoral circulation, and compare zones with different circulatory velocities. This technique opens a new field in the study of tumor circulation and allows comparison with the anatomo-pathological findings. We are now trying to characterize the curves and relate them to the various types of tumors and their evolution but as yet we have insufficient data.

LIPID ANALYSIS IN CYSTIC RENAL LESIONS

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Cystic lesions of the kidney are detected with steadily increasing frequency. Although most are benign, cause the patient no trouble and have significance only in the differential diagnosis of renal carcinoma, it must be accepted that about four per cent of renal carcinomas are cystic. Cystic renal tumours may not be possible to differentiate from benign cysts with modern radiological methods such as ultrasonography and computed tomography. The percutaneous puncture of the cystic lesion will therefore remain as an important technique in the differentiation of a benign cyst from a cystic renal tumour.

Histochemical examination of the lipid content of the cystic fluid was suggested as a possible way of differentiating malignant cystic tumours from simple benign cysts. To obtain a quantitative determination of the lipid content in renal cystic lesions 18 cystic renal tumours and 42 benign cysts were examined biochemically with regard to the total lipid and cholesterol content and the presence of atypical or malignant cells (1).

The total lipid and the cholesterol content was low in the benign cysts (0.30 ± 0.07 and 0.18 ± 0.07 mmol/l, respectively) and high in the cystic tumours (13.2 ± 3.3 and 8.5 ± 2.4 mmol/l, respectively). No cystic tumour had a total lipid value of <1.6 mmol/l whilst 29 of 30 benign cysts had a value of <0.8 mmol/l. The cholesterol contents of cystic tumours were always >1.1 µmol/l and those of benign cysts, with one exception, <0.7 µmol/l. Cytological examination of the fluid showed atypical cells in only two out of 11 cystic tumours.

It is concluded that analysis of the lipid content may increase

the accuracy in the differential diagnosis between renal cysts and cystic renal tumours. A low lipid value speaks strongly for a benign cyst. A high lipid value is likely to represent a malignant lesion, though a false positive lipid test may be obtained in inflammatory or hemorrhagic cysts. In this study the reliability of the cholesterol and total lipid estimation was equal and it therefore seems sufficient to analyze the cholesterol content of the aspirated cyst fluid, a technique which is available in most clinical chemical laboratories.

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CANCER OF THE KIDNEY: STAGING

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Staging methods for carcinoma of the kidney continue to be debated due mainly to the difficulty in characterising any deep seated tumour and especially one that is located in the retroperitoneum. The main staging system, widely used in Europe and N. America, was popularised by Robson et al (1). Four stages are described:-

Stage 1: Tumour within the capsule of the kidney
Stage 2: Tumour has invaded the perinephric fat but is confined within Gerota's fascia
Stage 3: Tumour involvement of regional nodes and/or renal vein and cava
Stage 4: Tumour involves adjacent organs or there are distant metastases

This staging system is based mainly on the pathological findings but it also relies to some extent on the description by the surgeon and the extent of the operative procedure in obtaining lymph nodes from at least the para-aortic region. But the final arbiter is the pathologist who can define precisely the extent of the renal capsular involvement and spread of tumour into the renal vein.

An alternative classification for this tumour was introduced by the UICC using the TNM categories.

The current TNM pretreatment clinical classification is as follows:-

TO no evidence of primary tumour.
Tl evidence of a small tumour without enlargement of the

kidney. There is limited calyceal distortion or deformity and circumscribed vascular deformities surrounded by renal parenchyma.

T2 evidence of a large tumour with deformity and/or enlargement of the kidney or calyceal or pelvic involvement. The continuity of the cortex is preserved on arteriography.

T3 evidence of spread into perinephric fat, peripelvic fat or hilar renal vessels.

T4 evidence of extension into neighbouring organs or abdominal wall.

TX the minimum requirements to assess the primary tumour cannot be met.

T CATEGORY

By definition, a T category is a clinical pre-operative assessment of a tumour but from the beginning it was evident that this would be difficult to apply. Urologists recognise the almost impossible task of assessing the size of a kidney clinically and even a kidney mass cannot be judged reliably by palpation; thus any attempt to obtain a T category by this method alone would be doomed. With the increasing use of arteriography and selective renal angiography it was decided to include this investigation in the minimum requirements for a T category when this tumour site was first classified by the UICC in 1973.

Few studies have looked at the usefulness of angiography in determining a T category, probably because in practice most urologists prefer to wait for the pathologist's report before giving a post surgical histopathological classification.

In 1977 Das et al (2) reported a study which examined the accuracy of renal angiography. Thirty-six patients underwent selective renal angiography prior to nephrectomy for a renal carcinoma. The radiological T category was then compared with the pathological findings in the specimen.

The results showed that there was agreement of T and P category in 21/36 (58%); there was false pre-operative overstaging in 8/36 (22%) i.e. $T > P$; there was a false pre-operative understaging in 6/36 (16%) i.e. $T < P$; in one patient the tumour was not diagnosed on the angiogram. There was therefore a substantial error when angiography was used to obtain an improved reliability of the T category.

Because selective angiography is now recognised to be so inaccurate, other imaging techniques have been suggested to improve the value of a T category. In recent years ultrasound has become a most important method for the investigation of renal masses but its

special value lies in the distinction of solid from cystic masses. Ultrasound can give useful information about other abnormalities in the retroperitoneum but cannot give the precision to the extent of tumour spread that is required for staging (3).

Computed tomography (CT) is increasingly used to determine the extent of renal tumours (4,5) and a recent report by Khoury (6) indicates an extremely high degree of reliability in staging renal tumours using CT alone.

In a comparative study of the use of CT and ultrasound for staging renal carcinoma Levine et al (7) showed that CT was capable of detecting tumour invasion of perinephric fat and adjacent muscles which could not (usually) be shown by ultrasound. Both CT and ultrasound demonstrated venous and retroperitoneal tumour extension but CT was more reliable because bowel gas often obscured the retroperitoneum for ultrasound scanning. These authors concluded that while ultrasound was helpful in determining the extent of a tumour, CT was the better method for staging purposes.

N CATEGORY

The minimum requirements for N category comprise clinical examination, urography and lymphography (8). Unfortunately, the reliability of these methods is seriously in doubt as indeed are most pedal lymphograms when it comes to providing a reliable measure of lymph node metastases.

In an early study by Hulter et al (9) the lymphographic findings correlated poorly with histologically proved metastases and false positive as well as false negative lymphographic signs occurred frequently. In this study of 22 patients, a retroperitoneal lymph node dissection provided information not only on the correlation with lymphography but also on the site of positive lymph nodes in relation to the renal tumour; the authors demonstrated that if lymph nodes are to be removed as part of a radical nephrectomy, then the dissection should include not only the lumbar nodes bilaterally but also the nodes along the common iliac vessels at least on the homolateral side.

More recent studies of lymph nodes (10) have shown the inaccuracy of pedal lymphography in renal carcinoma and emphasised that the information gained from lymphography has little effect on the treatment. Even the role of lymphadenectomy in renal carcinoma is debatable (11); the procedure provides staging information but whether or not it influences results is uncertain. Survival rates with and without lymphadenectomy suggest that lymphadenectomy enhances survival but the data suffers from being retrospective and not randomised. Thus, the use of node dissection for staging as the possible benefit in treatment has yet to gain general acceptance.

M CATEGORY

The minimum requirements for M category now comprise clinical examination, radiography and, in the more advanced primary tumours or when clinical suspicion warrants, radiographic or radioisotopic studies are recommended. Metastases from renal carcinoma arise most frequently in the lungs followed by the lymph nodes, bones and adrenals. Thus, radiological, and radioisotopic bone studies, are still the main methods for diagnosing and monitoring metastases.

There is at present no tumour "marker" that can be used in this tumour. A variety of abnormalities have been described in association with renal tumours (12) but either they are non specific or the incidence of the abnormality is too infrequent to be of clinical use (13,14).

CONCLUSIONS

The importance of staging tumours is not in doubt but it is evident that attempts to apply the TNM principles have added little to the reliability of staging renal tumours. Recent reports now show that CT provides the best information for a T category but it would make no sense to include this as a minimum requirement as CT is not yet widely available in the Western world and the accuracy of staging described, requires up-to-date CT equipment. However the TNM classification does allow for the inclusion of a C-Factor which refers to the level of certainty. It reflects the information at a point in time and according to the diagnostic methods employed. Degrees of C may be applied to the T, N and M categories and in this way those centres in which special diagnostic methods are available can identify the precision of their categorisation.

Both N and M categories are seen to be very weak in their diagnostic accuracy and reliability. It is hoped that in future years, it might be possible to incorporate some aspect of the paraneoplastic syndromes into diagnosis and monitoring of the tumour, or preferably, a specific tumour marker may be identified.

Meanwhile, the clinician must continue to use the best investigative techniques available to him in order to characterise the extent of the tumour prior to nephrectomy and then to use the evidence provided by the pathologist to place the tumour in the correct stage or pT category.

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HISTOPATHOLOGY AND NUCLEAR GRADING IN RENAL CELL NEOPLASMS

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INTRODUCTION

In most series of renal adenocarcinomas the overall ten year survival rate is between 18 and 27% (1). Considering that nephrectomy is frequently performed under adverse circumstances and in spite of metastasis at the time of surgery, the overall survival is still very high as compared to many other cancers with a similar clinical stage. This supports the belief that these tumors are usually slow growing. The poor prognosis is usually not solely attributable to aggressiveness but rather to the large sizes that these tumors can attain, the frequency of metastases being responsible for the presenting symptomatology, and the extensive delay before the patient seeks medical aid (2).

The most important gross features that these kidney tumors exhibit and which can be related to prognosis have been incorporated into a very comprehensive and relatively accurate staging system (3). These features relate primarily to the importance of renal vein and perinephric fat invasion. The most comprehensive staging system is that of Robson and associates (4). In their experience, perinephric fat invasion is less important than renal vein involvement; this is reflected in their staging system. We should consider their system in its entirety because it can be related to histological grading. We would like first to consider several features in relation to the gross specimen which should be described in the surgical pathology report because, in many instances, it does relate very closely to the prognosis.

SURGICAL PATHOLOGY GROSS SPECIMEN FEATURES

Number and Location of Tumors

The differences in number are not great and, at this time, the location of the tumors at either the upper or lower pole does not appear to alter prognosis. The presence of calcification is of very little prognostic significance (5).

Size

There are numerous reports demonstrating that the larger the renal tumor, the worse is the prognosis (6,7). However, the greater the size of the tumor, the greater is the likelihood that there will be vascular invasion, associated local extension and metastases to other parts of the body (8).

Local Extension

If the tumor is well circumscribed and expecially of low grade, then the patient's prognosis is very good. However extension beyond the renal capsule adversely effects patient survival. Kaufman has reported a 56% ten year survival in the absence of perirenal invasion as against 14% with invasion (1). The clinical outcome has an even more ominous prognosis when the tumor, either grossly or microscopically, is seen to infiltrate the surrounding renal parenchyma.

Renal Vein Invasion

Patients with renal vein involvement have a significantly worse prognosis than those whose tumors are confined to the kidney (8). Renal vein invasion however seems to be less important than perirenal invasion (1,5,6), contrary to the views of Robson et al (4).

Regional Lymph Node Metastasis

When the regional lymph nodes are involved, we can expect the same prognosis as that seen in the presence of renal vein invasion (4).

HISTOLOGICAL GRADING AND MICROSCOPIC FEATURES OF RENAL CARCINOMAS

It is generally agreed that compared to most other carcinomas, renal carcinomas are usually well differentiated with relatively little pleomorphism of their nuclei (2). The cells will usually demonstrate an occasional mitosis. Riches (9) graded 110 renal adenocarcinomas histologically and found that 45% were well differentiated, 36% were of intermediary differentiation and only 18%

were poorly differentiated or pleomorphic. There are many variegated patterns and sometimes histological features can be found. As a consequence, some authors (9,10) have tried to classify these neoplasms on the basis of cell type and light histology configurations. Many classifications have arisen and are based upon the presence of cystic, solid, tubular, as well as papillary patterns. None has shown an improved statistical correlation with survival. In addition a predominance of clear or granular cell types does not seem to have any prognostic significance (11). Bennington and Beckwith (2) in their extensive monograph on tumors of the kidney, renal pelvis and ureter, have also expressed the opinion that neither the presence of clear or granular cells nor the patterns of organization, i.e. cystic, tubular or papillary, has any prognostic significance. They are also of the opinion that histological grading can be used to assess prognosis but that it has no greater accuracy than staging. In their opinion and that of these authors, it usually requires extensive sampling of every tumor and considerable experience for the histological grade to be reliably assessed. Mostofi (12) has also extensively reviewed the subject of histological grading and he states that "grading is both difficult and of very little value" and as a result, he classifies renal adenocarcinomas as either well differentiated or not well differentiated.

In general, it appears that in the majority of kidney carcinomas which are not Robson stage 1, it is more important to assess prognosis on the basis of further staging, i.e. tumor size, extent of local invasion, vascular involvement and metastases. Many authors (13,14) are of the opinion that it requires less experience, is less time consuming and correlates better with survival than does histological grade. However, with the combined approach of careful physical workups, IVP and urinary cytology, it is possible to detect many more kidney tumors at an early stage. Consequently, there can be an increase in the detection of stage 1 kidney tumors, as has been seen at our institution in the last few years when compared to thirty years ago. It is hoped that quicker and more careful fixation of the surgical specimens will assist computer-based systems in analyzing the multiple variants found both in the gross specimen and in the histological and cytological features of the tumor (15). It is anticipated that there will be a greater degree of accuracy in estimating prognosis if one can numerically assign exact values rather than subjective values for grading renal tumors.

NUCLEAR GRADING AND STAGE IN PROGNOSIS

Three different groups of workers have attempted to correlate the various stages of kidney carcinomas with nuclear grade and size and to associate this with prognosis (16-18). Fuhrman and Limas (16) have reported recently that morphological parameters are of prognostic significance in renal cell carcinomas. Their study evaluated 76 cases of renal cell carcinoma. They classified their tumors in

regard to size, cellular arrangement and type (solid, papillary, tubular; clear versus dark) and nuclear grades from 1 - 4. The grade 1 tumors had small nuclei roughly the size of red blood cells with no nucleoli. Grade 2 tumors were twice the size of red blood cells and had very prominent nucleoli. Grade 3 were much larger and had very dark staining nucleoli. Grade 4 were spindle-shaped cells. In their hands, nuclear grading was superior to all other parameters in predicting the development of metastases following radical nephrectomy. When patients with stage 1 tumors were considered (43 cases), none of the grade 1 tumors (9 cases) developed metastases whereas 60% of the grades 2 - 4 did. It was also noted that there was a significant difference in the subsequent metastatic rates for stage 1 tumors when grade 1, as distinct from grade 2, neoplasms were compared (0% metastases for grade 1 and 50% metastases in grade 2).

Fuhrman and Limas also noted that there was an apparent relationship between cell type and prognosis but as a function of nuclear grade and not an independent variable. As an example - in clear cell carcinomas of the kidney, 68% were other than grade 1 and 44% developed metastases. Of the exclusively dark cell tumors, 92% were greater than grade 1 and 81% of them were metastatic. They also noted that their tumors were, in all instances, greater than 3 cm in size with 77% being over 5 cm and 36% being over 8 cm. There was a positive correlation between the size and stage of the tumor at the time of diagnosis but the size of the stage 1 tumors did not correlate with the subsequent development of metastases. Of the parameters which they studied, grade alone was significant in predicting the outcome of stage 1 renal cell carcinomas.

Gilchrist et al (17) retrospectively studied nuclear size as a prognostic discriminator in 44 renal cell carcinomas of Robson stage 1, 2 or 3. They wanted to determine if easily reproducible histological parameters could more finely discriminate for survival than pathological staging. Nuclear grading by relative size was examined at 10 - 20 magnifications. Similar nuclei were essentially equal in size but dissimilar nuclei were greater than twice the length or width of neighboring nuclei. Regions within one field of tumor necrosis were not studied. The designation of nuclear sizing in their study was based on the deviation in nuclear sizes after examination of every available histological section within the primary carcinomas. Correlation of nuclear size with numerous clinical features in their study was unrewarding. Their dissimilar nuclear sizes could not predict any possibility of distant metastases. In another study by Leiber et al (18), 90 cases of exclusively well differentiated eosinophilic granular renal cell carcinomas were studied. They excluded the higher grade tumors, i.e. grades 3 and 4. The clinical, laboratory, pathological and survival features of these patients were analyzed. Their tumors were identified mainly according to cytological features in terms of grading. Grade 1 oncocytic neoplasms or granular cell tumors were composed of closely similar cells

possessing round smooth nuclei and abundant eosinophilic cytoplasm. The grade 2 tumors had larger, more irregular nuclei and there was more variation among cells in size and configuration. The grade 3 neoplasms represented a further extension of the morphological departure from normal and featured very variable cells often with bizarre nuclear forms. Mitotic figures were abundant in this grade of tumor. The authors noted that 62 patients had grade 1 tumor and 28 patients had grade 2 tumors. None of the patients with grade 1 tumors developed metastases. However, 4 of the patients with grade 2 tumors died of metastatic disease. Survival curves showed no differences between patients with these renal tumors and age- and sex-matched controls.

SUMMARY

At the present time, not only is careful processing of the surgical specimens necessary for determining whether there is any renal parenchyma or fat invasion but the diagnosis should also be expressed in terms of grade, especially where the tumor is well encapsulated and a Robson stage 1. The burden of evidence at the present time seems to indicate that the higher the grade of the tumor, especially the stage 1 tumor, the greater is the possibility that these types of tumors will have a worse prognosis in the distant future.

With the advent and use of more definitive morphological instrumentation and analysis, differences in nuclear size and/or surface area can be computerized. The future use of this approach appears very hopeful. The prognosis of the various stages of the tumor as they correlate with nuclear grade and histological stage, provided there is adequate tissue sectioning, can be assessed more critically and in a more objective statistical fashion.

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PROGNOSTIC RELEVANCE OF CYTOLOGIC GRADING IN METASTATIC RENAL CELL CARCINOMA

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INTRODUCTION

In 1932 Hand and Broders stressed the importance in renal cell carcinoma (RCC) of tumor grading in predicting the course of the disease (1) and since then many authors have agreed with that point of view. This observation has induced a previous retrospective study at our department, which included 121 patients who had been treated by radical nephrectomy for RCC (2). At the time of diagnosis 52 patients had metastases. Of these 42 were operated on. In this study we have investigated the survival of these 42 patients in accordance with the histologic tumor grade. The Erlangen histological grading system, proposed by Hermanek and coworkers in 1976 (3), is based on the criteria shown in Table 1. Comparing these different histologic grades with regard to prognosis (fig. 1), we find that the one-year survival rate is significantly worse in G3 tumors than in G2. The difference between G1 and G2 tumors is not statistically significant, possibly due to the small number of patients with G1 lesions.

Thus, if the grade of tumor differentiation could be assessed pre-operatively, a high malignancy grade in metastatic RCC would be of great influence on further therapeutic procedures.

For pre-operative diagnostic assessment of the tumor grade, cytologic specimens obtained by percutaneous fine needle biopsy seems to be the most satisfactory (4), though the reliability of cytologic methods in relation to the histologic evaluation should first be examined. We have attempted to compare the cytological and histological findings in this study.

Table 1. The Erlangen System of Histologic Grading of Malignancy in Renal Cell Carcinoma.

Grade 1:	Solid tumors consisting of clear and intermediate cells, adenomas of doubtful malignancy.
Grade 3:	Tumors of glandular or glandular-papillary pattern, tumors showing exclusively granular cells or sarcoma-like areas.
Grade 2:	All other malignant parenchymal tumors.

MATERIAL AND METHODS

One hundred and five aspiration biopsies were carried out on the surgical specimens of 21 patients, who had had operations for renal tumors, using the technique of Franzén and coworkers (5).

The smear was stained according to the May-Grünwald-Giemsa technique and classified as proposed by Papanicolaou. Tumors were classified into two groups of low and high malignancy. The smear was then decolourised and stained with Feulgen-reagent. Thereafter DNA-single cell cytophotometry was performed on a Reichert-Densitometer(R) with connected Hewlett-Packard-computer(R).

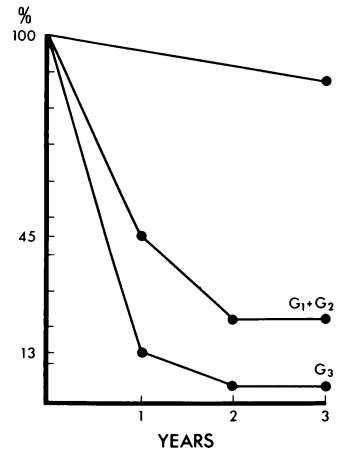

Fig. 1. Survival Rate by Grade in 42 Patients with Metastatic Renal Cell Carcinoma (Difference at one year, G2 versus G3, $p = 0.05$).

The classification of the histological specimens was made according to the Erlangen system as mentioned above (Table 1), but based on the non-significant difference in survival rates (fig. 1) grade 1 and 2 tumors were grouped together for correlation with the two classes of cytologic malignancy.

RESULTS

Twenty-one tumor specimens were evaluated. Tumor cells were found in 92 of 105 (87.6%) preparations. Cells of renal cell carcinoma were always identified without doubt.

In cytologic assessment there were 14 tumors of low and seven of high malignancy. On histologic grading, we found 13 grade 1 or 2 and eight grade 3 tumors. If we correlated these two examinations for each tumor, we found corresponding results in 12 tumors with grade 1 or 2 lesions and six in grade 3 tumors (fig. 2) to give a positive correlation rate of 85.7%.

In an attempt to obtain objective results DNA-cytophotometry was performed on cytological specimens. Table 2 shows postulated DNA-distribution for each malignancy grade, according to our previous work (6).

Grade 1 tumor cells (fig. 3) should have a DNA-distribution pattern like normal cells, e.g. leucocytes, in the histogram. The main DNA-content lies about the 2c-value and a tumor stemline cannot be identified. Therefore, SQ, the stemline quotient (which is the value of the tumor DNA-stemline divided by normal DNA-stemline) is about 1.0. The number of tetraploid tumor cells is not significant; therefore, the diploid deviation quotient (DDQ) is also about 1.0. (The DDQ is the average DNA-content per cell divided by normal DNA-value).

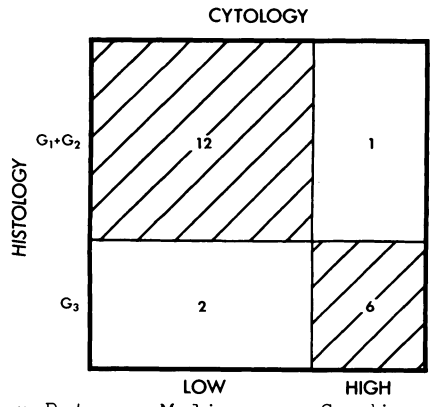

Fig. 2. Correlation Between Malignancy Grading, Obtained by Histologic Versus Cytologic Assessment in 21 Patients. A Positive Correlation was Found in 85.7% of Cases.

Table 2. Postulated DNA-Distribution Pattern According to Malignancy Grade in RCC

Grade 1 :	2c - (4c)	SQ = 1.0 $\pm$ 0.2	DDQ = 1.0 $\pm$ 0.2
Grade 2 :	2c - 4c	SQ = 1.0 $\pm$ 0.2	DDQ $\geq$ 1.2
Grade 3 :	.3c....6c..	SQ > 1.2	DDQ > 1.2

Grade 2 tumors (fig. 4) must also show a unimodal DNA-distribution with a SQ about 1.0, but as an expression of their higher proliferation rate, they should have a clearly distinguishable peak at the 4c-value (DDQ is higher than 1.0).

Finally, grade 3 carcinoma cells (fig. 5) are postulated to have a bimodal distribution pattern with an own tumor stemline, e.g. at the 3c-value (SQ higher than 1.2) and another peak for instance at the 6c-value (DDQ also higher than 1.2).

The two tumors of the study (fig. 2), which gave false negative results on cytological examination were checked by measurements of DNA-content. There we saw DNA-distribution patterns identical with those found in G3 tumors. This means that the high malignancy of these RCC was missed by standard cytology but was identified by cytophotometry. By combining cytophotometry with cytology, the correla-

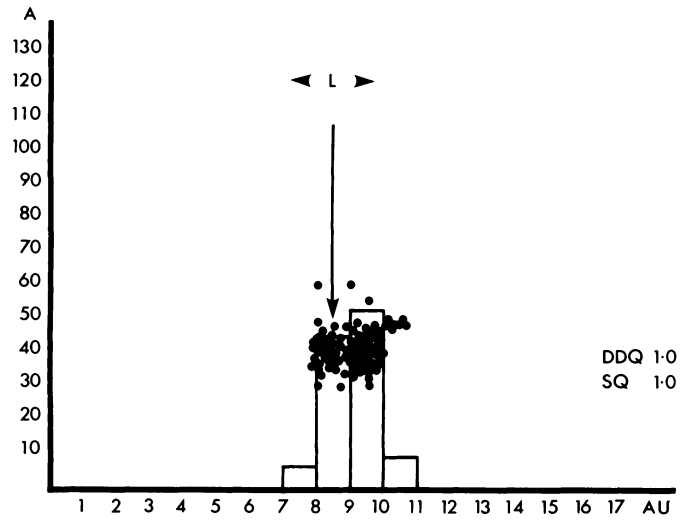

Fig. 3. DNA Cytophotometry in a Patient with a Gl Renal Cell Carcinoma.

