A SYNOPSIS OF CANCER

GENESIS AND BIOLOGY

BY

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PREFACE

THE disciplines involved in research into the genesis and biology of cancer are growing ever wider, and the detail of study is becoming increasingly deep. It is not surprising that the practitioner of medicine finds it difficult to maintain an appreciation of advances, and to co-ordinate and apply the results of basic research to his own sphere of work. Not only does this imply the possibility of deficiencies in therapy, but it results in a serious and fundamental loss to the sum total of possible avenues of exploration of cancer. The lack of application and correlation of the results of investigation and experiment to the observation and management of patients suffering from cancer detracts from the practitioner's understanding of the disease and reduces his potential contribution to knowledge of the subject.

The very idiom and terminology of many branches of cancer investigation are evolving along specific and unique lines. The inability to understand the new language is creating a deep rift between the practitioner at the bedside and the scientist at the bench, and, in fact, between some of the workers in different spheres of research. To bridge this rift is, perhaps, the basic minimum for consolidation of efforts in different fields of work towards the comprehension and the cure of cancer.

It is patently important to co-ordinate the various disciplines of research, to correlate them where possible, and to compare and contrast their findings and deductions. It is equally important to apply the same idea of co-ordination to different branches of medical practice. The usual methods of teaching students and postgraduates militate against such co-ordination: the standard orientation of teaching is based upon organ classification, so as to fit into the divisions of specialist medical practice. The orthopaedic surgeon teaches bone and joint tumours, the thoracic surgeon instructs in lung cancers, the gynaecologist is restricted to his particular subject, the urologist to his, and so on. This leads to pigeon-holing of ideas and knowledge, with the attendant danger of losing sight of the composite subject of neoplastic disease. An essential requirement is a reorientation of teaching. The system of instruction in organ-compartmented form needs to be augmented by presentation of the whole subject as a broad and compound picture, at postgraduate as well as undergraduate levels. Co-ordination in medical practice in all its branches may then be practicable without the obstacles and difficulties that exist today.

The present work is designed to meet some of these problems. Its framework of a synopsis is intended to include a review and appreciation of current knowledge, to suggest the areas of correlation in different disciplines of research and practice, to serve as an introduction to more detailed works of reference, and to provide a book that will excite the interest of student and practitioner in the genesis and biology of cancer, and yet remain a book of manageable proportions.

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To Ora, Janet, and Barbara
for the help and happiness
brought by your enthusiasm

FOREWORD

By

Sir ARTHUR PORRITT, Bt.

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THIS quite extraordinary little book styles itself a 'Synopsis' and is subtitled 'Genesis and Biology'. These two wide aspects of cancer underline the predominant trends in an incredibly comprehensive description of this dire disease.

Synoptic in form the book may be, but it avoids the soul-destroying itemization of many similar volumes. It is essentially readable, well paragraphed, and well illustrated—largely by clear and intelligible diagrams.

The author's desire to find a common ground between clinician and research worker and to treat the subject as a whole rather than to divide it into systematic and apparently unrelated parts seems to have been more than fulfilled.

Brief historical references abound and are linked to the most modern concepts of cancer aetiology as is shown by such excellent chapters as those on Heredity, Genetic Factors, the Mutation Theory, and the relation of Immunology to Cancer. Regional consideration of various cancers is by no means neglected but is interestingly linked with an assessment of the part played in carcinogenesis by the viruses, by hormones, by ionizing radiation, and by other agents.

Several chapters on geographical pathology are evidence of the author's very wide experience and even wider reading—and yet, appreciating this is a synopsis, good but short reference lists are included to stimulate the search for further knowledge.

The book is essentially descriptive rather than clinical—but it can only be of the greatest use and greatest value to the practising doctor. It most certainly and efficiently links (as the author says) the bedside with the laboratory. It is a book both to read and to keep available for ready reference; it is a veritable mine of information, a classical *multum in parvo*.

I am sure it will prove to have a wide popularity and to be deservedly successful.

London, December 1965

A SYNOPSIS OF CANCER
GENESIS AND BIOLOGY

CHAPTER I

THE NATURE OF CANCER

DEFINITIONS AND ATTRIBUTES

Neoplasm, or **New Growth**, implies the growth of newly formed cells derived from normal body cells or their preceding developmental cells of origin.

The new growth often, but not invariably, forms a lump or **tumour**, a term frequently used synonymously with neoplasm.

Benign Neoplasm, also referred to as 'simple tumour', indicates a tumour that does not of itself destroy the host. It may, however, cause such disturbance of function, e.g., bowel obstruction, as to be fatal. While growth in size of the benign tumour occurs, it does so by general expansion with fairly uniform enlargement of the whole tumour mass, maintaining its clear definition and remaining confined within a limiting capsule. It does not invade neighbouring tissue nor implant, i.e., metastasize, at distant sites. Its cells are mature in structure and arrangement.