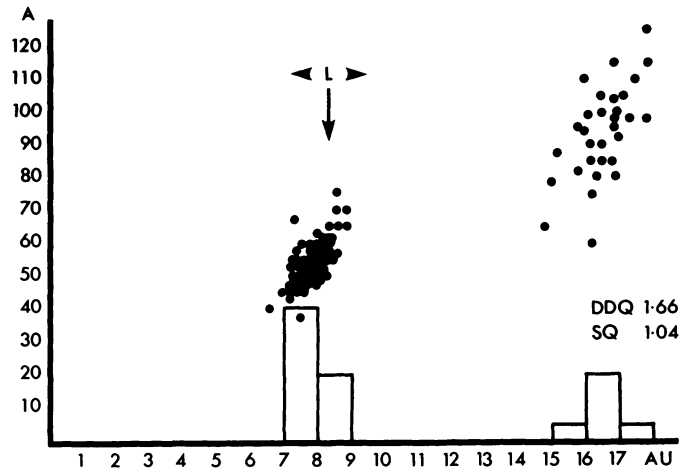

Fig. 4. DNA Cytophotometry in a Patient with a G2 Renal Cell Carcinoma.

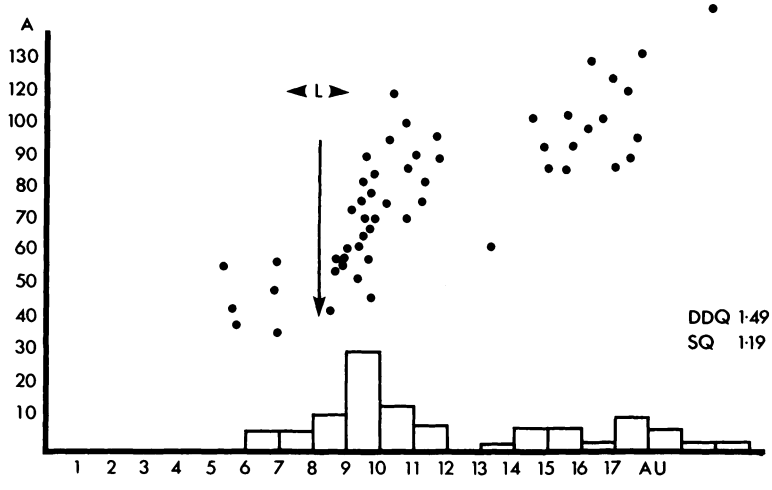

Fig. 5. DNA Cytophotometry in a Patient with a G3 Renal Cell Carcinoma.

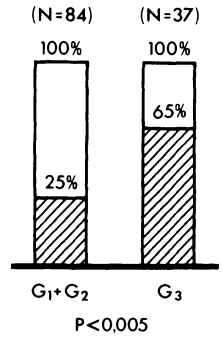

Fig. 6. Occurrence of Metastases by Tumor Grade.

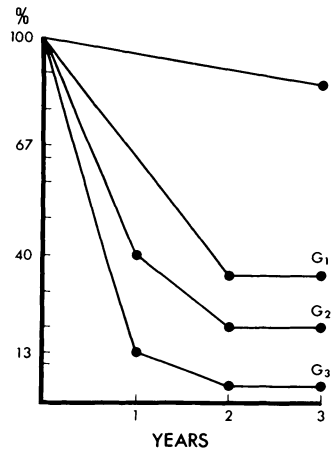

Fig. 7. Survival Rate in 42 Patients with Metastatic Renal Cell Carcinoma of Low Grade (G1 + G2) Versus High Grade (G3) Cancer (Difference at one year, $p < 0.05$).

tion of pre-operative cytological findings with those obtained by post-surgical histology can be greatly improved.

Percutaneous needle biopsy and DNA-cytophotometry are now routinely done in all patients with metastatic RCC with the goal of an adequate therapy planning.

In our clinical follow up study mentioned above we have also found a significantly higher tendency to formation of metastases in patients with highly malignant tumors (fig. 6). Considering this and the significantly lower one-year survival rate (fig. 7) in patients with metastatic G3 tumors compared to metastatic G1 and G2

tumors the importance of a reliable method for evaluation of tumor grading prior to any invasive therapeutic procedure is obvious. Possibly those patients with extremely poor prognosis (G3 tumors) should be excluded from surgical therapy except in cases of uncontrollable bleeding or pain.

CONCLUSIONS

In conclusion there are four points to be stressed:

1. tumor grade is an important factor for survival time.

2. percutaneous needle biopsy should be performed in RCC to assess tumor grade pre-operatively.

3. DNA-cytophotometry can improve cytological results, and

4. patients with poorly differentiated metastatic tumors should perhaps not be submitted to radical nephrectomy.

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PROGNOSTIC FACTORS IN RENAL CANCER

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In 1965, Rotterdam urologists and the Rotterdam Radiotherapy Institute started a prospective clinical trial in order to investigate the value of preoperative irradiation in renal cell cancer, to assess the value of the TNM classification of the International Union against Cancer, and to identify prognostic tumor and host factors. In a previous report, preliminary results have been reported (1).

Operable patients - without any evidence of metastasis - were randomized into either simple nephrectomy or preoperative irradiation immediately followed by simple nephrectomy. As a routine no lymph node evaluation was done. Preoperative irradiation consisted of 3000 - 4000 rad in 3 - 4 weeks depending on the side of the affected kidney; on the right side, the liver had to be considered. The irradiation field covered the kidney, the homolateral lymph nodes, and after 1970 also the contralateral lymph nodes.

The decision of "operability" was based on the results of physical examination, routine laboratory tests, routine X-ray investigations including chest X-ray, skeletal survey, intravenous pyelography, and in the majority of cases arteriography.

Routine histology of the operative specimen was done according to rules accepted by all Rotterdam pathologists; in 1977 review took place of all histological slides by one pathologist and of all intravenous pyelographies and arteriographies by one radiodiagnostician.

All patients have been seen at regular intervals for follow up examination. No patient was lost to follow up.

MATERIAL AND METHODS

A total of 206 patients were admitted to the trial. Table 1 lists the most important analyzed tumor and host factors. Thirty-two patients had to be excluded from further analysis for the following reasons: 15 appeared to have metastases prior to operation; five turned out to have a transitional cancer of the renal pelvis; 12 tumors appeared to be either a cyst, hemangioma, or fibroma. These patients were distributed equally over the two treatment modalities. Of the 174 remaining trial patients, 89 received preoperative irradiation while 85 underwent immediately simple nephrectomy.

There were 99 males and 75 females; both sexes had an average age of 59 years.

Table 1. Analyzed Tumor and Host Factors

Admitted to trial	206	P Categories	
Analyzed cases	174	P1	61
		P2	38
Males	99	P3	67
		P4	7
Females	75	Px	1
Preoperative X	89	Renal vein involvement	
No preoperative X	85	V-	105
		V+	64
T Categories		Not known	5
T1	14	Cell type	
T2	47		
T3	66	Granular	17
T4	39	Clear	123
Tx	8	Mixed	22
		Not known	12
ESR			
		Degree of differentiation	
<30	89		
≧30	83	High	21
Not known	2	Medium	81
		Low	60
		Not known	12

Based on intravenous pyelography and arteriography, the T categories of the primary were defined according to the rules of the International Union against Cancer (2); 14 belonged to the T1 category, i.e. small tumor without enlargement of the kidney; 47 were classified at T2, i.e. large tumor but cortex not broken; 66 were classified as T3, i.e. perinephric or hilar extension; 39 belonged to the T4 category, i.e. extension into neighboring organs. In eight patients no arteriography was done (Tx).

In 89 patients the sedimentation rate was less than 30 after 1 hour (ESR < 30); in 83 patients it was 30 or more after 1 hour (ESR ≥ 30). In two patients the ESR was not known.

The P categories of the UICC correspond to the T categories. Their assessment was based on the examination of the nephrectomy specimen.

In 105 patients no renal vein involvement was found in the nephrectomy specimen, whereas in 64 cases renal vein involvement could be demonstrated. In five instances this information was not available.

The distribution of the degree of differentiation and the distribution of the cellular type of the growths are also presented in Table 1.

A detailed statistical analysis was performed in order to assess the prognostic significance of each factor and in order to elucidate the interrelationship between the various analyzed factors.

RESULTS

The actuarial uncorrected survival rate according to Berkson and Gage (3) for all cases is about 50% after 5 years and about 45% after 10 years. Relapse-free survival is continuously 5 - 10% less. The expected survival of the same age group is about 40% higher (Fig. 1).

Preoperative irradiation had no bearing on survival.

The T categories had no bearing on prognosis except for T4 patients who had a poor prognosis (Fig. 2).

Prognosis of females was significantly better than prognosis of males (Fig. 3).

Patients with ESR <30 fared significantly better than those with ESR ≥ 30 (Fig. 4).

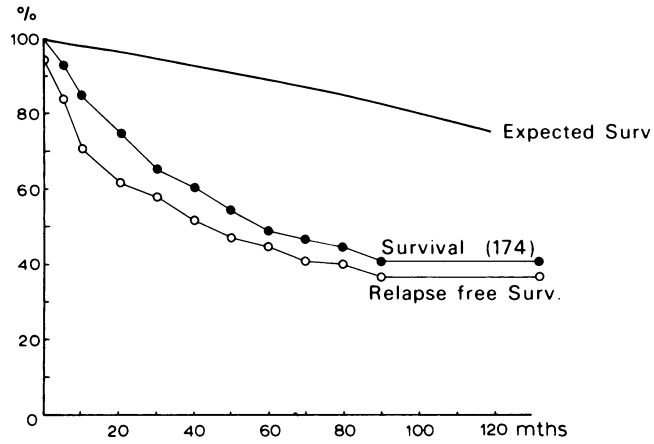

Fig. 1. Actuarial uncorrected survival.

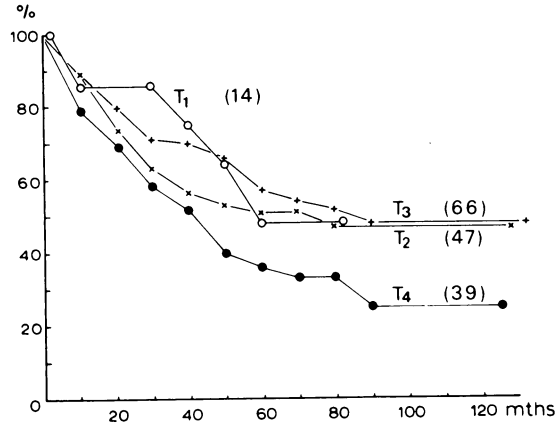

Fig. 2. Actuarial uncorrected survival by T category.

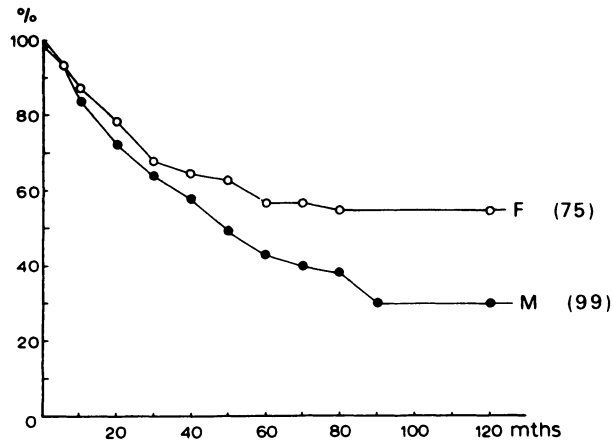

Fig. 3. Actuarial uncorrected survival by sex.

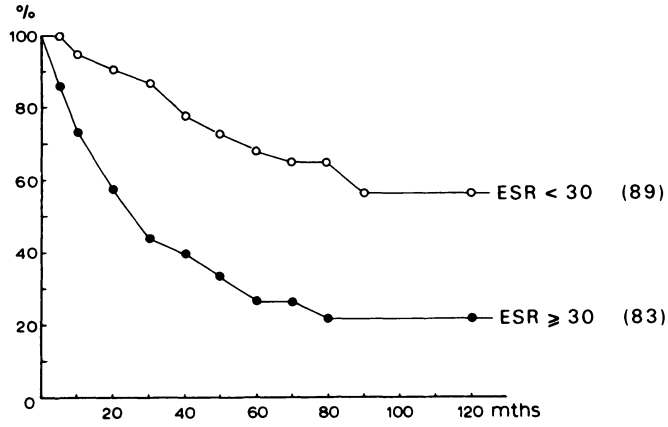

Fig. 4. Actuarial uncorrected survival by ESR.

There was no correlation between sex and T category or ESR and T category. In each T category, those with ESR < 30 had a better prognosis (Table 2). In case of ESR < 30 and especially in case of ESR ≥ 30, females fared better than males (Fig. 5).

With increasing P category, prognosis becomes worse; however, after 3 years, P1 and P2 patients show no prognostic difference (Fig. 6). Preoperative irradiation did not change survival in each P category.

Table 2. Average Actuarial 5 Year Survival by T Category and ESR

	ESR < 30		ESR ≥ 30	
	Number Treated	Survival (%)	Number Treated	Survival (%)
T1	11	59	3	0
T2	27	63	20	30
T3	30	85	35	30
T4	18	55	20	10

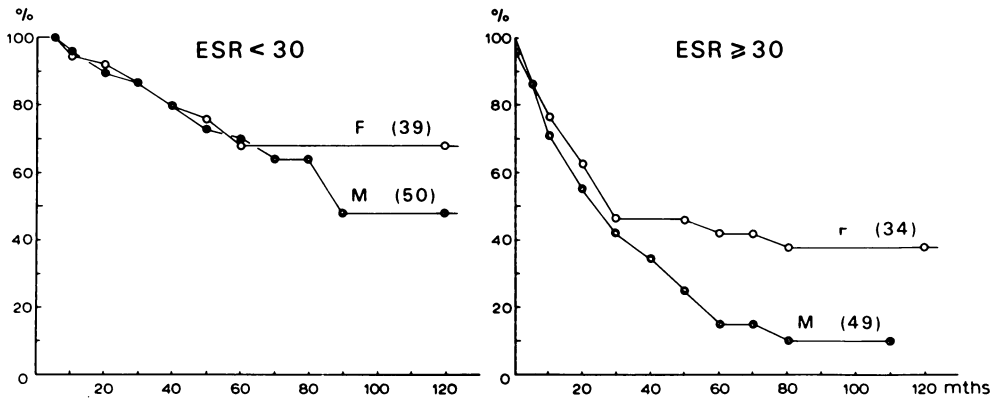

Fig. 5. Actuarial uncorrected survival by ESR and sex.

There was no correlation at all between P and T category: in about 60% of the cases P was smaller than T, in 20% P was larger than T, and only in about 20% P was the same as T (Fig. 7). This finding applied equally to the group with and without preoperative irradiation, indicating that probably preoperative irradiation did not influence visibly the extent of the growth.

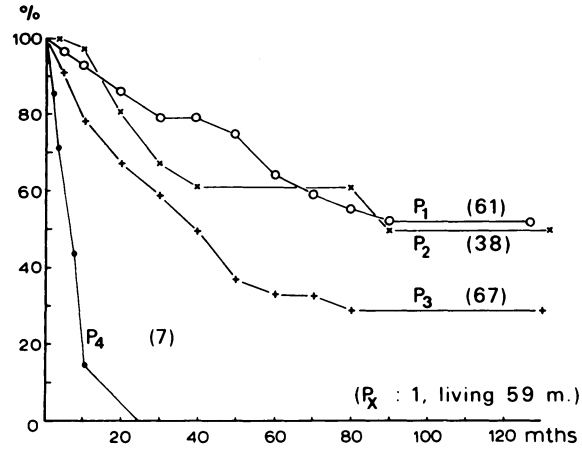

Fig. 6. Actuarial uncorrected survival by P category.

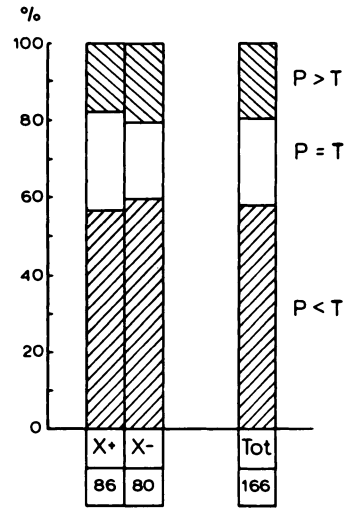

Fig. 7. Correlation of T and P categories (according to Preoperative Radiotherapy (X)).

Renal vein involvement significantly reduced the chance of cure: with renal vein involvement only 20% of the patients were alive after 10 years, whereas in case of no renal vein involvement about 55% were alive (Fig. 8). In the subgroups according to renal vein involvement, the P categories had no prognostic influence. As with increasing P category, there was an increasing incidence of renal vein involvement; apparently the influence of P categories on prognosis is derived from renal vein involvement (Table 3). Preoperative irradiation had no bearing on the prognostic influence of renal vein involvement.

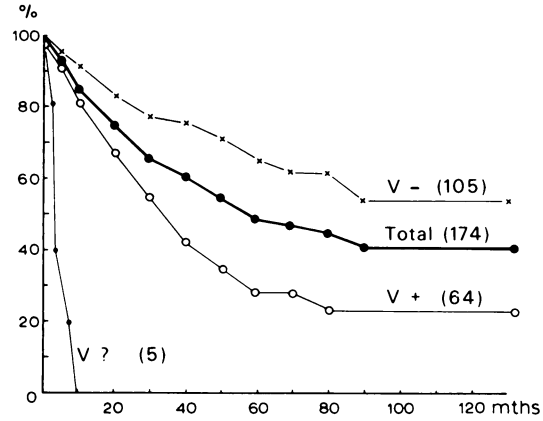

Fig. 8. Actuarial uncorrected survival by renal vein involvement.

Table 3. Prognosis by P Category and Renal Vein Involvement

	Number	5 Year Survival (%)	10 Year Survival (%)
P1			
V-	53	65	55
V+	8	40	30
P2			
V-	23	70	55
V+	15	45	40
P3			
V-	28	50	50
V+	39	20	15

Table 4 demonstrates prognosis according to sex, renal vein involvement, and ESR. Only in case of low ESR and no renal vein involvement do females and males have the same prognosis. In all other instances females fare better than males; in each sex, prognosis in case of no vein involvement and ESR ≥ 30 is comparable with prognosis in case of vein involvement and ESR < 30. Prognosis is worst in case of vein involvement and high ESR.

The cellular type, i.e. granular or clear cell carcinoma, had no bearing on prognosis.

The histological degree of differentiation influenced prognosis: with decreasing degree of differentiation survival also decreased. The degree of differentiation had no correlation with either renal vein involvement or sex; the frequency of cases with low degree of differentiation was significantly higher in case of ESR ≥ 30 than in case of ESR < 30. In each degree of differentiation the sedimentation rate determined prognosis. Analyzing the effect of degree of differentiation reveals that in each ESR group prognosis is identical for growths of high and of medium degree of differentiation, whereas a low degree of differentiation slightly worsens prognosis in both groups (Table 5).

In the P3 category, 36 patients had received preoperative irradiation. Four of them showed cancer in the plane of resection (11%), whereas of 31 P3 patients without preoperative irradiation 11 showed cancer in the plane of resection (35%). In spite of this apparent benefit from preoperative irradiation, due to small numbers it is not expressed in a better survival of the P3 category after preoperative irradiation.

Table 4. Average Actuarial 5 Year Survival by Renal Vein Involvement, ESR, and Sex

	ESR < 30		ESR ≥ 30	
	Number Treated	Survival (%)	Number Treated	Survival (%)
No renal vein involvement				
Males	38	90	22	20
Females	24	80	19	50
Renal vein involvement				
Males	12	18	26	10
Females	14	60	12	40

Table 5. Prognosis by Degree of Differentiation and ESR

Degree of Differentiation	Number	5 Year Survival (%)	5 Year Survival (%)
High	21	65	55
Medium	81	55	45
Low	60	40	38
ESR $<$ 30			
High	17	75	63
Medium	44	75	60
Low	24	60	44
ESR $\geq$ 30			
High	3	35	0
Medium	37	35	25
Low	36	26	25

There was no significant impact on prognosis either by the dose of irradiation (3000 or 4000 rad) or by the size of irradiation field (regional nodes included or excluded).

Apart from inoperable patients, where death is usually due to local growth with or without metastases, death usually is caused by distant metastases without local recurrence. In 38 instances pulmonary metastases were diagnosed during follow up: 23 showed progression; in seven cases there was dubious progression; and in eight cases (31%) there was spontaneous, complete, or partial regression. After pulmonary metastases, the next frequent site for metastasis were the vertebral bodies from cervical 4 to lumbar 2.

Table 6 presents the distribution of pulmonary and vertebral-body metastases according to renal vein involvement, sedimentation rate, and sex.

DISCUSSION

Of the preoperatively assessable tumor and host factors, only sex and sedimentation rate appeared to have a bearing on prognosis: women fared better than men, and patients with a sedimentation rate less than 30 after 1 hour had a better prognosis than those with a

Table 6. Hypothetical Benefit from Elective Irradiation by Renal Vein Involvement/ESR/Sex

	Number	I Pulmonary Mets (%)	II C4 + L2 Mets (%)	I + II (%)
Renal vein -				
ESR < 30	♂38	8	8	16 (40)[a]
	♀24	5	4	9
ESR ≥ 30	♂22	27	9	36 (50)[a]
	♀19	11	16	27
Renal vein +				
ESR < 30	♂12	67	8	75
	♀14	8	15	23
ESR ≥ 30	♂26	35	8	43
	♀12	50	25	75

[a]In case of low degree of differentiation.

higher sedimentation rate. Sex and sedimentation rate factors were not interrelated.

The T categories appeared to have no bearing on prognosis. It could be assumed that the accuracy of radiodiagnostic assessment is not sufficient. This suggestion is substantiated by the fact that there is no correlation between T categories and the more correctly microscopically assessable P categories. On the other hand, P categories also have no significant bearing on prognosis. The demonstrated prognostic influence of P categories is caused by the fact that with increasing P category the chance of renal vein involvement increases: patients with renal vein involvement have a significantly worse prognosis than those without renal vein involvement. Growths of low degree of differentiation predict a poorer outlook than those with a high or medium degree of differentiation. In each degree of differentiation the sedimentation rate overrules prognosis.

Prognosis in case of renal cancer - apart from inoperable or only partially removable growths - is mainly determined by the existence of micrometastases at the time of operation. Apparently renal vein involvement increases the risk of micrometastases (4,5,6),

and their existence is expressed by elevated sedimentation rate (5). A low degree of differentiation is not related to renal vein involvement but very much so to sedimentation rate; hence it apparently reflects the risk of micrometastases caused not only through renal vein involvement but also by other metastatic pathways (6). The better prognosis of women might be attributed to hormonal factors: hypothetically, hormones could suppress or even eradicate micrometastases in some instances and thus improve prognosis in circumstances otherwise comparable to those of males. Indirectly this hypothesis may be supported by the fact that once metastases are present, women respond less well to hormone therapy than men (7,8).

As prognosis at the time of operation is apparently already determined by existing subclinical metastasis, it is understandable that preoperative irradiation to the primary cannot contribute appreciably to improvement of prognosis.

Until now no form of chemotherapy has been found which can deal effectively with clinically established metastases; hence a trial using elective hormone therapy or chemotherapy in high risk patients (males with renal vein involvement and ESR $\geq$ 30) might be considered. On the other hand, as pulmonary metastases and metastases to the vertebral bodies from cervical 4 to lumbar 2 are frequent (Table 6), elective irradiation of these regions could be considered with a benefit ranging from 9% in the low risk group to 75% in the high risk group. Without great problems such an irradiation could be given by supervoltage machines.

SUMMARY

A total of 174 patients underwent simple nephrectomy in case of clinically operable kidney cancer without demonstrable metastases. Of these 85 received preoperative irradiation to the kidney and the regional lymph nodes (3000 - 4000 rad in 3 - 4 weeks). Prognosis was not influenced by preoperative irradiation. The preoperatively assessable prognostic criteria were sex and sedimentation rate: ESR $\geq$ 30 and being male worsened prognosis. The clinical T categories of the UICC were not related to prognosis. Of the microscopic examination of the nephrectomy specimen, renal vein invasion and to a lesser extent a low degree of differentiation appeared to worsen prognosis. The prognostic influence of the P categories was caused by a higher incidence of renal vein involvement in case of higher P category. The most important prognostic factors - ESR, renal vein involvement, and sex - were not interrelated. Elective chemotherapy, radiation therapy, and hormone therapy could be considered in certain high risk groups.

ADDENDUM (W.C.J. Hop, M.Sc.)

Based on the presented data pertaining to the relevant prognostic factors, a score for each factor was calculated by statistical methods (9).

The preoperatively assessable significant factors were sex and sedimentation rate. In case of female, no number is given; in case of male, 0.56 is attributed to the patient. To this initial figure the sedimentation rate score is added, obtained by multiplying 0.02

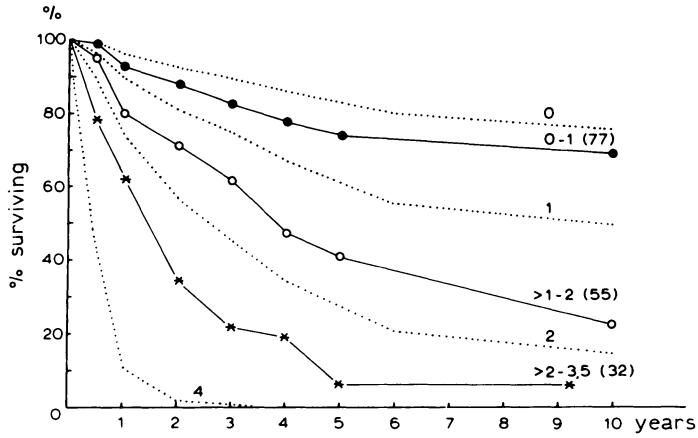

Fig. 9. Survival by preoperative scores: M = 0.56; ESR = 0.02 x value. T4 = 0.7. (...) Expected survival by score; (—) actuarial uncorrected survival by score.

with the sedimentation rate after 1 hour; and in case of T4, 0.7 is added. The sum of these two (or three) values is the final score to be used as an indicator of prognosis of patients. Grouping patients with a similar score resulted in survival curves as given in Figure 9.

After nephrectomy more relevant data are available and a more accurate prediction of prognosis becomes possible. The various scores are added and the sum of the scores can be used as a post-operative prognosticator. The postoperative scores are listed in Table 7. Survival curves according to score are given in Figures 10 and 11.

Table 7. Postoperative Scores for Prognosis

Sex	
Male	0.87
Female	0.0
ESR	0.02 x Value
Renal vein	
+	0.79
-	0.0
P-Category[a]	
"P"3	0.53
"P"2	0.0
"P"1	0.06
Degree of differentiation	
Low	0.39
Medium	0.0
High	0.14
Score	
Prognosis	

[a]Excluding "P"4

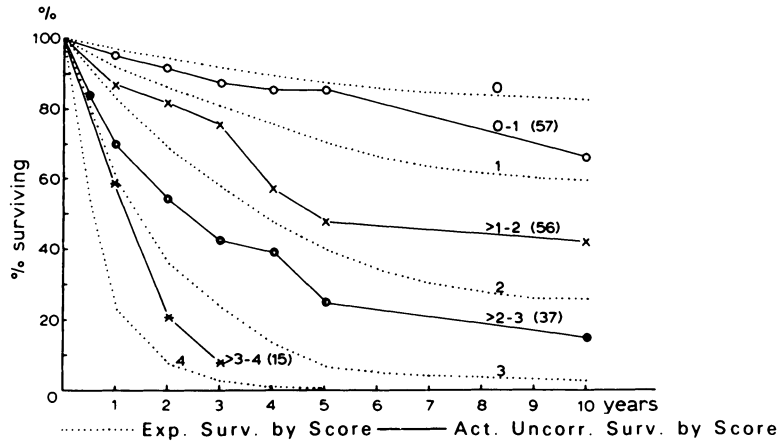

Fig. 10. Survival by postoperative scores (excluding "P"4): M = 0.78; ESR = 0.02 x value; V = 0.9.

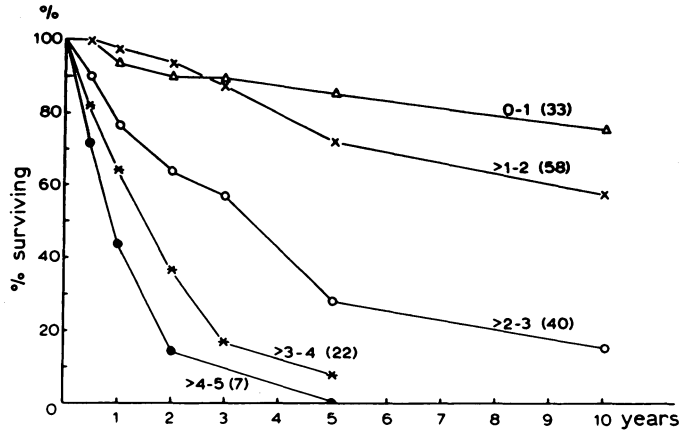

Fig. 11. Actuarial uncorrected survival by postoperative scores (excluding "P"4).

ACKNOWLEDGMENTS

The authors are grateful to all the Rotterdam urologists who contributed to the trial, to Miss P. de Haan who performed the secretarial work, to Mr. W. Hop (M.Sc.) and his department for statistical analysis, and to Mr. L. Ries and his department for drawing the graphs.

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RENAL CELL CARCINOMA: CLINICAL ASPECTS AND NATURAL HISTORY

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We teach our medical students that the presenting features of an adenocarcinoma of the kidney are haematuria, pain and a mass that has been noticed either by the patient or the doctor. We make the diagnosis by discovering a mass in the urogram that is solid in the ultrasound scan, typically vascular in the angiogram, or characteristically dense in the CAT scan. Pathologists tell us that one can tell a cancer of the kidney from a benign adenoma not by the histological features of either of them, but by their size - tumours smaller than 3 cm. in diameter being deemed benign, larger ones malignant. Treatment is nephrectomy: it is true that many of us have added other adjuvant agents from time to time, but it is equally true that none of them are (so far) of proven value. This is the conventional wisdom. We thought it would be of value to examine these well established truths in the light of the clinical experience of one centre over the last 15 years.

METHODS AND MATERIAL

A retrospective study was made of the records of 114 patients admitted to the London Hospital with an index diagnosis of adenocarcinoma of the kidney from 1964 through 1979. The list is probably incomplete, as we have had certain domestic difficulties in our records department: nevertheless the results may still be of interest.

It will not come as a surprise to find that there were nearly twice as many males as there were females, or that cancer occurred as often on one side as on the other (Table 1). It is equally unsurprising to see that cancer of the kidney was mainly a disease of older people (Fig. 1). We were surprised however to find that

Table 1. Present Series

	Right	Left	Total
Females	21	20	41
Males	38	33	71
Total	59	53	112*

(* Site of origin unclear at autopsy in 2 patients)

Table 2. Symptoms at Presentation to Hospital

Pain		11	9%
Haematuria		35	31%
Lump		7	6%
Combinations of pain, haematuria and lump		21	19%
Weight loss	2		
Proptosis	1		
Lung metastases	3	14	12%
Bone metastases	6		
Other metastases	2		
Hypertension		3	3%
Insufficient information		23	20%

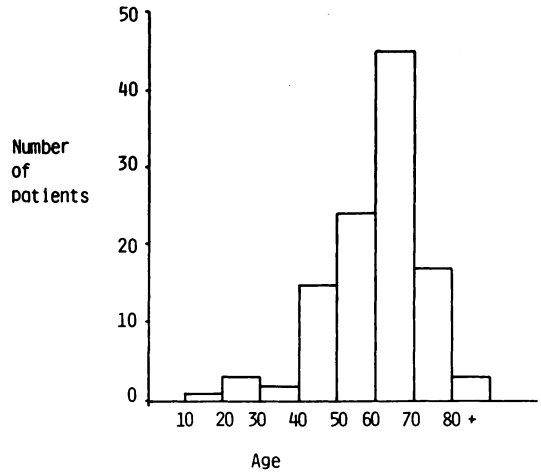

Fig. 1. Age distribution of 114 cases of renal cell cancer. (Adenocarcinoma; The London Hospital, 1964-69).

so many presented with clinical features of late or metastatic disease (Table 2).

Throughout this period, treatment was along conventional lines. Nephrectomy was performed whenever possible, even when there were distant metastases. Early in the series, when it was still accepted practice, our patients were given post-operative irradiation. Later, for a time, they received preoperative radiation as part of a clinical trial. Today they get no radiation unless there has been an obviously incomplete removal of the tumour though radiotherapy is used when appropriate in the treatment of metastases. Throughout most of this period our patients (when they have developed metastases) have been given medroxyprogesterone acetate, mostly because it is not known to do harm, rather than from any conviction that it would do any good.

We have tried to stage the tumours in accordance with the TNM system (1) but in doing so have probably erred on the side of understaging - since there were virtually no tumours in this series truly in pT1 where the renal contour was not deformed (Fig. 2) and most of our patients should perhaps really be placed in pT2 (Table 3). Prognosis was clearly related to the stage of the disease (Table 4, Fig. 3). Unfortunately only a small proportion of our patients were diagnosed when their tumours were in the early stage pT1 or pT2. As shown in an earlier study from this department (2) the outlook is good for tumours that are still confined to the kidney.

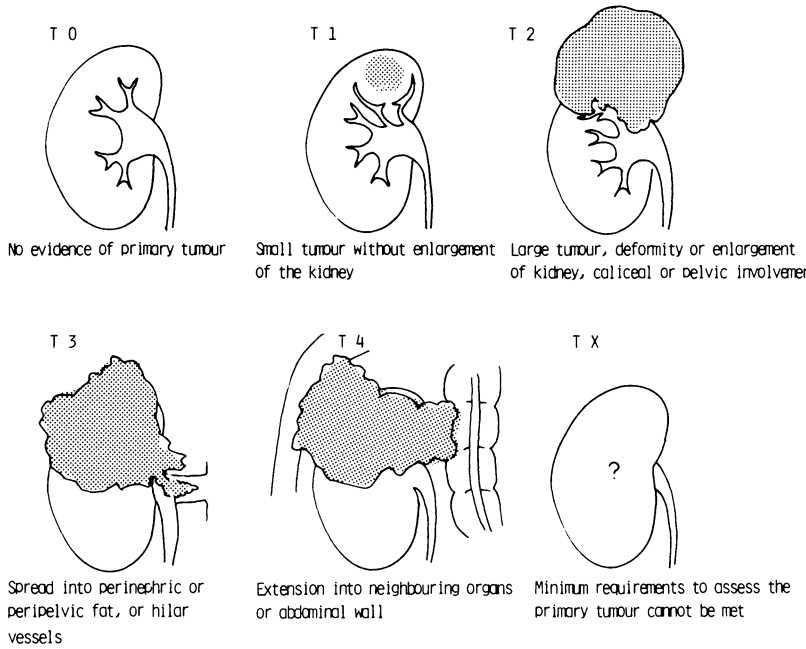

Fig. 2. T-staging of carcinoma of the kidney based on findings at operation. Diagram based on UICC TNM Classification of Malignant Tumours, 3rd Edition, 1978.

Table 3. pT Staging in 144 Cases of Adenocarcinoma of Kidney.

	pTX	pT1	pT2	pT3	pT4	Total	
Males	7	5	11	27	24	73	
M1	5	0	4	7	7	23	32%
Females	8	8	6	11	8	41	
M1	4	0	1	2	2	9	22%
Total	15	13	17	38	31	114	
M1	9	0	5	9	9	32	28%

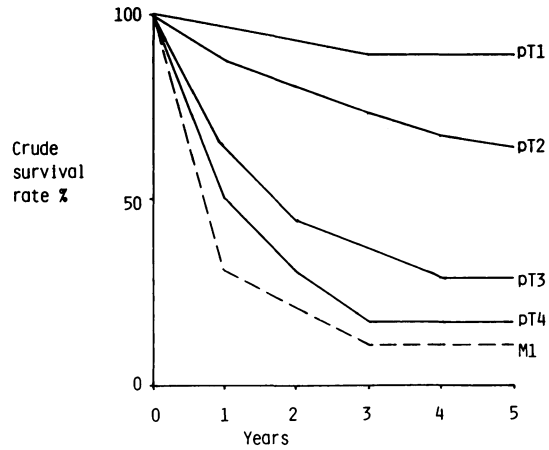

Fig. 3. Crude survival rate % according to pT stage of renal cell carcinomas. (Adenocarcinoma; The London Hospital, 1964-69).

Table 4. pT Staging and Survival

pT stage	Available for 5 Year Follow Up	Survived 5 yrs.	5 year Crude Survival Rate %
pT1	9	8	89%
pT2	14	9	64%
pT3	32	9	28%
pT4	31	5	16%
pTX	15	1	7%
M1	31	3	10%

Table 5. Tumour Grade and Prognosis

Grade	Number available For 5 Year Follow Up	Number Survived 5 Years and More	5 Year Crude Survival Rate %
G1	30	9	30%
G2	18	9	50%
G3	16	6	38%

Tumour grade could only be evaluated in a limited number of these patients (Table 5) and seemed to bear little relationship to prognosis, no doubt because of the small numbers, and the more important influence of tumour stage and tumour size (Table 6). Indeed, perhaps the most striking finding in this study has been the consistently enormous size of these tumours (Fig. 4). As a general rule, when they were relatively small, their outlook was good, but the very large ones did badly.

Table 6. Tumour Stage and Grade, Where Data Available

	Grade	pTX	pT1	pT2	pT3	pT4	Total	M1	
	G1	0	1	3	13	9	26	7	
Females	G2	0	2	5	4	6	17	3	
	G3	0	1	0	5	2	8	3	
	G1	1	4	2	3	2	12	1	
Males	G2	0	1	1	2	0	4	0	
	G3	2	0	2	1	3	8	2	
	G1	1	5	5	16	11	38	8	22%
All	G2	0	3	6	6	6	21	3	13%
Cases	G3	2	1	2	6	5	16	5	38%
Total		3	9	13	28	22	75	16	23%

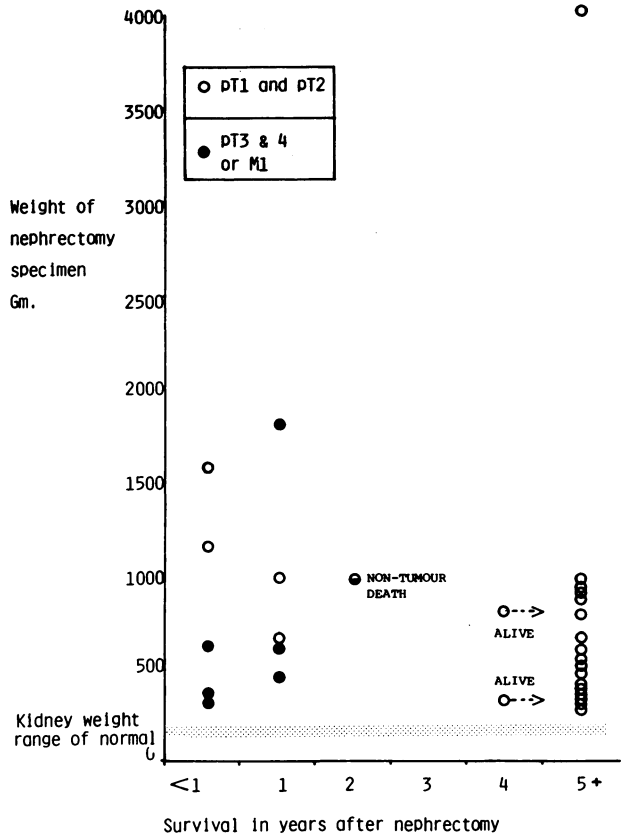

Fig. 4. Survival and weight of tumour specimen at nephrectomy. The shaded band indicates the normal range of renal weight. (Adenocarcinoma; The London Hospital, 1964-69).

DISCUSSION

There are no grounds for complacency in the contemporary detection of carcinoma of the kidney: we are only finding these cases when they are too late, and yet we already have the capacity to pick them up when they are so small (under 3 cm. diameter) as to fall within the pathologists category of benign adenoma, or at least into a critical limit less than which metastases virtually never occur. If detected at this level by chance radiography (3) the prognosis is excellent.

The expense and the difficulty of arranging annual radiological or ultrasound screening of the population at risk for cancer of the

kidney would be daunting, and the calculation has yet to be made as to whether its costs would exceed the very considerable costs of attempting to treat advanced cancer with surgery, radiotherapy and chemotherapy; let alone the hospital costs of caring for the patient dying with metastases. It could well be cheaper, let alone more humane. It would also be more effective in another way, for detection of renal cell carcinoma at an early size would permit conservative (4) resection of the tumour by partial nephrectomy. It is difficult to compute the value of a life: it is impossible to put a price on a kidney saved. Carcinoma of the kidney is now a preventable disease, and the question for tomorrow must be, "If preventable, why not prevented?".

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CARCINOMA OF THE KIDNEY: BIOLOGICAL MARKERS

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During the development and progression of malignant disease, certain biological products (markers) are produced by, or in response to, the tumour. They may be specific for a particular tumour, or non-specific, and have a potential value in diagnosis, screening, staging, prognosis, the choice of treatment, monitoring disease progression, and evaluation of the response to treatment. Improved biochemical and immunological techniques have made possible their identification, characterisation and availability for clinical use (1).

Ideal criteria for the reliability of tumour markers have been defined by Javadpour (2). These are specificity for malignant disease, detectability in body fluids and tissue extracts, short biological half-life and correlation with the presence of tumour.

The ideal tumour marker does not exist. Urologists are familiar with their clinical application in the management of carcinoma of the prostate (acid phosphatase) and testicular tumours (alpha-fetoprotein and human chorionic gonadotrophin). Measurable biological tumour markers include:-

- Foetal antigens
- Tumour associated antigens
- Hormone related substances
- Enzymes:-
  - Specific isoenzymes
  - Foetal isoenzymes
  - Placental isoenzymes
- Acute phase reactant proteins
- Polyamines

Tumour markers of carcinoma of the kidney have not been extensively investigated. This is probably because, as yet, no population group at particular risk of developing this disease has been identified and there is not yet available effective adjuvant or secondary therapy for relapsed cases. The progression of this malignancy is, however, unpredictable and a good marker has potential clinical value in forecasting prognosis and in detecting relapse.

During the last decade occasional reports have appeared in the medical literature identifying possible kidney tumour markers. The ideal marker should be specific for renal adenocarcinoma and because this neoplasm may be associated with systemic metabolic and endocrinological syndromes due to the excessive production of substances normally elaborated in the kidney, as well as the ectopic production of hormones such as chorionic gonadotrophin and parathormone, Surfin et al (3) investigated plasma hormones as tumour markers in renal cancer. They reported that Renin was elevated in 21 of 57 patients (37%) who had poorly differentiated, advanced clinical stage, poor prognosis renal cancer. Erythropoetin was elevated in 36 (63%) but its assay did not correlate with histological grade or clinical stage. Chorionic gonadotrophins (Total and B subunits) were not elevated in any of the patients. Renin and Erythropoetin may be of value as specific tumour markers, although Erythropoetin is sometimes elevated in other tumours, e.g. neoplasms of the brain, liver and uterus (4). Surfin and his colleagues could not identify the renal tumour as the source of these markers (3). It is possible that elevated levels are due to stimulation of the contralateral kidney or a decreased rate of degradation.

Another approach to identify a specific tumour marker is to measure antibodies to tumour associated antigens. DeKernion, Ramming and Gupta (5) measured such antibodies in patients with renal cancer and found them elevated in 94%. They were, unfortunately, also elevated in 20% of controls and 20-70% of patients with other tumours. Therefore, their sensitivity for kidney cancer is high (6% false negative) but specificity is low (20-70% false positives) and they have no value as specific markers. Their prognostic potential, however, needs further evaluation. In patients with metastatic disease their titre remains high in long survivors but falls with disease progression in poor prognosis patients.

Non-specific biological markers which have been investigated include carcinoembryonic antigen, polyamines, prostaglandins and acute phase reactant proteins. In 1974, Chu et al (6) reported that plasma carcinoembryonic antigen levels were slightly elevated in one of three patients with non-metastatic disease and significantly elevated in seven of eight patients with metastases. They were also elevated in four of 12 patients with local recurrence of renal carcinoma. Levels tended to fall following nephrectomy and during chemotherapy with CCNU.

Sanford et al (7) studied the urinary polyamines (putrescine, spermine and spermidine) in renal parenchymal carcinoma. In situations of rapid growth, these organic cations, which are related to ribonucleic acid synthesis, accumulate rapidly.

In the urine their concentration was increased in 9/11 patients with renal tumours. They are also concentrated in other urinary tract malignancies but not in benign urological disease. Therefore, as markers, their diagnostic value is limited but they may have a role in monitoring disease progression and response to treatment.

Prostaglandins can be identified in renal cell cultures, extracts of primary and secondary renal tumours, and in renal blood collected from the veins of kidneys with adenocarcinoma (8). Serum prostaglandin E is inactivated by passage through the lungs but has been reported to be elevated in two patients with pulmonary metastases and hypercalcaemia. It may, therefore, be a possible marker of renal lung metastases. Prostaglandin A was found to be elevated in a patient whose essential hypertension was suppressed when he developed a renal carcinoma (9). This prostaglandin was a potent antihypertensive agent (10). Further studies of prostaglandins as markers of renal cancer are indicated.

Acute phase reactant proteins are a group of proteins whose plasma concentrations are altered in response to such stimuli as tissue injury, acute and chronic inflammation, and neoplasia (11). One of these, haptoglobin, was studied by Vickers (12) in 36 patients with abnormal renal masses. None of ten patients with renal cysts had elevated levels. High levels were found in 14 of 16 patients with localised renal carcinoma and in all six cancer patients with visceral metastases. In two thirds of the cancer patients, a rise after treatment was diagnostic of recurrence.

The Yorkshire Urological Group (13) have studied the acute phase reactant proteins C-reactive (C-RP), α_1 chymotrypsin (ACTH) and α_1 glycoprotein (AGP) in renal adenocarcinoma. A progressive rise of these variants indicates progressive metastatic disease. Elevated but stable levels are found in patients with stable non-progressive metastatic disease. In the same group of patients the enzyme phosphohexose isomerase, which reflects glycolytic activity was measured in the plasma. This was elevated in 62% of advanced metastatic tumours and only in 17% of patients with localised disease.

CONCLUSIONS

This paper has reviewed some of the potential tumour markers for renal adenocarcinoma. The ideal marker has not yet been determined and the application of markers in clinical practice needs further investigation and evaluation. Potentially, however, biological markers, both tumour specific and non-specific, have an important role to play in the future management of renal cancer.

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REGRESSION OF HYPERNEPHROMAS

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Clear cell carcinomas of the kidney (hypernephromas) do regress within the kidney under certain circumstances. The Swedish pathologists, Bartley and Hultquist (1), have clearly shown this and illustrated it when they reviewed the literature and reported their own cases in 1950.

It is also clear that in some cases, although very few, distant metastases of renal cell carcinomas have been known to regress or disappear (2, 3). This has recently been summed up very clearly by Freed (4) who described 51 acceptable cases.

One of the clearly most acceptable cases with a reported autopsy showing regression was described by Jenkins (5) as a regression at eight years and then death at 13 years with an autopsy illustrating the regressed pulmonary lesions (6). Garfield and Kennedy (7) described a 16 year regression and reversal of liver disease. Braren et al (8) described reversal and regression of a skin lesion which they could clearly see and which they had biopsied.

Johnson et al (9) studied their cases and concluded that nephrectomy in the presence of metastases was justified only in cases with regression of bony metastases (2).

Usow and Silbar (10) reported regression of a pulmonary metastasis after nephrectomy and chemotherapy with 5FU, 600 mg. per week, Methotrexate, 25 mg. intravenously per week, Chlorambucil, 2 mg. three times a day and Provera, 10 mg. three times per day. Despite this encouraging report, there is no clear evidence that any chemotherapy is truly effective in renal cell carcinoma.

The growth of renal tumors and their metastases must be extremely variable and there are some reports of appearance of metastases at very long intervals after the original nephrectomy. Bradham et al (11) described a renal cell carcinoma metastasis 25 years after nephrectomy. They stated that the longest survival between recognition of a renal cell carcinoma and eventual death from metastasis is 37 years in a patient in whom the neoplasm was considered inoperable and left in place.

There has continued to be a great deal of speculation as to the possibility of some immunologic mechanism. There can be no doubt that there are well authenticated cases of regression, if not cure. The real unanswered question is "why and how", and what factors could be brought into play to make this phenomenon occur more frequently.

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SPONTANEOUS REGRESSION OF METASTATIC RENAL CELL CARCINOMA AND ITS SIGNIFICANCE IN ASSESSING RESPONSE OF SUCH PATIENTS TO CHEMOTHERAPY

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INTRODUCTION

Spontaneous complete regression of tumour metastases received recognition as a genuine phenomenon following the carefully documented study of literature reports by Everson and Cole (1). Renal cell carcinoma was the tumour most frequently reported to undergo spontaneous complete regression, although in that series for only four of 26 patients was there histological documentation that the lung shadows contained malignant cells. In the recent review by Fairlamb (2) at least 15/67 lived five years although half subsequently died of recurrence of their disease up to 15 years later.

Apart from a series reported by Werf-Messing and Van Gilse (3), who observed partial regression of lung metastases in 8 of 33 (24%) of patients when frequent chest X-rays were performed and the control series of Tykka et al (4), who observed partial regression of pulmonary metastases in one of 12 patients there are few carefully followed series of renal cell carcinoma in which it is possible to establish a reliable estimate of the frequency with which spontaneous regression occurs, although in reviews of the literature Bloom (5) reported three out of 606 patients and Fairlamb (2), two out of 173 patients undergoing such regression.

Equally, there is no series in which biopsy of probable pulmonary metastases has been performed systematically in patients with renal adenocarcinoma to establish the frequency of positive histology. Nonetheless, given the fact that there are histologically proven lung and extra-pulmonary metastases where spontaneous regression has occurred, it is sensible to take account of this possibility in assessing the response of this disease to any form of therapy.

It is the purpose of this paper to review our recent experience of chemotherapy, endocrine therapy and immunotherapy in the light of this fact.

CLINICAL MATERIAL AND TREATMENT

Twenty-four patients have been treated. All had bidimensional measurable lung or lymph node metastases; a few had in addition bone metastases. Although the majority had several examinations confirming that the metastases were growing, some patients, usually due to the presence of extensive metastases, were treated at first diagnosis.

Patients were treated between September 1977 and February 1981 and all have had a minimum of six months' follow-up. Because of the low frequency of referral, no single protocol has been followed and patients receiving chemotherapy were treated according to whatever therapy was currently under investigation in the department or suggested from literature studies current at the time the patient presented. Patients on Provera received 100 mg three times a day for a minimum of six weeks. Patients on BCG received 2 Heaf gun punctures through 40 μl of standard Glaxo BCG every two weeks for three months then monthly for one year or until there was evidence of tumour progression.

Assessment of response was by a greater than 50% reduction of the product of the two maximum diameters of all metastases for longer than one month. Patients with stable disease showed no measurable change in size of any metastases and no symptoms suggestive of new metastases for $\geqslant$ 6 months.

RESULTS

The response to treatment is shown in Table 1.

The single patient who 'responded' to BCG had a solitary metastasis 1 cm in diameter in the left lung present at the time of diagnosis. His primary tumour was removed one week after renal infarction using gelfoam. The metastasis took six weeks to regress completely but within three months of starting treatment the patient developed bone metastases and subsequently developed metastases in the opposite lung. Following radiotherapy to a lytic lesion in the right humerus, further metastases developed in the lung including one at the site of the original lesion which had regressed. He is currently still alive 11 months after diagnosis with progressively growing metastases.

As mentioned in the introduction, the chemotherapy used in this study was not standard. Table 2 presents results according to the type of drugs used and whether given as primary treatment or following disease progression after Provera or BCG. The majority of these

patients received single agent therapy, although one patient receiving Vinblastine received CCNU in addition (Table 2).

There were no responses and none of the patients had stable disease for six or more months as was seen for three patients receiving Provera.

Table 1

	N	Response	Stable Disease ⩾6 months
Provera	14	0	3
Chemotherapy*	7	0	0
BCG	3	1	0

* for details see Table 2

Table 2

	Previously untreated		Treated after progression on Provera and/or BCG	
	N	Response	N	Response
Cyclophosphamide (0.75 - 1G weekly)	2	0	1	0
Vinca Alkaloid	3*	0	8*	0
Chlorambucil	2	0	1	0

* Vinblastine n = 8, Vindesine n = 2, Vincristine n = 1
(6mg/m^2/2-3 wkly) (3-4mg/m^2/wkly) (2mg/wkly)

DISCUSSION

Recent reviews of endocrine treatment in patients with metastatic renal cell carcinoma have reported a regression rate of 7% in 643 cases while for chemotherapy the average for all cases reviewed (n = 486) was 10%, although for the best drug (Vinblastine) it averaged 25% (6,7). In contrast, for all series of patients receiving immunotherapy, either by BCG or by chemically altered tumour extract (n = 139), 27% have responded (8), and in the small series of 16 patients with lung metastases in the study reported by Tykka et al (4), 8 responded, 6 with total disappearance of all evidence of disease after treatment with a vaccine of autologous tumour cells polymerised with ethyl-chloroformate combined with tuberculin or candida albicans antigen depending on the state of pre-existing immunity in the patients.

Although the data presented in this paper are not adequate as a definitive study, they are worth reporting as they emphasise the need for accurate information about spontaneous regression and the incidence of prolonged stabilisation of disease - points which have not been adequately emphasised in the majority of previously reported studies of chemotherapy, endocrine therapy or immunotherapy in patients with renal cell carcinoma.

Prolonged stabilisation of disease is well recognised in patients with metastatic renal cell carcinoma even without active treatment. This observation, taken together with the less frequent but well documented occurrence of spontaneous regression, means that assesment of any treatment on the basis of regression rate or duration of response is invalid unless there are concurrent untreated controls who have assessment of their metastases as frequently as the treated patients. It is pertinent in this context to note that there was no evidence of stable disease amongst the patients receiving chemotherapy as primary or secondary treatment. In fact, one of the main reasons for the small number of patients treated by any of the drugs investigated was the extremely rapid progression of metastases which occurred in all patients once chemotherapy was initiated, almost as though the drugs were acting as fertilisers for tumour growth.

In the literature reviewed by Everson and Cole (1) only four of 26 cases of spontaneous regression had histological documentation of lesions which subsequently regressed. Although we did not obtain histological confirmation in the only patient who showed definite but short-lived regression, it is reasonable to accept this as a temporary tumour response since, when further metastases developed, the original lesion reappeared. Nevertheless, it would improve confidence on reports of tumour response if there was histological confirmation, as non-malignant conditions can produce changes similar to those produced by metastases. The increasing accuracy of fine needle aspiration biopsy should make this a realistic possibility in future studies.

SUMMARY

Retrospective analysis of the results of treating 24 patients with metastatic renal cell carcinoma emphasised the difficulty of using measurements of tumour response to assess the results of treatment and suggests that only by using large scale randomised control studies will bias due to chance inclusion of an occasional case of spontaneous regression be excluded. Equally, only by using randomised controlled trial will it be possible to exclude the possibility that treatment accelerates tumour growth.

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RENAL CELL CARCINOMA, SURGICAL TREATMENT

OF THE PRIMARY LESION

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Our management of the surgical approach to renal cell carcinoma is essentially the same as that outlined by Kaufman and Mims (1) in "Current Problems in Surgery, 1966". The most common approach was, with the patient in the flank position, to make an extraperitoneal, extrapleural, subdiaphragmatic approach through a flank incision below the twelfth rib. Although this is satisfactory, it does not really give good enough exposure for radical surgery, which is indicated in renal cell carcinoma which so frequently has extension or regional lymph node involvement. Modifications include removal of the twelfth rib or an incision between the eleventh and twelfth rib.

We believe that thoracoabdominal nephrectomy first described by Mortenson (2) is the approach of choice. The patient is placed in the flank position with the flank extended, and an incision is made between the tenth or eleventh interspace. After the pleura is opened, the diaphragm is divided far enough laterally so there is no injury to the phrenic nerve. The peritoneum is opened and the abdomen is inspected and palpated to determine the presence or absence of metastases.

Following this, the colon is reflected anteriorly toward the midline and the retroperitoneal space is entered. The kidney is pushed forward and the renal hilus is dissected free. The renal artery which lies posterior to the renal vein is identified and ligated. When the renal vein is occluded before the renal artery, there can be excessive bleeding. The renal vein is then exposed and should be palpated to determine the presence of a tumor thrombus before ligating it.

The renal artery is doubly ligated with 2 - 0 silk, and then the vein is doubly ligated and both structures are transected.

As Robson has pointed out, the best results are obtained in a truly radical nephrectomy when the entire kidney with Gerota's fascia intact is removed together with the regional lymph nodes. (3) When the tumor is excessively large, it is sometimes necessary to remove the kidney before dissecting the lymphatics which should be removed from the diaphragm down the the lower abdominal aorta. If there is involvement of the superior pole of the kidney, it is sometimes necessary to remove the adrenal with the "en-bloc" specimen. After the renal cell carcinoma is removed and the lymph node dissection is completed, the colon falls back into the empty space and the diaphragm is closed with interrupted sutures. The chest is closed in a conventional fashion, and I usually use a chest tube during the immediate post-operative period. The lungs should be inflated by positive pressure from the anaesthetist when the final closing sutures are placed.

The important thing is to remove all of the tumor after primary ligation of the renal artery and to do a dissection of the hilar lymph nodes and, if there is evidence of extension into the lumbar muscles or even into the diaphragm, to remove the areas of extension.

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Editorial Note (M.P.)

Dr Goodwin expresses his personal preference for the large thoraco-abdominal approach. Undoubtedly this route gives an excellent exposure and in my view is the approach of choice in the case of large cancers of the upper poles of the kidneys. Many surgeons are however inclined to believe that this is perhaps too extensive a procedure, which does not appear to be necessary in renal cancer of category T1 and T2, especially if it is located in the lower pole.

An extended lumbar approach still advocated by some authors, is probably adequate only for relatively small cancers. Many surgeons are nowadays in favour of an anterior transperitoneal route in most cases, with primary ligation of the renal artery at its origin from the aorta, followed by ligation of the renal vein, before mobilizing

the kidney en bloc with perirenal fat. Ligation of the renal vein prior to the artery is acceptable if preoperative embolisation has been performed. Some authors feel, however, that an attempt at primary ligation of the artery should be performed even after successful embolisation.

KIDNEY CANCER - SURGERY OF METASTASES

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The management of the solitary metastasis from renal cell cancer is not in dispute: it is agreed that where the anatomical site of the metastasis lends itself to surgical excision, the metastasis ought to be resected, and it is equally clear that when surgical removal is not feasible, then radiotherapy may give immediate relief of pain and sometimes prolonged freedom from recurrence. The only thing that perhaps deserves a comment, a propos solitary metastases, is their astonishing vascularity - a fact which is little mentioned in text-books and which seems to be one of those things which we all have to learn by experience. Nobody who has had to face the unbelievable torrent of haemorrhage that may follow biopsy of a metastasis from a Grawitz tumour will ever undertake such a minor procedure lightly, or without plenty of blood in reserve to meet such a dramatic crisis. The main topic for debate is the management of the patient who presents with multiple metastases: is it justifiable to attempt to take out the kidney? (1,2). The literature is unhelpful on the topic. Surgeons who have a patient whose metastases have disappeared after removal of the primary in the kidney tend to report such an unexpected miracle. Those of us who are less successful do not report our failures. There are two separate but cognate questions: (a) how often does removal of the primary cancer seem to prolong life or make metastases go away? and (b) how great a price does the patient pay in terms of pain and mortality for this small chance of benefit? It is to these two questions that we have addressed ourselves in this study.

CASE MATERIAL

In the 114 patients recently reviewed in our department 98 underwent nephrectomy, and in 12 others, an exploratory operation was

Table 1. The London Hospital. Adenocarcinoma of Kidney 1964-79.

		Nephrectomy	Laparotomy	Palliation only
Males	73	63	8	2
Females	41	35	4	2
Total	114	98	12	4

started in the hope that nephrectomy could be completed (Table 1). Overall, the operative mortality for nephrectomy in our hospital has been 2% when performed for carcinoma: one of these died four weeks after operation from his cerebral and other metastases and the other presented with a huge carcinoma in a horseshoe kidney which was impossible to remove completely, and whose attempted removal impaired the blood-supply to the remaining half-kidney. In neither instance was there an error of surgical technique, though with hindsight, there was certainly an error of surgical judgment.

RESULTS

In this series 34 patients had metastases at presentation (Table 2); of these we set out to perform a nephrectomy in 27. It was impossible in five, and we contented ourselves with a biopsy - often not a minor procedure - so that nephrectomy was achieved in 22 patients who presented with widespread metastases. As we have seen, one of these died of his cerebral and other metastases a month after operation. This gives 4% operative mortality for this "faint hope" operation. Most of the patients continued to go downhill and died of their metastases. But three survived for more than five years (11%) (Table 3). The first of these had shadows in the lung, never proven by biopsy, that remained unaltered for eight years until she died at the age of 83 from bronchopneumonia unproven by post-mortem examination. The second presented with a painful lump in the chest wall which was found to be a metastasis from a silent primary in the kidney, and lived for another five years after nephrectomy only to develop other and widespread metastases which swiftly killed him. The third patient survived for five years, but was intensively treated - though with radiotherapy and provera - now regarded as probably useless - only to die in the end of his disease.

The difficulty is to know whether these anecdotes mean anything. It is well known that patients can live a long time after their metastases have cropped up. We have three such patients (Table 4) who have respectively survived 12, five and four years from the emergence of their metastases, at varying intervals from nephrectomy of one to two years.

Table 2. The London Hospital. Adenocarcinoma of Kidney 1964-79.

Is it justified to attempt nephrectomy when there are distant metastases at the time the patient comes to Hospital? Nephrectomy for M1.

Patients presenting with advanced mets, terminal, or unfit to consider nephrectomy	7	
Nephrectomy achieved	22	(1 op. death, male 46 yrs. pT4.G1.M1 cerebral mets.)
Nephrectomy not possible biopsy only.	5	(Op. deaths nil)
Total operated on with intention of nephrectomy	27	(Op. mortality 1 [4%])
5 year survivals	3	(5 yr. crude survival rate 11%)

DISCUSSION

At a moment in surgical history when we have nothing else to offer the patient with widespread metastases from renal cell carcinoma an 11% chance of doing good - even if doing good only means prolonged life for five years - may seem worth while. Clearly it is not good enough. Equally clearly, there may be other reasons for trying to

Table 3. The London Hospital. Adenocarcinoma of Kidney 1964-79.

Unexpected prolonged survival of patients presenting with metastases (M1).

DP. f. 75 pT2. G2. M1.	Nephrectomy. 2 lung mets (not biopsied) remained unchanged for 8 years. Death from 'bronchopneumonia'. No P.M.
AM. m. 63 pT4. GX. M1.	Nephrectomy. Presented with lump in rib - excised, showed metastasis. Lived 5 years, then 2nd metastasis in femur. RT. Died 2 months later with widespread mets.
MP. m. 68 pT3. G3. M1.	Presented with supraclavicular nodes, biopsied then IVU revealed tumour. Nephrectomy, R.T. local lymph nodes N4. Provera, Prednisolone. Lived for 5 years, died with widespread mets.

Table 4. The London Hospital. Adenocarcinoma of Kidney 1964-79.

Prolonged survival after late appearance of metastases.

W. deS-L m. 55	1967	pT3.G3.MO Left nephrectomy. 760 g. post-op R.T.
	1969	Met. in right upper lobe of lung. lobectomy.
	1974	Mets. in left supraclavicular nodes biopsy and R.T.
	1981	Alive and well.
M.D. f. 53	1966	pTX.G2.MO Left nephrectomy. post-op R.T.
	1968	Chest mets. Provera.
	1972	Path. fracture left humerus. Mets left femur. R.T.
	1973	Died. Widespread mets. (7 years)
G.O. f. 64	1977	pT3.GX.MO. Left nephrectomy.
	1978	Chest mets. No treatment.
	1981	Chest clear. Alive and well (4 years).

take the kidney out - pain, haematuria, clot colic and so on. But the price of this small chance is a 4% operative mortality, added to the inevitable pain and suffering of a big transabdominal operation. After our review of our experience we remain optimistic, and unrepentant, but at the same time we are deeply aware that there must be something better just around the corner.

SUMMARY

Twenty-seven patients were explored with the object of removing the kidney for carcinoma that had already given rise to widespread metastases. One patient died (4%): three survived for five years (11%).

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ADENOCARCINOMA OCCURRING IN A SOLITARY KIDNEY

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Although many instances of adenocarcinoma occurring in the solitary kidney have been reported in the English literature, it has only been in recent years that the natural history and therapeutic options for this condition have been evaluated. To compare survival, prognosis and therapy, patients can be divided into three categories. The first group consists of those in whom one kidney was originally removed for a benign disease or those born with a solitary kidney. The second group includes patients in whom one kidney was removed at a previous time because of cancer (asynchronous renal carcinoma). The third group includes patients who have bilateral renal carcinoma at the time of diagnosis (synchronous renal carcinoma).

One of the first comprehensive reports on this subject was by Wickham (1). He reviewed the literature and evaluated data on 51 patients with cancer in a solitary kidney who had been diagnosed from 1942 - 1974 (1). There were 25 patients whose original nephrectomy was performed for benign disease. Seventeen (68%) were alive (mean time of 30 months), six (24%) had died of renal cancer (mean time of 8 months), and there was no follow-up on two patients. There were 27 patients whose contralateral kidney was removed because of renal cancer. Ten (37%) were alive (mean time of 26 months), 15 (55%) had died (mean time of 8 months), and there was no follow-up on two patients. Nineteen of the patients in this group had been diagnosed since 1968 but only seven patients in the entire group had surgical excision of their neoplasm in an effort to provide a cure.

This poor survival in patients with asynchronous renal cancer stands in contrast to the results reported by Novick et al (2) who reviewed the experience from the Cleveland Clinic. The records of the 353 patients with renal carcinoma were evaluated and 21 patients

with carcinoma in a solitary kidney were found who had received surgical therapy. There were ten patients whose other kidney was removed because of benign disease and five were alive five years after surgery with evidence of recurrence. Four of seven patients with asynchronous carcinoma had no tumor recurrence. It is important to note that the original nephrectomies in this group were performed 11 - 19 years earlier.

Schiff, Bagley and Lytton (3) reviewed the literature subsequent to 1968, and included seven patients of their own. These 62 selected patients had undergone surgical treatment that resulted in complete tumor removal. There were 48 patients (77%) who were alive without evidence of recurrence at an average follow-up period of 45.7 months. They found no differences in survival whether the contralateral kidney was involved with carcinoma or was removed for benign disease.

Jacobs and associates (4) reviewed the literature and the results of 61 patients with synchronous carcinoma who were treated with surgical excision. Hereditary types of carcinoma were found in 12 patients; six had Von Hippel-Lindau syndrome, five had familial renal adenocarcinoma and one patient had tuberous sclerosis. Ten patients had such extensive disease that bilateral nephrectomy was required. Six patients were placed on dialysis and four died of cancer while one had metastatic disease at the time of the report. Only two of four patients who had renal transplantation remained alive.

There were 51 patients who were candidates for total resection of their tumors with preservation of renal tissue. Only 11 patients died of their disease (79% survival). Ex-vivo surgery was utilized in 16 patients and 13 remained alive (82% survival). Of 34 patients with in situ surgery 26 remained alive at five years (75% survival).

Smith et al (5) reported on 33 patients treated at UCLA. Complete surgical excision was possible in 29 patients. There were 12 operable patients whose contralateral kidney was removed for benign disease. Ten remained alive without recurrence at five years. One death resulted from surgical complications and the others from recurrent cancer.

There were 12 patients with asynchronous cancer and eight with synchronous cancer. Three of these 20 patients had such extensive disease thay they were not candidates for surgical therapy. An additional three patients died from causes unrelated to their cancer. Eleven patients remained alive after five years following surgery, but two have evidence of metastases. There was no difference in survival between those with asynchronous or synchronous cancer.

Compiling the list of patients included in this report and other isolated case reports permits a calculation of crude survival data from a total of 182 patients (Table 1). There is an overall five

Table 1. Carcinoma in the solitary kidney; cumulative 5 year survival

Type of Carcinoma	No. of Patients	5-Year Survival	
		No. of Pts.	%
Solitary kidney*	60	45	75
Asynchronous	34	23	67
Synchronous	88	58	66
	182	126	69

*contralateral kidney congenitally absent or removed for benign disease.

year survival of 69%. This includes 45/60 (75%) patients with contralateral benign disease, 23/34 (67%) of patients with synchronous disease and 58/88 (66%) patients with synchronous carcinoma.

Patients with synchronous carcinoma occurring within five years of the contralateral nephrectomy for carcinoma have the poorest prognosis (47% five year survival). Early recurrence is generally associated with multiple sites of metastases. Late recurrence tends to be associated with a solitary metastasis and a good prognosis.

Both synchronous and asynchronous renal carcinoma represent metastatic disease. Therefore, it is not surprising that survival in these patients is not quite as good as those whose other kidney was removed for benign disease, although the differences are not statistically significant. All groups of patients without evidence of widespread metastatic disease deserve an attempt to eradicate their disease with surgery. Stage for stage, the disease seems to be no more virulent in a patient with cancer in a solitary kidney than in patients with two kidneys. The extent of the disease is far more important than the reason the other kidney was removed. Unfortunately, there is not any clear data which relates stage to survival but the presumption is that patients with low stage disease have longer survival than those with high stage disease. The good survival in these patients may be explained by the early detection of a solitary metastasis. Lung and osseous metastases are generally silent until they reach a large size. Renal metastases tend to produce hematuria early in the course of their development.

Current diagnostic techniques allow an accurate assessment of the extent of the tumor within the kidney and permit the surgeon

to carefully plan the operative procedure. We have found that upper pole tumors and extremely large tumors are easiest to approach through a thoracoabdominal incision. Transabdominal and extrapleural retroperitoneal approaches through the tenth and eleventh rib beds are also satisfactory.

Most carcinomas recurring in the solitary kidney can be managed by in-vivo surgery. Even though there has been a great deal of interest in 'bench surgery' in recent years, this type of surgical procedure is not usually necessary. However, the capability of performing ex-vivo surgery should be available before contemplating the removal of a large tumor or one that is located in the midportion of the kidney. The major disadvantages of ex-vivo surgery are the complexity of the technique, the need for vascular and possible ureteral anastomosis, increased operating time and the potential of ischemic damage to the kidney and increased patient morbidity.

Survival does not seem to be influenced by in-vivo surgery. Jacobs et al (4) found that there were only three cancer deaths among 16 patients treated with ex-vivo surgery (survival 82%), compared to eight deaths in 34 patients treated with in-vivo surgery (survival 76%).

The surgical principles which should be followed in the in-vivo excision of these tumors are those generally applied to all types of cancer surgery. The wound and surgical areas should be protected from possible tumor spill by appropriate drapes. All of Gerota's fascia can be removed but should not be stripped away from the tumor. The adrenal gland may need to be removed with the upper pole tumors since it is often a part of the tumor mass. When the adrenal is not removed, it should be carefully inspected for possible metastases. The renal artery and its major branches do not need to be stripped of surrounding tissues. This may induce serious vasospasm and compromise the residual normal tissue. The artery to the segment of the kidney containing the carcinoma may be identifiable and if so, can be ligated prior to occluding the renal artery; a Mannitol-induced diuresis is beneficial for the preservation of renal function. In some instances renal perfusion with cell-preserving agents such as those used in renal transplantation may be necessary. Generally, with the use of local hypothermia from iced saline slush, the renal artery can be occluded for an hour without serious long-term renal impairment. A Swan-Ganz catheter placed in the renal artery preoperatively can be used for vascular control as well as renal perfusion. Intraoperative angiograms may be necessary particularly with ex-vivo surgery. Delicate reconstructive surgery is sometimes necessary and is facilitated by the presence of appropriate instruments, magnifying loupes, or even an operating microscope. The use of Avitene on the cut surface of the kidney has been a benefit in providing hemostasis.

A staging regional lymphadenectomy is usually advocated to help determine prognosis. Removal of the ipsilateral nodal tissue adjacent to the kidney is sufficient. Following completion of the tumor removal and kidney reconstruction, the operative area should be thoroughly irrigated with distilled water.

CONCLUSION

Patients with carcinoma in a solitary kidney are potential candidates for curative surgery. Their prognosis depends on the stage of their disease and correlates with the cause of the contralateral nephrectomy. Those whose other kidney was removed for carcinoma do not have as good a prognosis as those whose kidney was removed for a benign disease. Patients with asynchronous tumors whose recurrences developed within five years of the original nephrectomy do not have as good a prognosis as those whose recurrence appeared later or those with synchronous disease in whom there are no other metastases.

Most operations can be done in-vivo. The operative team should be experienced in the various technical aspects, including ex-vivo surgery. Current diagnostic and operative techniques have improved the prognosis for patients with carcinoma in a solitary kidney.

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LYMPHADENECTOMY IN RENAL CANCER

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ABSTRACT

On the basis of the experience of the Urological University Clinic of Genoa (104 cases of renal cancer operated on in the last 10 years) the problems related to the rationale, the technique and the indications for retroperitoneal lymphadenectomy in renal cancer are reviewed and discussed.

It is concluded that, although lymphadenectomy adds little morbidity and constitutes a fundamental part of radical nephrectomy performed through an anterior transperitoneal approach, no actual benefit can yet be demonstrated.

The generally accepted management of renal cancer is radical nephrectomy, which includes perifascial nephrectomy through an anterior transperitoneal approach, adrenalectomy and retroperitoneal lymphadenectomy (1). However it is possible to question the benefits of lymphadenectomy, the technique and the extension of nodal dissection and the indications for nodal extirpation in high stage tumors. In this paper we shall discuss our experience at the Urological University Clinic of Genoa, based on 104 consecutive cases of renal cancer operated on during the last 10 years and we will attempt to define the role of lymphadenectomy in the treatment of renal cell carcinoma.

The rationale of lymphadenectomy

The indications for lymphadenectomy in every neoplastic disease are:

- to allow accurate staging, which is indispensable to the planning of the postoperative treatment and to the evaluation of long-term results, and
- to improve the radicality of surgery and therefore to improve the survival rate.

However, immunological studies in renal cancer patients have drawn attention to the fact that routine lymphadenectomy, by abolishing the regional lymph node barrier, could impair the immunological defences of the host against the tumor. As yet there are no convincing arguments which support this opinion and we are not able to shed further light on this matter.

Our figures which show a 77% overall five year survival in patients without distant metastases at the time of surgery, submitted to extended nephrectomy with lymphadenectomy reveal no evidence of any unfavourable influences of lymph node excision. Moreover, the fact that 34.6% of patients without distant metastases, but with nodal involvement, in whom nodal metastases were extirpated, survived five years suggests that retroperitoneal lymphadenectomy might have a beneficial effect.

Incidence of regional nodal involvement

It is well known that renal cancer metastasizes by both blood-borne and lymphatic routes. The generally reported incidence of positive regional nodes during radical nephrectomy is about 20% (2-5). In our patients we found 32.6% to have positive nodes. If we consider these data in relation to the stage (Table 1) we can see that positive nodes are present in 6% of low stage tumors, but in a higher percentage of high stage tumors. At present we have no data either about the number of involved nodes or about the magnitude of the tumoral infiltration, which varied from microscopic foci to massive invasion.

Table 1: Incidence of regional lymph node involvement in renal cancer patients submitted to radical surgery.

	P2	P3	M+	V+M+
N+/Total	2/33	13/28	13/21	6/9
	(6%)	(46.4%)	(61.9%)	(66.6%)

The extent of lymphadenectomy

The extent to which nodes should be removed is also controversial (1,6,7). The accepted steps of the involvement of regional nodes in renal cancer are - renal, hilar, ipsilateral para-aortic or paracaval and later the mediastinal nodes. However the pattern of the nodal involvement is unpredictable and although the iliac nodes are not involved unless by retrograde embolism, contralateral and supraclavicular metastases are not rare.

We assume that the very extensive lymphadenectomy advocated by Robson et al (6) and by Angervall et al (2) does not add greatly to survival and probably implies major morbidity for the patient. Our practice is to carry out a more limited lymphadenectomy, which extends from the diaphragm to the aortic or caval bifurcation and includes the ipsilateral lumbar nodes, the retro-aortic or retro-caval nodes and the interaortico-caval nodes (Fig. 1 a,b,c).

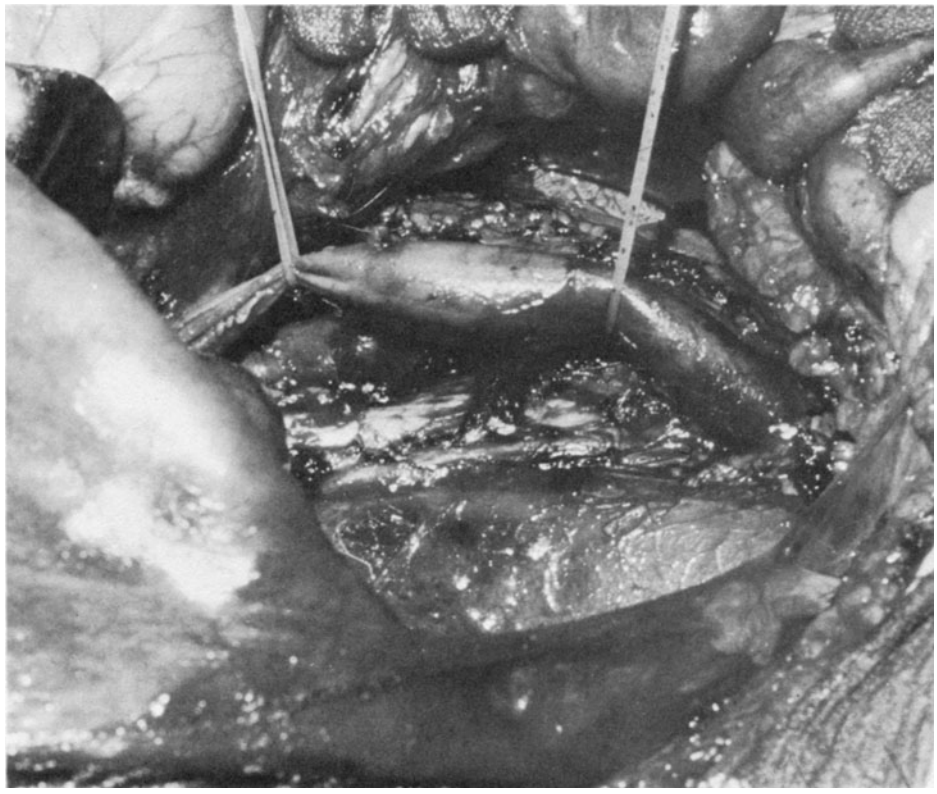

Fig. 1. Latero-caval lymphadenectomy

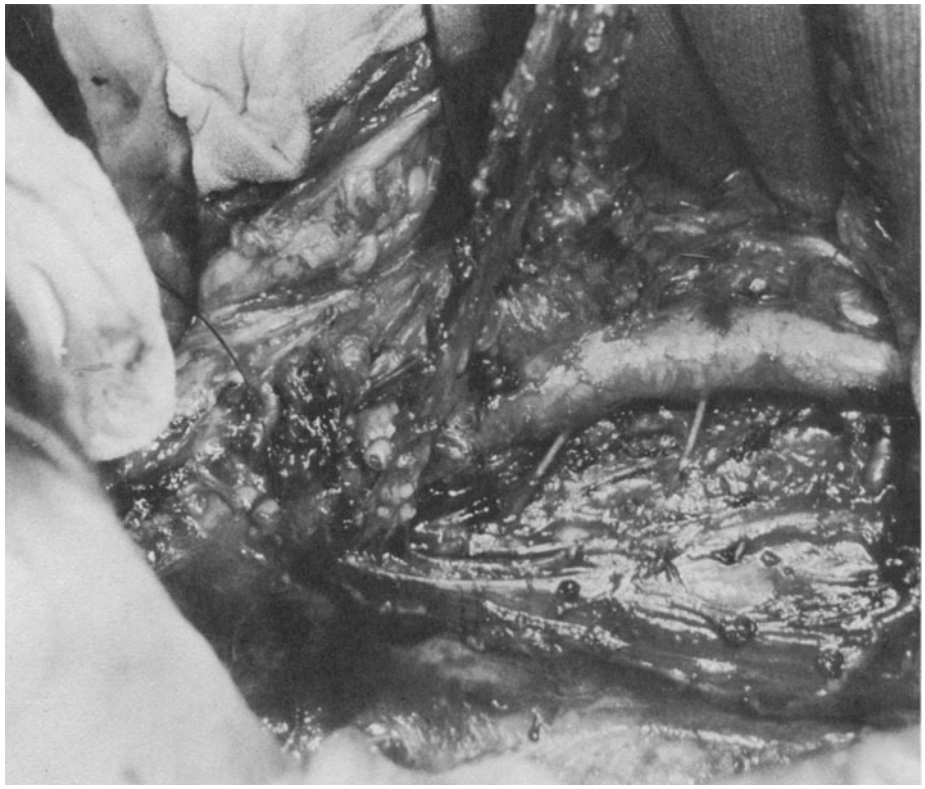

Fig. 1b. Latero-aortic lymphadenectomy

In our series we did not note any significant morbidity related to lymphadenectomy, which, on the contrary, represents an integral part of the intervention. Indeed the inter-aorto-caval lymphadenectomy is indispensable to correctly expose the anterior face of the aorta and vena cava, so allowing the surgeon to ligate first the renal artery at its origin from the aorta on either side (1). Lateral and retroaortic or caval nodes are excised after the nephrectomy. We do not perform an iliac node dissection, unless lumbar nodes are clearly involved. Contralateral nodes are not extirpated.

Lymphadenectomy in patients with distant metastases

Aggressive management of renal carcinoma in the presence of distant metastases has been advocated by several authors (1,5,8). However, in the majority of reports no mention is made of the practice of lymphadenectomy in these cases. In our patients nodal involvement in the presence of distant metastases was found in 52.4% of the cases.

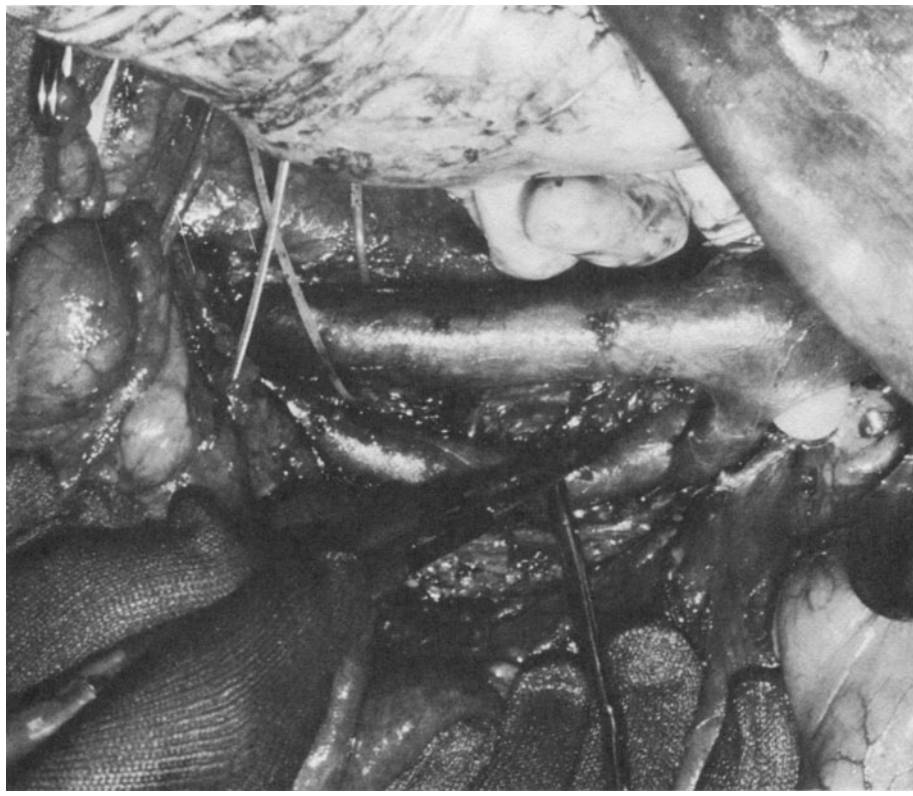

Fig. 1c. Interaortico-caval-lymphadenectomy

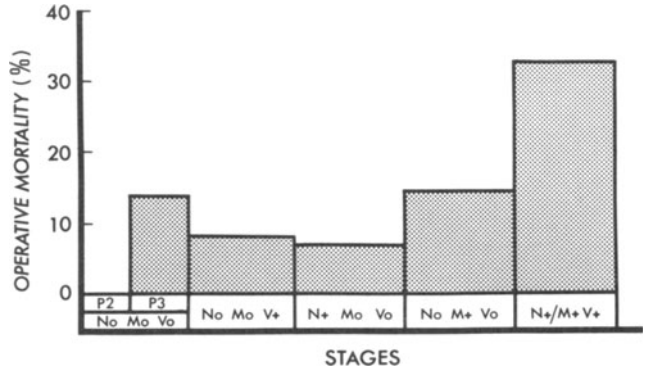

Fig. 2. Renal cancer - operative mortality rate according to the stage of the tumor

We are of the opinion that lymphadenectomy should be performed in these cases as part of the concept of reductive surgery, surgical toilette and prevention of local renal fossa recurrences. When we face a solitary metastasis, lymphadenectomy becomes of greater importance in the effort to achieve a radical excision. In the presence of multiple metastases, its goal is to give better possibilities to chemotherapeutic treatment. On the other hand, it is true that operative mortality is increased in patients with distant metastases from 9.6 to 14% (Fig. 2).

Conclusions

We continue to advocate and to practice lymphadenectomy, although no actual benefit from it can be demonstrated. On the other hand, we have never observed any significant morbidity which could be attributed to it and in skilled hands it adds little time to the operation. However, there are as yet no reports in the literature which satisfactorily define the problems related to its practice. Additional information regarding the metastatic patterns of renal cancer and tumoral lymphatic drainage and the effective contribution of lymphadenectomy to the survival of renal cancer patients are needed and we hope that a prospective clinical trial to provide information on the role of lymphadenectomy will soon be started.

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TECHNIQUES OF EMBOLIZATION IN RENAL CANCER

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ABSTRACT

Embolization has now been widely accepted as a step in the management of large kidney tumours. Herein the technical problems of embolization are described and discussed.

As yet Cyanoacrylates are the best substances available for kidney embolization. A coaxial catheterisation system is not always necessary. Isobutyl-2-cyanoacrylate (IBC), which is the most used acrylic resin, can be made radiopaque by mixing it with Lipiodol Ultrafluid.

Radical nephrectomy should be carried out within 24 hours of embolization. The complications of embolization of kidney tumors are, in our experience, very few.

INTRODUCTION

Transcatheter embolization of renal tumours is a procedure that has gained more and more worldwide acceptance among urologists and its indications are now well defined (1,2). What is still debated is the technique of embolization, and especially the choice of embolic material to be used. A variety of different embolic materials have been utilized including finely fragmented pieces of autologous muscle (3), particles of gelatin foam (1,4), microspheres (5), special devices such as the Gianturco coil (6), complex apparatus such as an electromagnet in ferromagnetic silicone vascular occlusion (7) and finally the tissue adhesives (2,8,9).

Table 1. TNM Categorization of 62 Renal Tumours treated by Preoperative Embolization at the Urological University Clinic of Genoa from 1975-1980.

T_1.... 0	V_0....39
T_2.... 8	V_1....16
T_3....41	V_2.... 7
T_4....13	

We have investigated, developed and perfected this last technique (2,8,9) and we report herein on the technical aspects of 62 cases of renal tumour embolization by cyanoacrylates performed at the Urological University Clinic of Genoa during the last five years (1975-1980). (Table 1)

USE OF TISSUE ADHESIVES

Tissue adhesives are acrylic acid derivatives which, liquid in a monomer form, rapidly polymerize, instantly solidifying as soon as they come in contact with blood or other biological or electrolytic fluids. Owing to their fluid nature they can be injected even through very small catheters and the embolus can be brought downstream in the arterial tree to the capillary bed, so producing a complete and distal embolization by forming a non-resorbable "cast" of the arterial tree. This offers undoubted advantages over other embolic materials such as gelatin foam, which is quickly reabsorbed, Gianturco's device, which only produces a proximal embolization and is difficult to place correctly in certain cases as for example when there is early bifurcation of the renal artery. In selected instances the use of a balloon catheter can prove very useful, but generally it is not necessary. The recourse to a complicated system of a detachable balloon to be used as an embolus (10) is still in the experimental phase and, in this type of embolization, does not seem to offer particular advantages.

Pitfalls in the use of Tissue Adhesives

The only disadvantage of the acrylic emboli is that they are not easy to handle and do not allow any mistake, so that a very experienced operator is needed. Previous practice in laboratory animals is highly advisable.

The Choice of the Catheter: Coaxial or Conventional System?

Initially we thought that a coaxial catheterisation system was necessary (9) for fear that the catheter's tip might remain trapped within the sticky thrombus and owing to the need to control the effect of embolization by a subsequent injection of contrast medium through the outer loading catheter, since the inner one was occluded by the polymerization of the acrylic fluid and the cyanoacrylates are radiolucent substances.

The former danger proved to be groundless, whilst the latter problem could have been overcome by a trick that made the substance radiopaque.

How is one to make IBC radiopaque? In order to make IBC radiopaque, so facilitating the control of the embolization procedure, Dotter and Rösch proposed the use of tantalum powder (11). However this substance is not easily available and is difficult to sterilise and we have proposed and adopted the addition to the IBC of an equal quantity of Lipiodol Ultrafluid® (2), which, being a non-ionic substance, does not cause IBC polymerization.

Technique of Embolization

It is extremely important to flush the catheter carefully with five per cent dextrose in water prior to embolization (2) in order to prevent polymerization within its lumen. After careful and prolonged flushing of the catheter, a 2.5 ml plastic syringe containing the embolic mixture - 1 ml of IBC plus 1 ml of Lipiodol - is connected through a two way plastic stopcock and the embolus is slowly and completely injected under fluoroscopic control.

Owing to the radiopacity of the embolus, the entire procedure can be very easily controlled. The IBC Lipiodol mixture is quickly transported by the blood flow downstream to the finest arterial branches, so producing a very distal embolization.

In order to eject completely the embolic material out of the catheter a further small amount of five per cent dextrose in water is injected, until the circulation appears completely stopped.

The catheter is then withdrawn, while the radiopaque cast persists unmodified and clearly evident within the renal artery (Figs. 1a,b,c). Operation is usually carried out 24 hours after embolization.

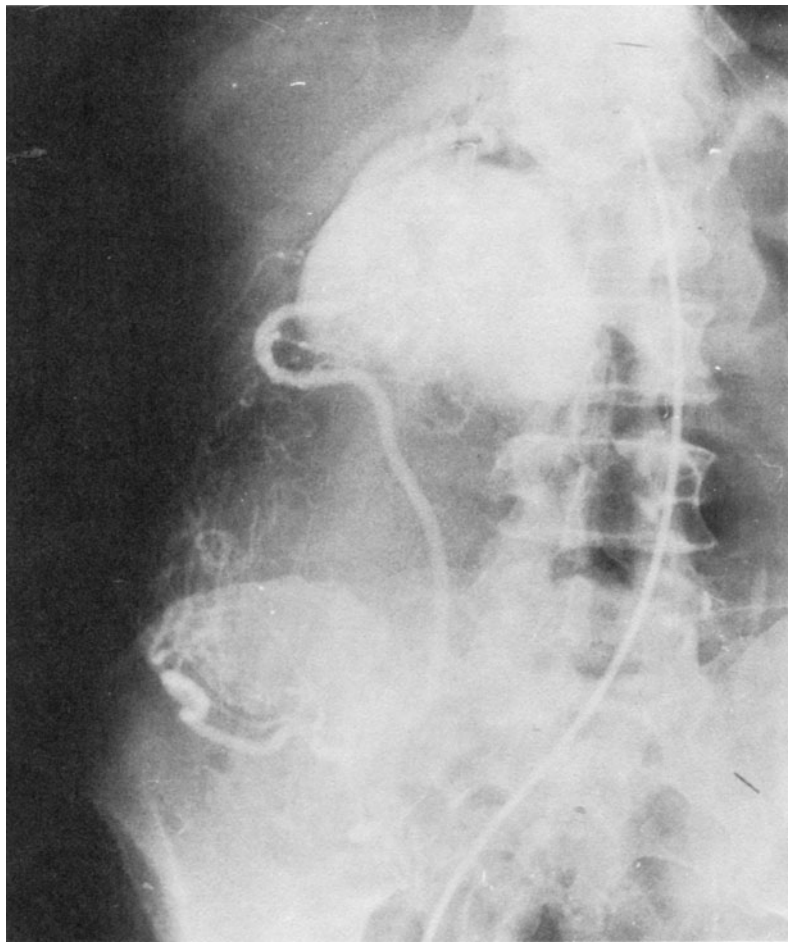

Fig. 1a. Angiography and embolization of a very large right renal tumour.

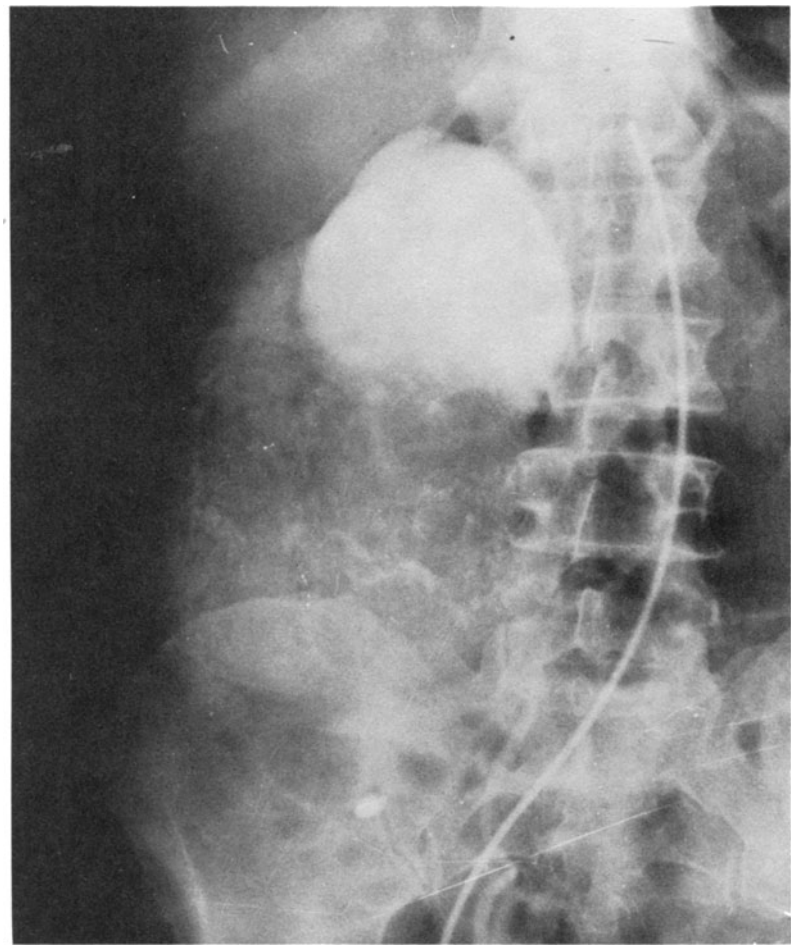

Fig. 1b. Parenchymal phase.

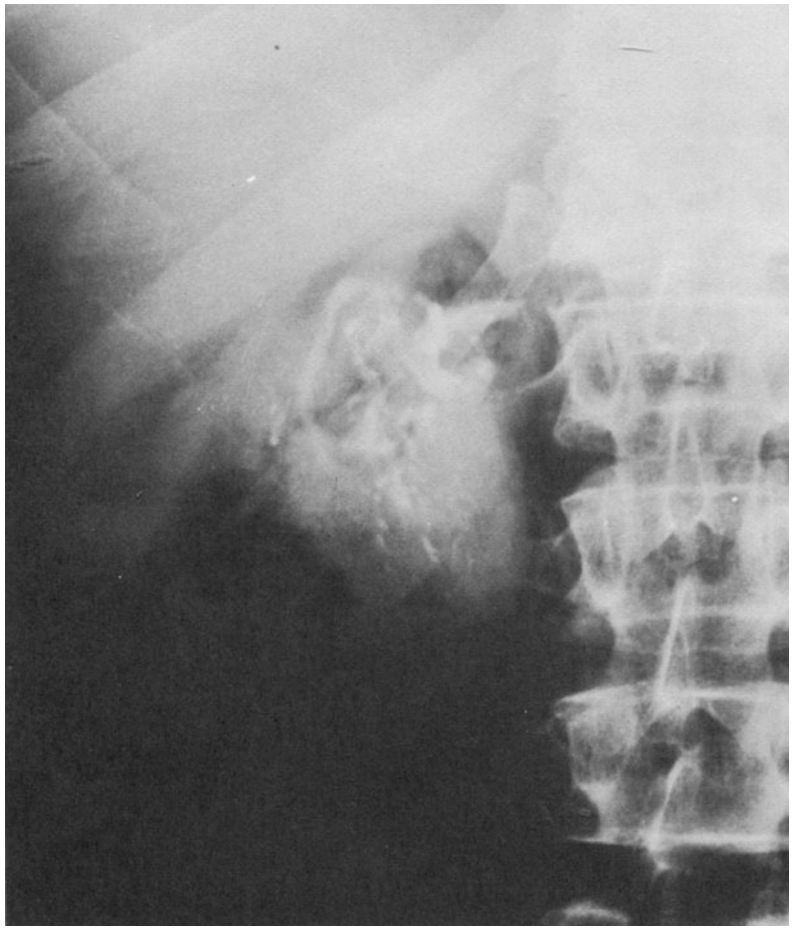

Fig. 1c. "Cast" embolization of the renal artery with Isobutyl-2-Cyanoacrylate.

COMPLICATIONS

The only complication we have seen is a certain degree of lumbar pain, which generally subsides following the administration of analgesics. Mild hyper-pyrexia was observed in 30 per cent of cases.

The search for new materials which give complete and lasting embolization is fully justified, because the "ideal" embolic material has not yet been found. In our opinion, and experience, IBC represents the best currently available embolic material for renal tumour embolization.

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HOW OFTEN IS EMBOLIZATION OF RENAL CANCER USEFUL NOWADAYS?

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Renal tumor embolization has many advantages which are summarized in Table 1. Embolization alone does not affect paraneoplastic manifestations (1) and any immunological advantages are still hypothetical. Embolization destroys normal kidney tissue but induces little or no histological change in the tumor itself.

Unfortunately embolization also has disadvantages:-

1. It is an additional procedure, especially since arteriography is no longer necessary for the diagnosis of kidney tumors.

2. One should embolize all arteries going to the kidney and since there may be several and also, because embolization to be effective must be as distal as possible to the main artery, it is sometimes a lengthy procedure.

3. Embolization lengthens the period of hospitalisation and adds to the costs.

4. It also has complications including pain, fever and ectopic embolization. Pain always occured in our cases, obliging us to keep patients under sedatives and to program operation 24 to 48 hours after embolization. This might disturb the organization of busy operating room schedules and we are now doing renal embolization under epidural anaesthesia, leaving the epidural catheter in place to maintain anaesthesia until the time of operation. Fever is also a common manifestation after embolization.

Ectopic embolization (in areas other than the diseased kidney) is sometimes a very serious complication. Even in the best hands

this complication still happens. In our experience it has occured in 3% of cases.

In arteriosclerotic patients (as most of them are) the manipulations may provoke endoarterial lesions. Embolization needs an experienced vascular radiologist if a prohibitive level of complications is to be avoided.

The only aim of embolization is to enable the surgeon more easily to remove the diseased kidney. In these circumstances any disadvantage detracts from the value of the technique. The benefit of embolization is marginal in T1 and T2 tumors, which account for approximately 40% of all our patients. We have therefore always limited our indications to cases where local difficulties at surgery are to be expected and until 1977 we selected for embolization patients who had one or more of the criteria listed in Table 2, which encompassed nearly 30% of all patients. Embolization was very often combined with the diagnostic arteriography.

With time, our enthusiasm for embolization has decreased. We now rely on CT scan for the final diagnosis of kidney tumors in 95% of cases and very rarely carry out arteriography for diagnostic reasons. We now believe that the usefulness of embolization is limited to certain patients with T3 and T4 tumors.

Our criteria of selection have thus changed in the last two years and, apart from very large tumors, a major lymph node involvement at CT scan or massive thrombosis of vena cava or renal vein, our indications are based on the combination of more than one of the conditions stated in Table 2. Our most common indication is the association of a large tumor with lymph node involvement or renal vein thrombosis.

Given this more selective attitude our indications for embolization dwindled down to 12% in our last 100 radical nephrectomies, as compared with 26 in a previous series of 100 cases.

This personal opinion might change with different local factors and is dependent upon the skill of the radiologist, the possibility of a rapid appointment for embolization, and whether one still depends on arteriography for diagnosis. However even in the most optimal conditions we do not believe that embolization is of any great value in more than 20% of all cases.

Embolization is sometimes indicated as a palliative measure when surgery is not practicable, e.g. in cases of intractable haematuria for unresectable tumors. In some such cases control of haematuria was satisfactory in five. In cases where patients had metastases no regression of these lesions was noticed.

Table 1. Advantages of Renal Tumor Embolization

1. Reduction of the size of the tumor.
2. Permits ligation of renal vein prior to ligation of artery.
3. Reduction of distension of capsular veins.
4. Decrease in operative blood loss.
5. Improves surgical dissection planes.

Table 2. Indications for Embolization

1. Large tumors.
2. Massive renal vein thrombosis and vena cava extension.
3. Enlarged nodes on CT scan.
4. Palliative procedure in non resectable tumors with intractable haematuria.

Embolization also has experimental indications. We are now working on the elaboration of microcapsules of antimitotic agents having a slow release effect, and we are doing animal experiments, trying to embolize the kidney and other organs with these microcapsules. The major drawback in kidney tumors is that we do not have a potent antimitotic agent at the moment and that the use of non potent agents intra-arterially is not likely, in our opinion, to change the outcome and the potency of the drug.

In some cases kidney tumors have been embolized by radioactive material. This obviously can only be a palliative procedure and we do not believe that any palliative effect can justify the potentially dangerous use of isotopes as a treatment modality. In addition, some researchers in California are embolizing kidney tumors with a ferrite compound and will use an external magnetic field to heat the kidney. They call this technique electro magnetic surgery and will report their results at the coming meeting on kidney tumors in Paris next November.

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THE EFFECT OF EMBOLISATION/NEPHRECTOMY FOR RENAL CARCINOMA ON THE PARANEOPLASTIC SYNDROMES

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INTRODUCTION

The presentation of renal carcinoma may be considered under four headings:-

1. A triad of loin pain, mass or haematuria. The components of this triad more commonly present separately; thus haematuria is the presenting feature in 62%, loin pain in 50% and a renal mass in 34% (1). It is unusual for all three features to occur together, and most series report the triad occurring in approximately only 10-15% of cases.

2. Metastatic disease. There is almost no organ in the body where a metastasis from renal carcinoma has not been found and in many of these it is the presenting feature of the tumour. A secondary tumour in a bone is the most common site but the diagnosis is not usually made until the biopsy is examined.

3. Autopsy findings. It is well recognised that a renal carcinoma may be an incidental finding at autopsy. This incidence is low, $<1\%$ of all autopsies (2). However, in a recent study by Hellsten (3) a detailed study of kidneys in an area with a very high autopsy rate showed a much higher incidence than previously reported.

4. Paraneoplastic syndromes. A very wide range of clinical and laboratory abnormalities have been described in association with renal tumours (4). The diversity of these abnormalities has led them to be described either as systemic effects or as tumour markers. A classification of these paraneoplastic syndrome is as follows:

1. Non-specific (toxic) syndromes

   - Haematological syndromes (e.g. anaemia)
   - Biochemical syndromes (e.g. abnormal liver function tests)
   - Metabolic syndromes (e.g. fever)
   - Immunological syndromes (e.g. neuromyopathy)

2. Specific endocrine (humoral) syndromes

   - Hypersecretion of a substance usually associated with the kidney (e.g. renin, erythropoietin)
   - Hypersecretion of substances not normally associated with the kidney (e.g. parathormone, gonadotrophins)

3. Miscellaneous syndromes

   - including mucin secretion and salt losing nephritis

The clinical importance of recognising these abnormalities is that they are often the early signs or symptoms of a renal carcinoma. It is well recognised that many of these apparently non-specific abnormalities can lead to extensive medical investigation before the link with a renal tumour is established. What is less well recognised is that the presence of these abnormalities does not necessarily mean that there is metastatic renal carcinoma. For example, the abnormalities of liver function, first described by Stauffer in 1961 (5), are almost always not due to metastases because they disappear following removal of the tumours. Using $\alpha 2$ globulin as an example, the abnormal level of this substance usually returns to normal after nephrectomy but can again rise with the development of a metastasis. On the other hand, if the $\alpha 2$ globulin level does not return to normal after nephrectomy this is good evidence of metastatic disease.

Thus some of the paraneoplastic syndromes can be useful not only for diagnostic purposes but also for monitoring the course of the disease.

The possibility that embolisation and/or nephrectomy may have specific immunological effects has been raised but not well substantiated (6,7,8). Assessment of anti renal carcinoma antibodies by microcomplement fixation assays has suggested that a high percenage of these patients mount an adequate antitumour response which falls only when the malignancy becomes overwhelming; persistently elevated titres are associated with the longest survival (9).

We have studied the effect of embolisation and/or nephrectomy on the paraneoplastic syndromes and extended the study to examine the effects especially on the immunoglobulins.

PATIENTS AND METHODS

Between 1978-80 20 patients (14 male, 6 female) presented with renal carcinoma. Half of these patients had metastatic disease at presentation. The presenting symptoms and signs were: Haematuria (6), haematuria + loin pain (5), haematuria, pain and mass (1), mass (2), tiredness (2), dysuria (1), polycythaemia (1), hypertension (1), bone pain (1).

All these patients were assessed by urography, chest x-ray, ultrasound, Technetium bone scan and renal arteriography. Laboratory measurements consisted of haemoglobin, white cell count, ESR, blood urea, lactic dehydrogenase, bilirubin, alkaline phosphatase, calcium, phosphate, total protein, globulins, and immunoglobulins.

The management policy for these patients consisted of renal arterial embolisation at the time of renal arteriography with nephrectomy, (3-5 days later), at which time the kidney was removed with its envelope but no formal node dissection was made. The decision to undertake nephrectomy in those patients with metastases at initial presentation depended upon the general state of the patient; this was an arbitrary decision and was influenced by the age of the patient and the activity level, thus two of the patients were treated by embolisation alone.

RESULTS

The initial assessment showed the following abnormalities:

Haematological

ESR	75%
Anaemia	50%

Liver function

Lactic dehydrogenase	35%
Alkaline phosphatase	20%

Bone

Calcium ↑	20%

Proteins

Albumen ↓		55%
Globulins	$\alpha 2\uparrow$	55%
	$\beta\uparrow$	60%
	$\gamma\uparrow$	20%
Immunoglobulins	IgM	40%

There was no difference in the number of abnormal measurements in patients with metastases and without metastases.

The initial data and the follow up of six patients who underwent embolisation and nephrectomy are detailed in Table 1; none received progestogen. In all six patients at least one of the "marker" measurements was abnormal. At three months, nearly all of these abnormalities had returned to normal. Two patients had a raised ESR, three had mildly raised globulin fractions and two had raised IgM levels. Three patients had a raised gamma globulin at three months (but not pre operatively).

At six months, the two patient with persistently raised IgM showed further abnormalities (Table 1). One patient developed a raised serum calcium, then paraesthesia of the right hand followed by a lytic lesion of the first rib. Another patient developed a raised alkaline phosphatase and ESR at six months: one year later she had developed liver metastases.

In the two patients treated by embolisation alone, the one with bony metastases developed a marker elevation of gamma globulin, IgG and Ig M three months after embolisation but these had returned to normal by six months. This patient remains alive after 15 months but developed a positive bone scan one year after embolisation. The other patient with soft tissue metastases showed no response to embolisation and was started on chemotherapy.

DISCUSSION

The role of the paraneoplastic syndromes in renal carcinoma has not yet been clarified. Despite the range of abnormalities, most of them are non specific and can occur in association with other malignancies and other chronic diseases. The specific humoral abnormalities are uncommon and their exact incidence is uncertain since it requires renal arterial, venous and tumour tissue concentrations to be certain of an abnormal production by the tumour of a hormone. It is possible that these abnormalities are more common than reported, but few centres are able to pursue these studies in the detail required to answer this question.

A raised sedimentation rate remains the most common abnormality (10,11) but this is often found in other malignancies and may be associated with other medical conditions. The abnormalities of liver function described by Stauffer (4) are common in renal tumours, but they can also occur in the non-malignant condition of tumefactive xanthogranulomatous pyelonephritis. A raised $\alpha 2$ globulin spike has been described as a characteristic association with renal carcinoma (12) and this is probably the most useful 'marker' for renal tumours; changes in the $\alpha 2$ globulin correlate well with the course of this tumour.

Table 1. Abnormal serum tumour markers pre and post embolisation and nephrectomy

Initial Abnormality*	At 3 months: Normal	Still Abnormal	Recent Abnormality	Outcome: Metastases	Outcome: No metastases
1. E.S.R	E.S.R	IgM	Ca^{++} γ globulin	Bone 8/12 Died 15/12	
2. IgM		IgM	6/12 alkaline phosphatase E.S.R.	Liver 18/12	
3. Haemoglobin E.S.R. Albumin↓ α2 & β globulin	Haemoglobin Albumin α2 globulin	E.S.R. β globulin			9/12
4. E.S.R. L.H. Calcium↓ β globulin IgM	L.H. Calcium	E.S.R. β globulin IgM	γ globulin IgA		12/12
5. Anaemia Calcium↓ Albumin↓ Bilirubin	Calcium Bilirubin	Albumin↓	γ globulin IgA		8/12
6. Anaemia E.S.R. Calcium α1 α2 β γ globulins IgA	Anaemia E.S.R. Calcium α1 α2 β γ globulins IgA		L.H.		6/12

* "Markers" represent increased concentration of the relevant substance except when specified (↓)

An extra renal triad that occurs commonly with renal tumours comprises fever, anaemia and hyperhaptoglobinemia (13). However many patients do not have all components of this triad so that its usefulness is limited.

In the present study we have looked for changes that might correlate with the treatment by embolisation/nephrectomy. There was no special pattern seen in these data as a group but the numbers are few and the follow up limited. However, each patient acts as his own control so that serial monitoring identifies marker changes. This study needs to be extended before a conclusion can be reached. In addition further studies of the paraneoplastic syndrome need to be undertaken so that a more complete picture of their role in renal carcinoma can be determined. A reliable tumour marker, or a pattern of markers is a great advantage in the diagnosis and treatment of an increasing number of tumours; the aim must be to define those paraneoplastic syndromes which can be of use in the management of renal carcinoma.

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RADIOTHERAPY OF RENAL CANCER: PRINCIPLES AND INDICATIONS

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INTRODUCTION

This paper will deal with the question of the usefulness of radiotherapy in renal adenocarcinoma (hypernephroma), and will not be concerned with renal sarcoma or with Wilms' tumor. There is a lack of definitive knowledge about the role of radiation therapy in the treatment of renal cell carcinoma (1-4). There are several reasons for this:

(1) This uncommon tumor accounts for only 2% of all malignant tumors in man.
(2) Definitive studies have been few, and the design of many which have been done was compromised by the low incidence of the disease.
(3) Pretreatment staging procedures have not been perfected, further hampering the design of careful research studies, and making comparison of results from different studies difficult.
(4) The liver, the gastrointestinal tract, and the spinal cord, because of their radiosensitivity and their close anatomical relationship to the kidney, present barriers to aggressive pre- or postoperative radiotherapy.
(5) Renal adenocarcinomas are considered to be relatively radioresistant.
(6) Diagnosis is established late in the disease, partially due to the inaccessible anatomical location of the kidney, and partially due to the lack of cardinal symptoms, such as pain, swelling, or other obvious manifestations.

Earlier reports of the use of radiotherapy (whether pre- or postoperative) are conflicting (5-9). The small number of

TABLE I

Comparison: Preoperative Irradiation + Surgery vs. Surgery alone in the Treatment of Renal Cell Carcinoma Without Metastases at Time of Treatment.

Major Study Location: Title	Author	Date of last report	No. Pts. randomized (evaluable)	Radiation dose	Differences in 5 year survival
U.S.: The Genito-urinary Group	Cox, C.*	1981	150	4500	No differences.
Holland: Rotterdam Carcinoma of Kidney Trial, 1965	Van der Werf-Messing, B.	1973	141	3000	No differences (but decrease in renal fossa recurrence & in distant mets in P3 patients).
Finland: Helsinki University Central Hospital Clinical Trial, 1968.	Juusela, H.	1977	88	3300	No differences.

* Personal communication

patients and lack of consistent staging, or of controls, make these earlier reports difficult to evaluate.

PREOPERATIVE RADIATION AS A SURGICAL ADJUVANT

In the 'sixties and early 'seventies several more stringent clinical trials were carried out. Three studies, all of which were designed to compare radical surgery plus preoperative radiation versus radical surgery alone, have been completed in the last decade (see Table I) (10-13). All three were done on patients with no evidence of local or distant metastases at the time of treatment. Comparable numbers of patients were studied, and radiation doses ranged from 3000 to 4500 rads.

These recent studies were well designed in that three important criteria were met. The patients were studied prospectively, they were properly randomized into the two treatment groups, and they were not lost to follow-up.

Results of Prospective Studies: Overall, actuarial survival at five years was not affected by preoperative radiotherapy in any of the studies. The usual pattern of decreasing survival with increasing P category was observed (Table II). The P1 survival rate at 5 years was generally very high, around 90%, followed closely by the P2 group, who, at 5 years, were at the 75% level. In the P3 group, a much poorer result, about 25% survival, was typical. These five year survival figures agree with most studies of this tumor after surgery alone.

TABLE II

Definition of stages of renal cell carcinoma by P-categories of the U.I.C.C. 1978*

P CATEGORY	DEFINITION
P_1	Tumor confined within the kidney
P_2	Extension of tumor beyond the kidney
P_3	Tumor involves vascular or lymphatic structures

* U.I.C.C. 1978 TMN Classification of Malignant Tumours, Geneva.

The early postoperative course of the disease was however affected by radiotherapy, according to van der Werf-Messing (13). In the P3 group of that study, residual tumor occurred frequently in the surgery alone group and there was clearly improved survival during the first 18 months postoperatively in the irradiated patients who had metastatic disease discovered postoperatively. Of 33 patients with such metastatic disease, 100% of those irradiated versus 62% of those who had surgery only were alive at 12 months post treatment. At 18 months post-treatment the figures were 78% survival for the combined treatment versus 28% for those with surgery alone. The explanation for this was thought to be that the follow-up procedures in the radiotherapy unit were better at identifying metastases earlier than in the surgical clinic, where those treated by surgery alone were followed.

POSTOPERATIVE RADIATION

The literature on the use of postoperative irradiation illustrates that it has been less well studied. Tumors which are found to extend beyond the kidney (Stage II or P2), or which extended to the regional lymph nodes (Stage III or P3), can be presumed to have residual microscopic disease after surgical removal of the diseased kidney. There are numerous reports of the use of radiation in such cases (9,14-17), but most of these are hampered by the lack of controls. Overall, it appears that radiation is best used postoperatively to treat residual disease in the renal fossa or for selected attack on solitary metastases.

CONCLUSIONS

Overall, the data on the outcome of radiation therapy in renal cell carcinoma are too few to warrant strong recommendations. Surgical treatment is still recommended as the primary treatment (1,2).

Radiation may be indicated (a) for residual disease after surgical resection and (b) for selected solitary metastases found postoperatively.

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COMBINED CHEMOTHERAPY AND IRRADIATION OF PULMONARY METASTASES IN PATIENTS WITH RENAL ADENOCARCINOMA

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INTRODUCTION

In the records of patients treated for renal adenocarcinoma and followed at the Radiumhemmet in Stockholm since 1940, no cases of spontaneous regression of metastases have been observed, either in the lungs or elsewhere. All patients with metastases died of their malignancies. During one period in the 1960s, hormone therapy, mainly provera in high doses, was given to patients with metastases from their primary kidney tumor. A short term objective regression was observed in 1.7% of patients.

In 1975 a chemotherapy pilot study was started in Stockholm in which patients in whom progressive pulmonary metastases appeared following primary nephrectomy for renal adenocarcinoma were treated with vincristine, bleomycin and irradiation.

All patients had bilateral, multiple metastases in the lungs which had been shown to be progressing on two chest X-rays taken prior to therapy. In some patients fine needle aspiration biopsy had been carried out to verify the diagnosis.

METHOD

Combined therapy with vincristine, bleomycin and irradiation was given twice weekly. Vincristine 0.5 mg IV was administered, followed six hours later by bleomycin 15 mg i.m.

Irradiation was delivered by a conventional 200 kV X-ray unit with a central dose in both lungs of 1000 rad (10 Gy) given in one month five days weekly; when the patient received the chemotherapy

irradiation was given one hour after the bleomycin.

Chest X-rays have been performed every one to two months after the completion of therapy.

RESULTS

Of twenty patients treated, partial remission of the metastases has been observed in twelve and a stabilization of the lesions in seven. In one patient there was no effect observed. The mean duration of partial remission and stabilization was 5 months (1 - 24 months).

The subjective response in terms of pain relief and improved well-being was good and the side effects of the combined therapy were negligible.

CONCLUSION

Short term objective remission of pulmonary metastases have been seen following combined treatment with vincristine, bleomycin and irradiation in 12 of 20 patients treated. Subjective improvement also occurred.

On the basis of this investigation a randomized study has started, to compare the above-mentioned combined schedule with inferferon given in daily doses of 8 million international units.

Editorial Comment (P.H.S.)

A 60% partial response rate following chemotherapy and radiotherapy of pulmonary metastases in an institute in which spontaneous regression of metastases has not been observed for forty years is truly remarkable and deserves to be repeated, preferably with further evidence of the details of the subjective responses seen.

PREOPERATIVE IRRADIATION AND NEPHRECTOMY IN POORLY DIFFERENTIATED CARCINOMA OF THE KIDNEY (A PRELIMINARY STUDY)

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INTRODUCTION

In the late 1960's we started to investigate whether post-nephrectomy survival in renal carcinoma is improved by preoperative irradiation. It was intended to study especially those patients with poorly differentiated cancer. However, a few cases of moderately or even well differentiated cancer were included in the investigation.

METHODS AND MATERIAL

Malignancy Grading

Fine needle aspiration of the tumor was performed after the diagnosis had been established by urography and nephroangiography. The puncture was done under television - monitored fluoroscopy and with the patient in the prone position. The kidney was located by intravenous urography.

Irradiation

The irradiation was given by a 6 MeV linear accelerator with a three-field technique to give an average irradiation dose to the kidney tumour of 3,500 rad, in about 35 days. Extrafascial nephrectomy was carried out 3 - 4 weeks after cessation of irradiation. No extensive lymphadenectomy was done.

Clinical Material

This investigation comprises 34 patients with carcinoma of the kidney operated on in the period 1968 - 1971. The cytologic smears

were reviewed by one cytologist (S.F.). In 24 cases the cancer was initially evaluated as poorly differentiated, in 9 as moderately well and in one case as well differentiated.

RESULTS

In 11 cases judged as poorly differentiated carcinoma before irradiation, only moderately well or well differentiated carcinoma was found in the operative specimen. In one case diagnosed as moderately well differentiated cancer before irradiation only well differentiated tumour was found in the operative specimen. In another case of moderately well differentiated cancer in the biopsy, poorly differentiated tumour was found in the specimen. In the remaining cases there was either the same malignancy grade in the biopsy and operative specimen, or incomplete data were available (9 cases).

There was no operative mortality in this series. The postoperative survival of the 24 patients with poorly differentiated carcinoma is presented in Figure 1. The postoperative survival of 103 patients with poorly differentiated carcinoma, subjected to nephrectomy in the same hospital without previous irradiation and previously reported by Arner, Blanck and von Schreeb (1) is demonstrated for comparison. In the latter series, operative deaths are excluded from the calculations.

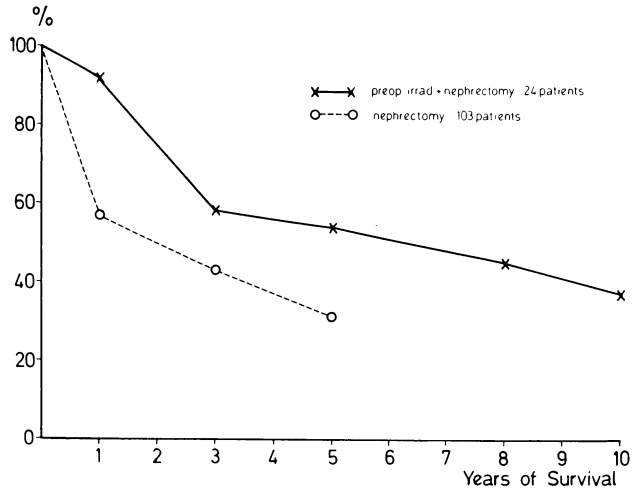

Fig. 1. Postnephrectomy survival in patients with poorly differentiated renal cell carcinoma after preoperative irradiation, compared with survival in patients treated by surgery alone.

Average five year survival in the patients given preoperative irradiation was 54%, in the other group 31%.

The group of moderately well differentiated carcinoma was too small, only 9 cases, to allow any definite conclusions. With this reservation in mind, it may be mentioned that survival in this small group was approximately the same as survival rate in a series of 55 non-irradiated cases of moderately well differentiated carcinoma reported by Arner, Blanck and von Schreeb (1). Five year survival in the patients given irradiation was 67%; in the other group 63%.

With the technique used here there was no damage to the contralateral kidney but some patients with right-sided tumours had a slight to moderate, but temporary, disturbance of liver function following irradiation.

DISCUSSION

It is noteworthy that in 12 cases the kidney tumour had a lower malignancy grade at nephrectomy a month after irradiation than on the preirradiation biopsy. Whilst allowance must be made for certain personal variations in the evaluation of cytologic and histopathologic patterns, a previous investigation (2) has shown, however, that with the technique used in this study there is good conformity between the percutaneous aspiration biopsy and the histopathologic findings of the nephrectomy specimen. It would therefore appear that in certain cases the preoperative irradiation may eradicate cell populations of high malignancy grade.

From this small series it would also appear that in cases of poorly differentiated renal carcinoma, preoperative irradiation may improve postoperative survival. Investigation of a larger patient series is in progress.

SUMMARY

Thirty-four patients with carcinoma of the kidney were treated by preoperative irradiation (3,500 rad) followed by nephrectomy 3 - 4 weeks later. In some cases the malignancy grade of the operative specimen was lower than on puncture biopsy of the tumour before irradiation, indicating possible eradication of highly malignant cell populations. The postoperative survival in cases of poorly differentiated tumour cases was higher than in a previous series of patients operated on without preoperative irradiation. In a small number of patients with moderately well differentiated carcinoma there was no difference between patients given preoperative irradiation and those treated by surgery alone.

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Editorial Note (M.P.)

This paper by Professor Andersson and his associates is closely related to another work by the same group, published in this book under the title of "Aspiration biopsy and cytology of renal cancer".

These stimulating articles raise some questions. The indication for a fine needle aspiration-biopsy of renal cancer lies in the fact that the finding of a high grade tumor should favour the decision of performing preoperative irradiation prior to nephrectomy. The validity of this approach will become clear if some doubts can be solved. In the first place, the accuracy of a grading solely based on cytology from fine needle aspirates does not yet appear to be established with certainty. As the authors point out from their own personal data, which stem from the work of very experienced cytologists, the correlation between cytological and histological grading is far from being satisfactory. Agreement was obtained only in 36 of 47 cases, i.e. there was disagreement in 23.4% of all cases. The disparity was sometimes of one, but occasionally even of two grades. Cytology tended to produce an "overgrading", as shown by the fact that only four cases were classified as "high grade" by histology, whereas eight cases were assigned to the high grade category by cytology. As the grading was determined by the highest malignancy grade found in both the histological specimen and in the cytology smear, it is obvious that a larger number of cells can be visualised by histology, so that even small foci of more atypical cells can be identified. This seems to amplify the risk for "overgrading" by cytological technique in this particular tumour. This conclusion is supported by the fact that the percentage of high grade tumors in the present series based on cytology (70.6%) is much higher than that reported in other reports (see Tannenbaum and Tannenbaum in this volume: only 18% of G3 renal adenocarcinomas). If these considerations are correct, then the finding of a lower malignancy grade at histology of the nephrectomy specimen as compared to the higher grade on cytology of the pre-nephrectomy aspirate can hardly be considered as a criterion for response and the suggestion that the preoperative irradiation may eradicate populations of high malignancy grade remains hypothetical. On the other hand the fact that survival was better in this series of patients treated with

preoperative irradiation followed by nephrectomy than in a previous series of patients operated upon without irradiation, is not a definitive argument in favour of preoperative irradiation, but suffers from the weakness inherent in all comparisons based on historical controls. Therefore, neither the need for needle aspiration biopsy nor for preoperative irradiation is based, in my opinion, on unshakable evidence. However the idea is very attractive and further studies are certainly warranted. Their results will be awaited with the greatest interest.

It should be emphasized that no untoward effects were produced by percutaneous puncture of renal tumors, contrary to a widespread fear. This is certainly a very important observation.

IMMUNOTHERAPY FOR RENAL CARCINOMA

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Adenocarcinoma of the kidney may be uniquely suited for attempts at immunotherapy. The natural history of this neoplasm is unusual because these patients may have long periods of time between excision of the primary tumor and development of metastases. We have observed patients in whom the primary tumor existed for 15 to 20 years before treatment was initiated. Following removal of the primary tumor, metastases rapidly appeared. Temporary regression of metastases and long term stabilization of tumors has been described. These findings suggest the presence of immune response which is able to control the development of the tumor.

Considerable evidence has been amassed to suggest the presence of cellular and immune response in patients with renal cell carcinoma (1). These observations, along with the usual clinical behavior of the tumor which suggest an important role of host immunity, prompted a variety of immunotherapy trials.

The first efforts at passive immunotherapy of renal carcinoma were in the form of serotherapy. Horn et al (2) infused plasma from a patient previously cured of renal carcinoma into a family member with diffuse metastases. The patient remained clinically free of tumor for 20 months, after which he died of cerebral metastases. Another patient with extensive pulmonary metastases received plasma infusion from a family member who previously had undergone curative nephrectomy for a renal carcinoma. One pulmonary metastasis completely resolved and two others remained stable. The serum of the treated patients showed significant levels of serum blocking activity prior to immunotherapy, but were demonstrated to have unblocking activity during periods of regression or stabilization of tumor. The serum donors also appeared to have specific unblocking

activity (3). These preliminary attempts at serotherapy have not been expanded into formal clinical trials. Adoptive immunotherapy in the form of transfer factor and immune RNA have been used more extensively than any other form of immunotherapy for renal carcinoma. Montie et al reported early results in a few patients with metastatic renal carcinoma treated with transfer factor (4). Although they reported some objective responses, which included stabilization of measurable lesions, the number of patients was too small to make a true assessment of the value of this agent.

In a subsequent report from the same institution, transfer factor was combined with other modalities (5). These were not randomized trials but three different regimens were employed at different times. Nine patients received transfer factor alone, of which one had a complete response. There were no partial responders. A second group received transfer factor plus Tice strain BCG by scarification. Two of the eight patients treated had partial regression of measurable metastases. A third group received transfer factor plus CCNU plus Megace. Of the 14 so treated, one had a complete response and four had partial responses. Toxicity was apparently acceptable. Before attributing the regressions to the therapy, it will be important to perform randomized clinical trials. Furthermore, the duration of response and survival of treated patients must be compared either to untreated controls or to historically matched controls from the same institution. Nonetheless, the incidence of complete response (two out of 31) and partial responses (six out of 31) is higher than would be expected from that due to inherent natural history characteristics. It is also interesting to note that in this study and in those by Schapira et al (6) and Tykka et al (7) most patients who responded to therapy were those with pulmonary metastases.

DeKernion described experiments using immune RNA in the treatment of renal cell carcinoma. Initially, lysis of human renal carcinoma tumor cells by allogeneic peripheral blood lymphocytes which were stimulated with immune RNA were reported (8). Subsequently, 20 patients with metastatic renal cell carcinoma and nine patients with minimum residual disease who had a high risk for recurrence following nephrectomy received weekly intradermal injections of 4 mg. of immune RNA. The RNA was extracted from lymphoid organs of sheep which had been immunized with human cell carcinoma. Toxicity of therapy was minimal and consisted of erythema and a flu-like syndrome in six patients. Total doses of over 700 mg. and single doses of 64 mg. did not produce severe toxicity. Complete tumor response was not observed in any patients with metastases. Seven patients had a partial response which was defined as less than 50% regression of stability of measurable, previously growing tumors for over three months (9).

The most commonly used agents have been non-specific immuno-

stimulants, especially BCG. We have treated 12 patients with far-advanced renal cell carcinoma and compared survival to 10 patients who did not receive immunotherapy (10). Although survival of the treated patients was somewhat increased, the difference was not significant. Eidinger and Morales treated eight patients with metastatic renal carcinoma with BCG (11). They reported objective improvement in five of eight patients, although the criteria for improvement included stabilization of growth of metastatic foci in one patient. Also, all of these responses were transient in this non-randomized study and the mean duration of follow-up was brief.

Lange treated patients with metastatic renal carcinoma by multiple intradermal BCG injections after removing the bulk of the tumor (12). Tumor stabilization or regression occurred temporarily in only 15% of patients, and they concluded that survival was not increased by this method of surgery plus immunotherapy. Minton et al reported clinical responses in four of nine patients with metastatic renal carcinoma to the lungs treated with intradermal BCG (13). Antibody-dependent cell cytotoxicity was correlated with clinical control of the tumor. Sera from the responders induced lysis of old tuberculin-treated red blood cells by normal human peripheral lymphocytes, whereas sera from non-responders did not.

Tykka et al treated 31 patients with metastatic renal cell carcinoma with active specific immunotherapy after palliative nephrectomy (7). A soluble fraction of autologous tumor prepared by hypotonic cell lysis was polymerised into small particles by addition of ethylchlorformiate. The polymerised tumor was injected intradermally with PPD tuberculin or candida antigen as adjuvants. Survival of treated patients was improved over that of controls, although only 20% of treated patients were alive at five years. The major effect was noted in patients with pulmonary metastases, which disappeared in six of 16 treated patients.

Although these results appear to be encouraging, it is unclear whether patients were prospectively randomized or selected for reasons such as unavailability of tumor or other criteria. Some patients had only microscopic tumor, others had solitary lesions which were excised, and some had no measurable disease. Although control and treatment groups were similar with respect to stage of disease, it is not stated whether they were matched with respect to site and number of metastases and the use of polymerised tumor. Anti-tumor antibodies could not be detected in the sera of treated patients and no difference in migration inhibition assay was noted between treated patients and controls. It is therefore not possible to conclude from these data that this form of immunotherapy is effective. However, results are sufficiently better than those expected for patients with this stage of renal carcinoma to warrant a prospective randomized trial.

Several reports of active immunotherapy with allogeneic lymphocytes (14) and microsomal renal carcinoma cell fractions (15) were reported to show possible efficacy in renal carcinoma, but have not been expanded into clinical trials from which definite conclusions could be derived.

DeKernion from UCLA has begun a study in cooperation with investigators from Roswell Park and the University of Rochester. The Method of immunotherapy proposed in this study is based on the combination of a tumor cell vaccine with a non-specific adjuvant. One of the classic immunologic techniques for priming the immune system is to inject intradermally, the foreign antigen bathed in an adjuvant such as Freund's adjuvant. This is particularly useful when the antigen itself is poorly immunogenic. The adjuvant concept was applied to animal neoplasia beginning in the early 1970's. When poorly immunogenic animal tumors are prepared as a cell suspension in medium containing an adjuvant and injected intradermally, a systemic, long-lasting antitumor effect can consistently be produced. Bartlett and Zbar demonstrated that BCG could be effectively used as an adjuvant in their model of guinea pig hepatoma (16). Scott demonstrated that C. parvum could be used as the adjuvant in a mouse mastocytoma system (17). When tumor cells and a bacterial adjuvant are injected, two distinct categories of an immune response occur: a local inflammatory response and a systemic tumor-rejecting specific immunity which is cell-mediated.

C. parvum was selected as the adjuvant for this trial because of the possibility of decreased toxicity in human trials. Based upon animal studies, variables have been observed which determine success or failure of specific immunotherapy with bacterial adjuvants. These are: a) proper ratio of C. parvum to tumor cells: b) serial immunizations rather than a single dose: c) adequate quantities of tumor cells in the immunizing doses: d) an effective method of cryopreserving tumor tissues. Others have demonstrated that this immunizing procedure is highly specific for the individual tumor and required autologous (syngeneic) rather than allogeneic tumor cells (18).

The phase II study in metastatic renal carcinoma included 16 patients (19). Objective regression of metastases occurred in five patients and a sixth had symptomatic improvement and no progression of tumor for greater than 27 months. Six patients survived 20 months or greater which is better than one would expect in that group of patients.

The major shortcoming of this modality is the necessity of obtaining autologous tumor cells. In view of the current practice of palliative nephrectomy in patients with metastases, removal of the primary tumor is a reasonable approach. In addition to the expectation for palliation and perhaps temporary cessation of growth

of metastases, further justification is cited for the prospect of offering the patient an immunotherapeutic modality.

Allogeneic cells do not seem to be appropriate in such a study. Animal data suggests significant antigenic disparities among various tumors of similar cell types, in spite of the reported evidence of the presence of shared tumor associated antigens. In addition, the potential for toxicity, including hepatitis, is increased with the use of allogeneic cells.

Brown and co-workers have recently reported their experience using a tumor vaccine consisting of a single cell suspension of tumor cells prepared by mechanical disaggregation and complexed with dimethyldiotadecyl ammonium bromide (DDA) (20). DDA is a potent macrophage activator and depending on the techniques of administration gives some selectivity in promoting delayed hypersensitivity or humoral responses.

Twenty-seven patients with metastatic carcinoma were treated with radical nephrectomy followed by specific immunotherapy. They received monthly injections of a vaccine which was placed intradermally near regional lymph nodes. A vaccine dose of 25 mg. of autologous or allogeneic tumor protein complexed with two micromoles of DDA was used.

The longest follow-up was 131 weeks with a median follow-up of 41 weeks. Seven patients have died of their disease. Three patients have developed complete remission but the duration of this remission is not reported. Utilizing life table analysis, Brown et al reported a one year survival of 69% with a predicted median survival of 131 weeks. This group of patients was compared to two groups of historical controls. Thirty-six patients who received nephrectomy alone had a one year survival of 48% and a median survival of 36 weeks. The second group received no surgery and only palliative therapy. Their one year survival was 32% with a median survival of 18 weeks.

Neidhart et al report their results in 24 patients with metastatic renal carcinoma who were treated with a tumor vaccine similar to that described by Tykka et al (21). Aggregated soluble tumor antigens were admixed with tuberculin or phytohemagglutinin. Initially the patients own tumor was used to prepare the antigen but later allogeneic cells were used. In contrast to Tykka's study, scarification with BCG was added to ensure maximum reactivity to tuberculin.

Two patients demonstrated a complete response to therapy and were tumor free after four and 56 weeks. Two patients had a partial response at 12 and 32 weeks and 11 patients had stable disease at

14-111 weeks. These responses are similar but not quite as good as those of Tykka.

All of these various immunotherapy studies in patients with metastatic disease indicate that there are patients who may have a beneficial response to selective immunotherapy. The results are as good as, and in some cases better than, current chemotherapy.

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CHEMOTHERAPY OF ADVANCED RENAL CELL CARCINOMA: RESULTS OF TREATMENT WITH METHYL-GLYOXAL BIS-GUANYLHYDRAZONE (methyl-GAG): AN EORTC STUDY

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INTRODUCTION

The range of drugs which have been thoroughly tested in advanced renal cancer is still limited. Few oncologists have been able to accumulate sufficient experience of the treatment of adequate numbers of patients. Results of single agent therapy have recently been reviewed by Bodey (1) and de Kernion (2). It is uncertain whether any agent yet tested can be regarded as effective but there is some evidence to suggest that vinblastine may be, Hrushesky and Murphy (3) reporting a 25% objective response rate - including three complete remissions of up to four years duration - in a series of 135 patients. Other reports on the activity of vinblastine have been less encouraging (2). In recent studies, methodichlorophen has been shown to be inactive (4) as has 4-epi-adriamycin (5). A variety of chemotherapeutic combinations have been investigated with disappointing results. A combination of adriamycin, vincristine and medroxyprogesterone acetate with BCG was reported as giving a 33% overall response (6) and vinblastine plus CCNU a 24% response (7). There is no evidence that any combination studied is superior to vinblastine when used as a single agent. It is clear that there is a continuing urgent need to evaluate untested, especially new, chemotherapeutic agents in renal cell carcinoma. It may then be possible to study the most promising agents in combined schedules. Further investigation of the vinca alkaloids, in particular, the newer analogue vindesine, seems indicated. Study of combinations with other agents found to have significant activity would then be a logical next step.

Methyl-GAG was first synthesised in 1958 and was used in the

treatment of the leukaemias in the early 1960's (8,9). Toxicity with daily administration was considerable but recently, weekly administration schedules have been shown to cause much less toxicity and to produce responses in a variety of solid tumours, including kidney cancer (10-14). The drug has an anti-proliferative effect on cancer cells which probably reflects two principal properties (15, 16): selective binding to mitochondria with resulting structural and functional damage; and inhibition of polyamine synthesis, notably the inhibition and depletion of spermidine.

It was against this background that the EORTC Urological Group formulated a protocol for a phase II study of the anti-tumour effect of methyl-GAG in patients with renal cell carcinoma with measurable metastases not amenable to surgery. A secondary objective was to assess the morbidity of the treatment.

MATERIAL AND METHODS

It was required that the patients should be under 75 years of age with a Karnofsky index $> 60\%$. Concurrent, but not previous, treatment excluded, as did the existence of brain metastases and evidence of significant bone marrow depression or renal failure. Measurable metastases of histologically or cytologically proven renal cell carcinoma were necessary.

Methyl-GAG (supplied to the EORTC for the study by Riom Laboratories, France) was administered weekly in a dose of 500 mg/m^2 by intravenous infusion over 30 minutes. Dose modifications were not allowed but delay of up to 3 weeks was permissable in the event of bone marrow depression (wbc $< 3000/mm^3$) or renal dysfunction (serum creatinine $>$ 1.5 mg/100 ml.).

Physical examination, whole blood count, biochemistry profile and roentgenographic examinations were done before the start of therapy and at regular intervals during treatment. Full description and measurement of indicator lesions were recorded. Investigations necessary for the assessment of indicator lesions were repeated as appropriate but were required in all cases after seven treatment cycles.

WHO criteria (17) were applied with complete remission necessitating disappearance of all lesions and partial remission a 50% or more decrease in the product of the two largest perpendicular diameters of all measurable lesions. Complete and partial remissions had to be maintained for no less than four weeks with no new lesions appearing. Stable disease and progression were the other two recognised categories. Arbitrarily patients were considered evaluable for response if they had received a minimum of four treatment cycles with methyl-GAG.

RESULTS

Forty-five patients were entered into the study. Only three of these were completely non-evaluable because of lack of data or protocol violation. Thirty patients had received four or more treatment cycles, and were evaluable for response. Twelve patients were only partially evaluable, i.e. for assessment of toxicity.

Of the 30 fully evaluable patients, three achieved partial remission, 11 showed no significant change and in 16 the disease progressed. No complete remissions were observed (Table 1). The duration of the partial remissions in no case exceeded eight weeks.

The nature and severity of the toxicity encountered in 42 patients are presented in Table 2. The commónest side effects were anorexia, nausea and vomiting (43%), moderate or severe in 19% of all patients. Neuropathy, myopathy and myalgia also proved troublesome in some patients and was encountered in 21% overall (moderate or severe in 14% of all patients). Mucositis was moderate or severe in 7%. Six patients had to be taken off treatment because of polyneuropathy (two), mucositis (one), arthralgia (one), nausea, vomiting and diarrhoea (one) and vasculitis (one). Evidence of bone marrow depression was unusual and leucopenia necessitated delay in treatment in only three patients.

DISCUSSION

The results of this EORTC study add to information which is accumulating as to the effects of methyl-GAG in advanced renal cancer. The data from SWOG furnished by Knight et al (10-12) indicated significant responses to methyl-GAG in a weekly dose of 500 mg/m^2 with 100 mg/m^2 dose escalation per treatment cycle, occuring both within four and after four treatment cycles. One complete and two partial remissions were seen in 23 patients. Todd et al (13), who also gave 500 mg/m^2 weekly but with 50 mg/m^2 escalation, reported one complete and three partial remissions among 18 patients. All these remissions occurred within four weeks. However, in a more recent study, Zeffren et al (18) observed no remissions in 30 patients, in whom toxicity was apparently severe - including muscle weakness, lethargy, myalgia, mucositis and skin rashes. In the study reported here, where a fixed

Table 1. Results of Methyl-GAG in Metastatic Renal Cell Cancer

Patients Evaluable/ Patients Entered	Partial Remission	Stable Disease	Progression
30/45	3	11	16

Table 2. Toxicity of Methyl-GAG 500 mg/m^2/wk. (42 patients)

Side Effects	Number of patients		
	Mild	Moderate	Severe
Anorexia, nausea, vomiting	10	5	3
Neuropathy, myopathy, myalgia	3	4	2
Mucositis	3	2	1
Skin reactions	2	2	1
Leucopenia	2	2	0
Diarrhoea	2	1	1

dose was adopted, three partial remissions have been recorded among the 30 patients receiving at least four treatment cycles. The toxicity observed was moderate to severe in some patients, necessitating cessation of treatment in six patients. Knight et al (11) and Todd et al (13) encountered rather more side effects presumably because of their dose escalation schedule.

Currently, there is good evidence that methyl-GAG has limited activity of short-term duration in renal cell cancer. However, the degree of toxicity encountered with the use of this drug may well preclude further study.

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4'-EPI-ADRIAMYCIN IN METASTATIC RENAL CANCER

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INTRODUCTION

4'-epi-Adriamycin (4'-epi-ADR) is a stereoisomer of Adriamycin Preliminary observations have indicated cytostatic activity of 4'-epi-ADR in metastatic renal cancer (1). A phase II study was therefore performed in order to define the efficacy of 4'-epi-ADR in this malignancy.

MATERIAL AND METHODS

During a nine month period 21 consecutive patients with histologically proven metastatic renal cancer were treated with 4'-epi-ADR (75 mg/m^2 intravenously as a push injection, together with a 500 ml normal saline infusion, repeated every third week). All patients had at least one bidimensionally measurable tumour manifestation for monitoring the response to 4'-epi-ADR treatment. Details concerning the patients are given in Table 1.

After at least two treatment courses efficacy has been evaluated using the WHO criteria for response (2). If progression occurred, 4'-epi-ADR was discontinued after the second course. In case of stable disease after two treatment courses the patients received an additional (third) injection of 4'-epi- ADR. If no tumour regression was observed in these patients after three courses, 4'-epi-ADR was discontinued in most of the patients.

The toxicity of 4'-epi-ADR treatment was evaluated using the WHO recommendations (2). Hemoglobin, leukocytes, thrombocytes,

Table 1. Details of 21 Patients with Metastatic Renal Cancer Treated with 4'-epi-Adriamycin

Total No. of Patients			21
Age (years)		Mean:	60.1
		Range:	40-70
Karnofsky Index		Mean:	84.5
		Range:	60-100
Interval between initial diagnosis and demonstration of metastases (months)		Mean:	15.7
		Range:	0-76
Interval between initial diagnosis and start of 4'-epi-ADR treatment (months)		Mean:	23.0
		Range:	1-92
No. of nephrectomized patients			18
No. of patients with previous cytostatic treatment			8
	CCNU-Vinblastine		4
	Methyl-GAG*		3
	Ifosfamide		1
Localization of indicator lesions:	Lung		12
	Retroperitoneal and/or mediastinal lymph nodes**		5
	External lymph nodes		2
	Kidney (primary tumour)		2
	Cutis/subcutis		1
	Os pelvis***		1
Progression of indicator lesions prior to start of 4'-epi-ADR:	<2 months		8
	<6 months		1
	>6 months		2
	Unchanged within 6 months		7
	Not evaluable		3

* Methyl-Glyoxal Bis-Guanylhydrazone
** Measurable by CT Scan
*** Surrounded by a soft tissue tumour, measurable by CT scan.

hematological liver function tests and serum creatinine were analyzed prior to each treatment course. In order to define the myocardial toxicity of 4'-epi-ADR an electrocardiogram was performed before each 4'-epi-ADR injection. Furthermore, the systolic time interval (STI) was determined prior to each treatment course and at treatment discontinuation. The grade of gastrointestinal toxicity and the degree of hair loss were also estimated regularly during the treatment period.

RESULTS

One patient died after the first treatment course due to progressive disease (early death). For the remaining twenty patients the response rates are given in Table 2. No tumour remissions were obtained. Four patients received 4 - 6 courses without reduction of the indicator lesions. "No change" was observed in two of eight patients with indicator lesions which were progressing during the two months preceding treatment with 4'-epi-ADR.

Toxicity was generally mild to moderate (Table 3). In particular, no major myocardial toxicity was observed. STI remained unchanged during 4'-epi-ADR treatment in all patients. Two patients however complained of increased angina pectoris after two and three courses of 4'-epi-ADR respectively. One of these patients died of myocardial infarction three days after the third treatment course. The lowest values of leukocytes were observed in patients who had received cytostatic treatment prior to 4'-epi-ADR therapy. Anemia was most frequent and most severe in patients with widespread metastatic disease, and was not clearly related to the accumulated total dose of 4'-epi-ADR. The degree of hair loss increased with increasing total accumulated doses of 4'-epi-ADR.

DISCUSSION

This study did not confirm previous preliminary results showing anticancer activity of 4'-epi-ADR in renal cancer (1). Objective response was not observed in any of the twenty evaluable patients.

Even in untreated patients with metastatic renal cancer periods of progressive disease may alternate with intervals of stable disease lasting for several months. Therefore "no change" of even previously progressive metastatic renal cancer observed during treatment with an anticancer drug does not necessarily indicate activity of the cytostatic drug. In renal cancer the efficacy of a cytostatic drug can only be demonstrated by the frequency of clearly defined objective remissions (partial response, complete response) obtained in measurable indicator lesions.

Used at the above dose schedule the overall acute and subacute toxicity of 4'-epi-ADR seems less than the side effects of Adriamycin

Table 2. Response to 4'-epi-Adriamycin in Patients with Metastatic Renal Cancer

Evaluation	C.R.	P.R.	N.C.	Progression	No. of Observed Patients
After 2 courses	0	0	13	7	20[x]
After 3 courses	0	0	11	1	12

[x] One additional patient died after the first course due to progressive disease (early death).

C.R. = Complete Response
P.R. = Partial Response
N.C. = No Change

Table 3. Acute and Subacute Toxicity in 59 Courses of 4'-epi-Adriamycin (75 mg/m^2) i.v. q 3 weeks

	Grade 0	Grade 1	Grade 2	Grade 3
Anemia	29	12	16	2
Leukopenia	45	7	4	3
Thrombopenia	59	-	-	-
Nausea Vomiting	-	41	18	-
Alopecia	-	23	26	10
Cardiac Arhythmia	59	-	-	-
Cardiac Dysfunction	59	-	-	-

in comparable doses. It is unlikely that the ischaemic heart disease observed in two of our patients (angina pectoris, myocardial infarction) was due to treatment with 4'epi-ADR. In general, anthracyclines do not cause this type of cardio-toxicity. These drugs lead to a primary damage of the myocardium with the clinical symptoms of congestive heart failure (3).

However, we cannot define the long-term toxicity of 4'-epi-ADR as most of our patients did not receive more than two to four courses. As with Adriamycin, cardio-myopathy may represent a problem when higher accumulated doses of 4'-epi-ADR are used (4). Furthermore, STI determinations probably do not represent the optimal method of determining anthracycline-induced myocardial toxicity, biopsies from the myocardium (4) or assessment of the radionuclide ejection fraction (3) being modern techniques to evaluate anthracycline-induced cardiomyopathy.

CONCLUSIONS

4'-epi-ADR does not seem to have a cytostatic effect in metastatic renal cancer. Due to the relative mildness of toxicity, 4'-epi-ADR seems to be especially useful for the cytostatic treatment of out patients. 4'-epi-ADR should therefore be evaluated more extensively in other malignant diseases.

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IS RENAL CANCER A HORMONE-DEPENDENT TUMOUR AND HOW DOES IT RESPOND TO HORMONAL TREATMENT? ROUND TABLE REPORT

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Despite the lack of clinical data to support the concept of renal cell carcinoma (RCC) as a "hormone dependent tumour" - the title of a paper from Concolino in Cancer Research in 1978 (1) - hormone therapy is still being used in many institutions.

There are three simple reasons -

1. In metastatic RCC there are few, if any, therapeutic alternatives.

2. Hormone treatment is easy to administer and produces few adverse side effects.

3. It often produces a remarkable improvement in the well-being of the patient.

The recent availability of synthetic ligands for receptor measurements is another reason for the renewed interest in the endocrine relationships of this rather relentless tumour.

THE NORMAL KIDNEY AS ENDOCRINE TARGET ORGAN

There is some evidence that the normal kidney is a target for hormones of the pituitary, adrenal, parathyroid and gonad. It contains receptors and enzymes capable of degrading testosterone. However, the progesterone and estrogen receptor concentrations in the normal is low, as is the capability to degrade testosterone (2).

HORMONE RELEASE OF THE NORMAL KIDNEY AND RCC

The kidney is an active endocrine organ secreting erythropoietin, parathormone-like compounds and PGE2. The secretion by RCC of a variety of hormones has been demonstrated. Of interest in this context is the ectopic secretion of HCG, Prolactin and ACTH in 5% of the afflicted patients.

Dunzendorfer et al (3) measured the circulating peptide hormones in 25 patients with RCC. None of the patients had a paraneoplastic syndrome. 47% had increased prolactin (PRL) values which dropped to normal in MO disease after nephrectomy. TSH which is controlled like PRL by a releasing hormone was high in 29% without correlation to PRL.

ETIOLOGY OF RCC: HORMONES?

There is circumferential evidence that hormones have some influence, since RCC has not been observed at times of endocrine hyperactivity, e.g. pregnancy, and is less common in younger women (see below).

M : F = 3 : 1 in pts <40 yrs, but

M : F = 2 : 1 in pts >40 yrs.

Van der Werf-Messing reported a M : F ratio 1.2 : 1 in 174 MO-RCC (4), but the women had a significantly better prognosis than the men. There are three case reports suggestive of an estrogen induced RCC in men being treated for prostatic cancer: one by Bellet et al (5) and two by Nissenkorn et al (6), but no obvious cause-and-effect relationship could be demonstrated.

Finally, if vital isolated RCC cells were exposed to estradiol 5/10 were stimulated, but the other five were inhibited as determined by H^3-thymidine-incorporation. The same applied to progesterone (7). Thus, there is no strong evidence that hormones are involved.

RECEPTOR CONTENT OF RCC AND ITS SIGNIFICANCE IN PREDICTING HORMONE RESPONSIVENESS

Some RCC contain low concentrations of high affinity binding sites to estrogen and progesterone. Estrogen treatment does not induce receptors in humans, as opposed to the finding of Li et al in hamsters (8). Androgen and glucocorticoid receptors binding to gestagen have recently also been demonstrated.

Pizzocaro (I.N.T. Milan) reported that 12/31 RCC had estrogen receptors. Of five RCC patients with metastases, two had stable

disease whilst on medroxyprogesterone acetate. In a randomised trial they collected 62 Mo-RCC, of whom 10% had estrogen and androgen receptors. Of the 46 evaluable Mo patients, four relapsed, of whom three had been treated with MPA. All MO - locally extensive disease - relapses occurred in receptor-negative cases. Twelve Ml-RCC were treated with MPA: four patients had stabilization, but no objective response was seen. There was no correlation between receptor content and stabilization. Concolino (Rome) found estrogen and progesterone receptors in 17 of 55 RCC without clear relation to hormone treatment response.

Macchia (New York) developed a system in which testosterone is bound to albumin and labelled by fluoresceine. Subsequent Immunofluorescence is regarded as an indicator of binding. Cyproterone acetate reduces the visible fluorescence probably by competitive inhibition. Thirty-six RCC were studied: nine showed no binding, four low binding for estrogen, one moderate and eight high binding (= more than 50% cells fluorescent). Determining specifity and intensity appeared to be problematic. Furthermore the binding sites cannot as yet be defined.

HORMONAL TREATMENT OF RCC

It was emphasized that stabilization does not constitute a response. Furthermore, spontaneous regression, though infrequent (0.4% of 1340 RCC from the literature) could be present.

RESULTS

Hrushesky and Murphy (9) observed that, between 1967 and 1971, 228 patients were treated with Progesterone with 17% remissions, but of 415 patients treated between 1971 and 1976 only 2% had a remission. The difference is due to a different definition of remission rates. In a recent prospective trial of progesterone plus androgens versus no treatment, De Kernion treated 110 patients without a single remission. The South West Oncology Group Study of Tamoxifen showed 4% remissions but 28% subjective improvement (1). Finally, there is a report of the Eastern Cooperative Oncology Group, where 41 RCC have been randomized to MPA or Nafoxidine, producing 5% and 15% partial remissions, respectively (11).

IMMUNOTHERAPY AND HORMONES

Ishmal et al (12) treated 31 patients with Vincristine, Adriamycin, BCG + Provera and claimed a 33% response. At the Cleveland Clinic a regimen of Megestrol acetate + CCNU + Transfer Factor + BCG was used in 33 patients (13). Six (19%) had a partial remission of short duration.

CHEMOTHERAPY AND HORMONES

In the literature are reports of 11% responses with MeCCNU + MPA in 38 patients and of 8% responses with VLB + MPA in 38 patients (14).

CONCLUSIONS

There is some evidence that a weak hormone dependence may exist in RCC, but at the present time hormonal treatment does not induce remission in M1-RCC. However, MPA is well tolerated and leads to a remarkable subjective improvement.

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STRATEGY OF THERAPY IN RENAL CARCINOMA - ROUND TABLE REPORT

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Schmidt began with a comprehensive review of the state of the art, drawing attention to some of the more notable gaps in our contemporary approach to renal cell cancer. This was followed by a lively discussion, which fell into three parts:

1. The need for earlier diagnosis

There was universal agreement that the diagnosis was too often being made too late, and that we should aim to detect these tumours when small and non-invasive, and before they had metastasized, ie., pT1/pT2, M0, but nobody had any new notion how this was to be achieved. Clearly to screen the entire population at risk by ultrasound, CAT or urography would be logistically impossible. Smith reminded us again of the fallacies of conventional diagnosis, illustrating this by one example where a huge tumour remained unchanged for many years, and another in whom an enormous mass appeared within a few months. In the face of this unpredictable rate of growth, conventional clinical signs and symptoms were of even less value. Nor were the para-neoplastic syndromes of any greater value: certainly internists needed to be reminded of their existence, but it was seldom that they denoted early disease. Those alterations of the plasma albumen/globulin ratio including elevation of the α_2 globulin fraction that resulted in a raised ESR, were not features of early disease. Van der Werf-Messing found that elevation of the ESR signified a poor prognosis and appeared to denote occult micro-metastases. We still have no easy means of detecting these tumours at an early stage, and none that could be used on a large scale.

2. Management of the local lesion in the kidney

(a) A no-nephrectomy group? We discussed the interesting suggestion of Denis that there was a place for refraining from nephrectomy in patients over the age of 75 who had no symptoms. Considerable anxiety was expressed about the risk of untoward late morbidity. Perhaps this might form the subject of a well designed clinical trial.

(b) Embolisation The initial burst of enthusiasm for pre-operative embolisation had obviously been tempered by experience; now only a minority of urologists continued to employ angiography, let alone embolisation, for every case. It clearly had value in limiting blood loss when the tumour was very large or where there was evidence of caval invasion, in which case a cavogram should be added.

(c) Lymph node dissection There was even more discussion as to the place of lymph node dissection. Its enthusiastic advocacy by Carmignani received little support. Pavone questioned whether the inevitable morbidity of this procedure in the hands of the average surgeon could ever be justified when lymphadenectomy could never be more than a staging manoeuvre. To this Goodwin retorted that some patients might benefit by removal of a single lymph node metastasis: perhaps a more limited node resection could give equally useful staging information without heroic dissection behind the great vessels. The suggestion was made that the advantages now being claimed for node dissection needed to be tested for the only hard data that were available (1) came from a retrospective study of a very selected series in which node dissection appeared to confer an advantage (3 year actuarial survival 73% versus 64%). This is certainly a question that will not go away and if we do not set about answering it in the near future, it will continue to hang around and worry us for years to come.

(d) Radiotherapy - not a dead issue? There was general agreement that there was no place either for pre-operative or post-operative radiotherapy, but Andersson caused us to discuss whether in the anaplastic group (detected cytologically by fine-needle aspiration) pre-operative radiotherapy might deserve a second look. When tumour was known to be left behind after nephrectomy, radiotherapy was probably justified, particularly when modern techniques were used, and the residual mass was carefully monitored by CAT scanning. At the other end of the scale it was again suggested that we should reconsider the role of partial nephrectomy for pT1 tumours.

3. Management of metastases (M1)

(a) Hormone therapy In the absence of any acceptable

evidence to support the use of androgens or progestational agents in patients with metastases, de Vere White pointed out that this futile practice was hindering the active search for more effective treatment, and perpetuated the myth of the 20% response to hormone therapy. Clinical trials should put an end to this practice. No alternative hormone therapy appeared to be on the horizon, and anyway the evidence that kidney cancer is hormone-sensitive appears to be limited to one species.

(b) Chemotherapy Stoter's grave recapulation of the scrupulous EORTC and other trials of single-agent chemotherapy showed that no agent so far tested was effective. Nevertheless the method of the clinical trial, discouraging as it sometimes seems, is the only means whereby these desirable agents can ever be detected.

(c) Interferon In the face of the news media excitement about Interferon Edsmyr warned us against unjustified euphoria and observed that much more work was needed.

(d) Immunotherapy Equal caution was necessary in interpreting the results of immunotherapy. The claims for pre-nephrectomy embolization followed by BCG needed to be ratified in carefully supervised trials before it was adopted or abandoned. Brosman pointed out that the promising autologous tumour vaccine developed by Tykkä et al (2) was still very experimental. Premature enthusiasm, even for clinical trials, would risk premature disrepute. In our quest for immunological suppression of tumour growth we must never forget that immunology is two edged, and that its undesirable edge was tumour enhancement.

Before the session closed de Voogt entered an impassioned protest at the "negative" attitude, expressed by the many participants who had listened to such a long and dismal recitation of negative results. He urged a more "positive" view and reproached us for failing to give sufficient attention to cell kinetics from which he sensed, alas without any evidence, that something good would come.

Summary

It seemed that five "questions for today" could be identified:

1. The need for some method, whether a "marker" or a method of screening, which could pick up the early pT1 M0 cancer.
2. Whether or not it was legitimate to refrain from nephrectomy in the symptomless patient over 75 years of age.
3. To test in properly designed clinical trials the current claims that lymphadenectomy improved survival.
4. To continue the painstaking search, by means of well-regulated

clinical trials, for effective chemotherapeutic agents. The search was likely to be long - but this was no good reason for giving up. Rome, let alone Erice, was not built in a day.
5. Current claims for immunotherapy needed to be tested by similarly well-controlled clinical trials.

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TREATMENT OF RENAL CANCER

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The therapy of renal-cell carcinoma has evolved slowly. The earlier reports of increased benefit from radiotherapy, as adjuvant to nephrectomy, were tempered by the improved survival afforded to patients using a radical or extrafascial nephrectomy, (1-6). In this procedure the kidney, with its tumor, is removed along with the accompanying adrenal gland, perinephric fat and perinephric fascia of Gerota. An ipsilateral regional lymphadenectomy, removing tissue from the diaphragmatic crus to the bifurcation of the aorta or vena cava has been espoused by many. At this point, lymphadenectomy can be considered important for more accurate staging and therefore of prognostic value, but it is unlikely that many patients with tumor involvement in the regional lymph nodes (Stage III) are cured by this manoeuvre alone. Pre- or postoperative irradiation is not indicated.

Radical nephrectomy is best performed via a transperitoneal or thoracoabdominal route. Simple nephrectomy via a flank or retroperitoneal route is generally reserved for the older patient with a renal cancer who is not a good risk for the extrafascial procedure. Operative mortality for radical nephrectomy should be less than 5%.

The value of surgery in renal cancer has been emphasized in patients with multiple tumors, bilateral tumors or a tumor in a solitary kidney. Rather than total or bilateral nephrectomy requiring hemodialysis and possible transplantation, renal conserving operations have benefited many patients. Choices available to the urologist have included partial nephrectomy, wedge resection of renal parenchyma and local excision (Table 1).

An exciting adjuvant to radical nephrectomy is the use of

TABLE I

Surgery for Renal Cell Carcinoma

Local Excision

Partial Nephrectomy

Simple Nephrectomy

Radical Nephrectomy

Regional Lymphadenectomy

Resection of Metastases

angiographic embolization and infarction preoperatively (7). We currently employ this technique in large and medium-sized hypernephromas, usually the day prior to or on the morning of the planned surgery. The shrinkage of the kidney and tumor has been impressive (Figures 1-2). The surgery is facilitated as 1) the mass is smaller, 2) the renal artery or arteries are

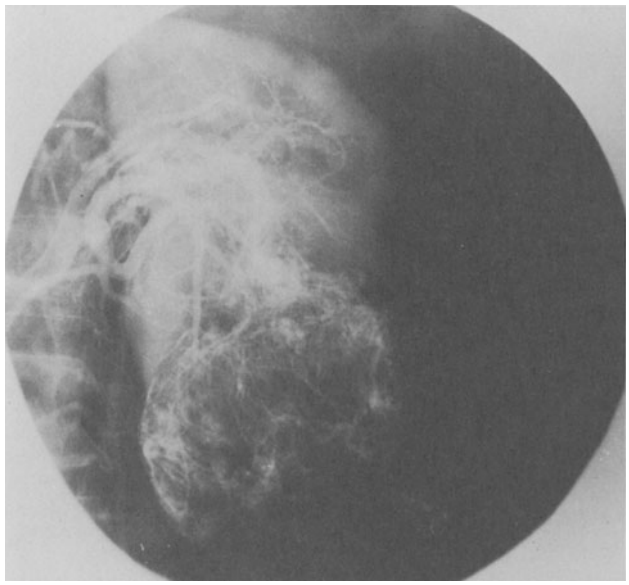

Fig. 1. Magnification aortogram demonstrating typical hypervascular left lower pole mass.

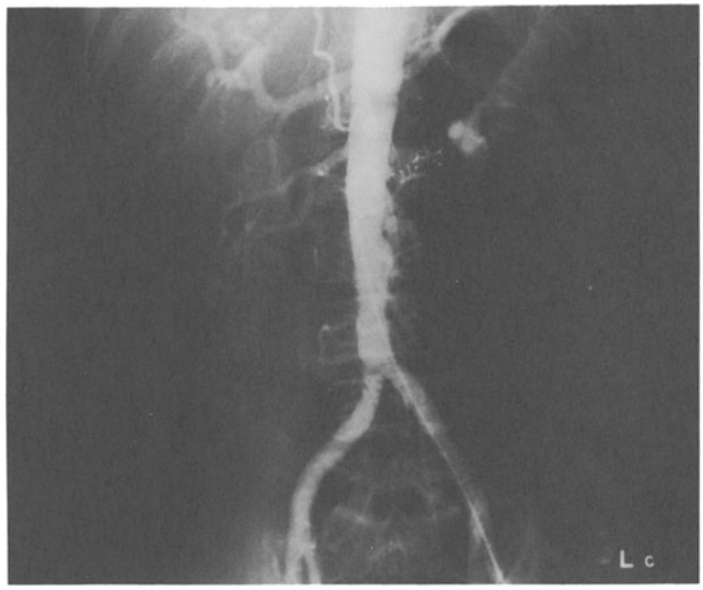

Fig. 2. Aortogram of same patient as in Fig. 1. following embolization using Gianturco coil.

already occluded allowing the early ligation and division of the renal vein, and 3) the dissection seems to be improved by the reactive edema created.

In patients who have not undergone operation immediately there has been a variable degree of ipsilateral flank pain, fever and ileus related to the infarction. Materials used for the angio-infarction include the Gianturco coil, particles of Gelfoam and Ivalon sponges. We have also utilized angioinfarction in place of surgery in a few instances: in patients refusing surgery or in those with advanced disease having complications such as severe renal hemorrhage.

HORMONAL AND CHEMOTHERAPY

Progestational agents have been associated with a small but significant response rate in advanced (Stage IV) renal cancer (8-9). We recommend the use of medroxyprogesterone acetate (Depo-Provera), 400 mg intramuscularly twice weekly or 800 mg IM once weekly for twelve consecutive weeks. Although the use of androgen therapy, for example testosterone proprionate, 25-50 mg two- to three-times weekly, has also been reported to be occasionally effective, this author has not seen such responses. Progestational agents are relatively nontoxic and thus well accepted by the patient.

Over the last ten years numerous studies have been reported regarding chemotherapy of advanced renal cancer. To date there is no clear-cut evidence that any single drug or combination of agents is effective. Yet responses have been documented for vinblastin sulfate (Velban), 5-10 mg intravenously weekly for twelve weeks and for methyl-CCNU or CCNU 130-200 mg/m^2 orally every six weeks. Of significance are the disappointing results using cis-platinum (DDP), a drug shown to be of value in both prostatic and bladder cancer. It is postulated that the tendency for hypernephroma to be slow-growing explains its relative radioresistance and resistance to chemotherapy.

IMMUNOTHERAPY

Scattered reports continue to be published regarding the effective use of adjuvant immunotherapy, generally in advanced renal cancer. These agents include immune-RNA and Bacillus Calmette-Guerin (BCG) (10). Although of interest, for the most part these studies have not been randomized or controlled. The employment of angioinfarction seven to ten days prior to radical nephrectomy may be considered a form of immunotherapy when the infarction of both normal and neoplastic tissue may incite an immunological response. Such a study is currently underway in the United States. Although the theory is inviting, there has been no evidence that spontaneously induced tumor infarction as evidenced by fever has been associated with regression of metastases or improved survival.

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RENAL CANCER - CLINICAL STUDIES IN THE U.S.A.

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SURVIVAL FACTORS

The five year survival for untreated renal cell cancer is approximately 2%. This increases to 30 - 47% following simple nephrectomy whereas the overall five year survival following radical (perifascial) nephrectomy is 57 - 80%. Ten year survivals for renal cell cancer overall are 17 - 27% for simple nephrectomy and 40 - 66% following radical nephrectomy (1-5).

Employing the staging system as seen in Table 1, the five year survival rates for radical nephrectomy stage by stage are: Stage I 59 - 80%, Stage II 51 - 64%, Stage III 34 - 48% and for Stage IV 5% (5,6). The ten year survival rates for radical nephrectomy are for Stage I 39 - 70%, Stage II 17 - 66%, Stage III 20 - 38% and for Stage IV 2%. The addition of regional lymphadenectomy may increase these figures by another 6 - 9%.

Survival has also been related to histologic cell type. Pure clear cell carcinoma accounts for 25% of all cases of hypernephroma; five year survival for pure clear cell tumor is approximately 58%. Granular and mixed cell types account for 15 and 46%, respectively, of all renal cancers; the five year survival for the granular cell or mixed type is 46%. Finally, spindle cell tumors account for 14% of all renal cell cancers and are associated with a five year survival rate of only 23%. The lesser survival of spindle cell, granular cell and mixed cell types relates more to these tumors being of higher grade compared to the lower grade propensity of pure clear cell lesions.

Table 1. Comparison of Robson and TNM Staging for Renal Cell Carcinoma

Robson	TNM Clinical	TNM Pathological	Extent of Tumor
I	T1	pT1	Tumor within capsule (small)
	T2	pT2	Tumor within capsule (large)
II	T3	pT3	Tumor contained within perinephric fascia
III	T3	pV1	Tumor in renal vein
	N1-N3	pN1-N3	Tumor in regional nodes
	-	pV2	Tumor in vena cava
IV	T4	pT4	Tumor in adjacent organs
	M1	pM1	Distant metastases
	N4	pN4	Tumor in nodes beyond regional group

The current concepts for renal adenoma and oncocytoma are that the tubular adenoma should be considered a small malignancy whereas the oncocytoma, in spite of its often attaining a large size, is a benign tumor. The "spoke wheel" pattern on angiography earlier thought to be pathognomonic for renal oncocytoma is no longer felt to be specific for this lesion.

Studies of inferior vena caval involvement reveal this to occur in 5 - 10% of all patients with hypernephroma, but this incidence increases to 25% in the group of patients with renal vein involvement. Of interest, the five and ten year survival statistics for patients with vena caval involvement and no other involvement are similar to Robson Stages I and II: 55% at five years and 43% at ten years.

CLINICAL PRESENTATIONS

About one third of patients present with some conbination of flank pain, hematuria, and abdominal mass. Yet this "classic triad" is seen in only 10% of all patients with hypernephroma. Another one third present with symptoms from metastatic disease. The remaining one third have symptoms of a constitutional nature, e.g. fever or

weight loss. A varicocele, mostly left-sided, has been noted in only 1% of cases.

Spontaneous regression of the primary tumor is rare. Spontaneous regression of metastatic lesions has been reported in over 50 cases; 88% have involved pulmonary metastases and three fourths of cases have been in men, about equal to the usual sex distribution of hypernephroma. Few of the cases of spontaneous regression of metastases have had histologic confirmation of their malignant nature. Regression has generally been related to nephrectomy but has also been reported without such surgery. To date there is little evidence that a nephrectomy influences survival in the face of metastatic disease; however, there may be a slight advantage in survival for patients with osseous metastases.

It is of interest to compare the interval between nephrectomy and the appearance of metastasis and subsequent survival. If metastases occur within six months post-nephrectomy, survival has been only 8% at two years. On the other hand, if metastases occur 24 months or longer after operation, the two year survival measured from that point is increased to 32% (5).

SPECIFIC CLINICAL TRIALS

In the late 1960's the Renal Cell Carcinoma Cooperative Study Group began a trial comparing radical nephrectomy alone to the combination of 4500 rads of external beam irradiation in 4 - 6 weeks followed by radical nephrectomy for clinically localized hypernephroma. Although early results seemed to favor the combined treatment, later data showed no significant difference in survival (7). However, external beam irradiation to the tumor may shrink the lesion, making nephrectomy possible if not easier.

At the M.D. Anderson Hospital and Tumor Clinic, a study of angio-infarction related to nephrectomy and other treatments is currently being pursued in patients with varying stages of renal cell cancer (8). Immunologic studies so far have demonstrated an improvement in the impaired baseline immunocompetence measured by delayed hypersensitivity following angio-infarction. However, concomitant decreases in cellular immunity as measured by lymphocyte blastogenesis have been reported by the same team of investigators (9,10). Humoral antibodies to the renal cell cancer have been identified in 59 - 94% of patients studied (11).

In the presence of metastatic disease, angioinfarction is followed by medroxyprogesterone acetate (Depo-Provera) with or without an interval nephrectomy. The interval between angioinfarction and nephrectomy has generally been two to ten days. Of 36 evaluable patients with pulmonary metastases, responses have been seen in 21 (58%). Six responses have been complete; five have been partial

(50% or greater reduction in size of metastases). Nine patients have either remained stable or demonstrated a less than 50% shrinkage in the size of pulmonary metastases. Survival has been as long as thirty months in patients with a complete response following angio-infarction, nephrectomy and progestational therapy. The early results in 25 patients treated with embolization and hormones (no nephrectomy) are less optimistic (9).

The use of immunotherapy in metastatic renal cell cancer has been received with less enthusiasm recently. Using immune xenogenic ribonucleic acid (RNA), no complete or partial regressions have been reported (12). Stable disease has been noted in 35% of patients with Stage IV tumors. In another study, transfer factor plus either Bacillus Calmette-Guerin (BCG) or CCNU and megestrol acetate (Megace) has been associated with a response rate of 26% with a ten month median duration of response (13).

SUMMARY

The mainstay of therapy for renal cell carcinoma continues to be surgery. The earlier reports of increased survival associated with combinations of irradiation and simple nephrectomy have been replaced by unassisted radical nephrectomy (perifascial nephro-adrenalectomy) and/or regional lymphadenectomy. Current efforts consist of evaluation of 1) angioinfarction techniques with either nephrectomy or progestational agents as well as with the combination of nephrectomy and hormones, 2) immunotherapy and 3) chemotherapy.

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CONSERVATIVE MANAGEMENT OF RENAL TUMOURS

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INTRODUCTION

Although the conservative treatment of renal tumors is an option attracting increasing interest, it is still, in our experience, an uncommon event. During the last eight years we have carried out 250 nephrectomies for tumours in our institution and we have adopted a conservative approach in a further 11 cases (less than 5% of the cases).

This form of treatment was initially reserved for those patients in whom the preservation of functioning renal parenchyma seemed more relevant than radical surgical excision, ie, in patients with bilateral renal cell carcinoma and in cancer in a solitary kidney. The favourable results obtained have allowed us to extend the indications to include patients with contralateral renal damage. Conservative surgery may also be performed for small tumours discovered within renal cysts, or on the surface of kidneys operated upon for different reasons. The available surgical techniques consist of polar resection and tumour enucleation; both may be performed either in-situ or extracorporeally. Each technique finds its indications according to the size and location of the neoplasm.

The rationale for performing conservative surgery is related to the unusual way in which renal tumours grow, at least in the initial stages, since they are often surrounded by a pseudocapsule of fibrosis and compressed tissue.

PATIENTS AND METHODS

Indications for conservative surgery in our 11 patients were:

simultaneous bilateral tumours in two cases, tumours in solitary kidney in three (one surgical, two functional), tumours with a damaged contralateral kidney in three cases (one UPJ obstruction, two stones), two small tumours associated with a cyst, and a pseudo-encapsulated small tumour with normal contralateral kidney. Polar resection was performed in six patients and tumour enucleation in five. Pathology revealed nine renal cell carcinomas (three consisting of granular cells), one oncocytoma and one renal adenoma.

All patients are alive, with a follow-up ranging between two months and eight years (mean: three years and six months). The first eight cases were reviewed in September 1980 (mean follow-up at that time: 44 months) and were without evidence of metastases. No significant impairment of renal function was observed post-operatively.

DISCUSSION

When one has to face the difficult choice between radical excision of a neoplasm and preservation of renal function, various therapeutic solutions have been proposed. We believe that only surgery can be considered as an effective treatment.

A radical procedure, rendering these patients anephric, commits the patient to subsequent chronic dialysis and possible renal transplantation, which nowadays implies a 40 to 50% 5-year survival. This is definitely better than has been observed in untreated renal cancers, but worse than the results reported for conservative surgery (1).

Polar resection, removing the tumour with an area of surrounding normal tissue, is apparently safer, but presents some drawbacks, such as difficulties in haemostasis, possible infarction of the underlying parenchyma and at times excessive sacrifice of functioning tissue. Enucleation could theoretically leave behind some neoplastic cells, but presents the advantages of being a simple and fast technique which seldom requires hypothermia and pedicle clamping and sacrifices a minimal amount of normal tissue. In our opinion it is always worthwhile to try it first (2).

Extracorporeal surgery finds it main indication in the treatment of central bulky renal masses; this procedure allows efficient haemostasis, meticulous reconstruction of the excretory system and ample time for pathologic reports on the section surface. It is often used by surgical teams familiar with transplant techniques.

In patients with bilateral renal tumours we favour a midline transperitoneal approach, first removing the smaller tumour conservatively, then performing a radical contralateral nephrectomy during the same procedure.

The results of conservative surgical management of renal tumours seem promising. Palmer and Swanson (3) reported a 78% survival after an average follow-up of 45 months which compares favourably with a 65% 5-year survival rate for radical nephrectomy in cases of cancer confined to the kidney. Obviously a longer trial period is still required, particularly if we consider the unpredictable behaviour of renal tumours. An accurate pathological review is also necessary, since we cannot group renal cell carcinoma with other tumours which have a more benign prognosis.

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