Malignant Neoplasm, or **Cancer**, if left untreated, destroys the host. The tumour may be encapsulated for a limited period, but it ultimately infiltrates and grows through such confines to invade neighbouring tissues. Metastases (distant secondary sites) are characteristic. Its gross and histological characters are commonly irregular and degenerative changes are frequent. Malignant tumours arising from epithelial cells are called 'carcinomas'; those from connective tissues are called 'sarcomas'.

The attributes defining malignant neoplasms are elaborated in subsequent sections, but it is useful at this stage to tabulate them to indicate the main differences between benign and malignant tumours.

ORIGIN OF TUMOURS

All tissues in the body are liable to undergo malignant change, and all cells have an inherent potential for the development of cancer.

The origin of cancer may be unicentric or multicentric:—

1. Unicentric, or unifocal, origin implies that one cell or a localized group of cells becomes malignant and that all subsequent cancer cells are descendants from this focus. Neighbouring cells are not

Origin of Tumours, *continued.*

Table I.—Comparison of Properties of Benign and Malignant Tumours

Property	Benign	Malignant
Destruction of host	Absent	Present or potential
Gross form of tumour	Regular and defined	Irregular and without definition
Character of growth (i.e., increase in size)	Concentrically uniform	Lacks uniformity, and growth is from periphery
Rapidity of growth	Usually slow	Often rapid
Encapsulation	Characteristic	Absent or soon destroyed
Infiltration	Absent	Present
Dissemination	Absent	Present or potential
Cellular maturity	Mature and resembles tissue of origin	Varies: the more malignant, the more immature
Cellular morphology	Regularity of structure	Pleomorphism is common
Composite arrangement of cells	Adult type of organization	The more malignant, the less the resemblance to the adult arrangement
Cellular activity	Mitotic activity not increased	The more malignant, the higher the mitotic index
Mitotic pattern	That present is regular and resembles the normal	Often abnormal and irregular
Degenerative changes	Unusual	Common

themselves altered or added to the initial centre. This concept of the origin of tumours is subject to doubt. It certainly does not apply to all tumours: in virus tumours, there is evidence of affection of neighbouring cells, and in many parts of the body, e.g., in the tongue and colon, cells adjacent to clearly malignant sites often appear to be undergoing changes.

2. The multicentric, or multifocal, concept implies a simultaneous origin of cancer at a number of sites in one organ. There is a sound basis for this theory in regard to a number of cancers, but its universal application has not sufficient supporting evidence.

Malignant change appears to be more liable to occur in those cells which, in the normal course of activity, undergo repeated phasic and replacement alterations. Blood-cells which have a limited life and are constantly replaced by newly formed elements; epithelium which is constantly being cast off superficially and replaced from deeper layers;

endometrium and breast parenchyma, which have cycles of alternating quiescence and hyperactivity, are all examples of cells which carry a high potential danger of cancerous development.

The change from normal to malignant cell appears to be a sudden transformation, although a long period of induction may have preceded the change. Defined stages in such change have not been recognized.

GROWTH

Malignant tumours have the power of continuous growth. The cells increase in number, reproducing cells of a like character, whether in the primary site of origin or in secondary, metastatic sites.

Many tumours appear to grow without restriction or control, differing from normal cell growth which replaces effete cells, repairs injuries, or hypertrophies as a reaction to 'work demands'. Such normal cell increase is limited to the functional and morphological requirements of the particular tissue affected, whereas neoplastic cell growth is independent of such restraint and possesses a far greater degree of autonomy. However, this autonomy is not absolute, nor is it of uniform grade in different tumours. Some tumours, e.g., of the breast and of the generative organs of males and females, are known to be partially dependent upon hormone production by the host.

The rate of growth varies in different tumours from very slow to very rapid. Generally, the more rapid the growth, the more malignant is the tumour. Variation may also occur in a single tumour in that its rate of growth varies at different times.

The rate of growth is reflected in the increasing size of the tumour mass and also, microscopically, by:—

1. Active cell division, i.e., mitotic activity. This may be expressed as a mitotic index: a high proportion of dividing and multiplying cells giving a high index, and, by contrast, sparse mitoses are equivalent to a low mitotic index.
2. The maturity of the cells. Mature cells, i.e., resembling developed adult cells, are significant of benign tumours or relative benignance of a malignant tumour. By contrast, immature or primitive cells, also known as anaplastic cells, indicate malignancy of a grade directly proportional to the numbers of such cells. Broders's* classification of grades of malignancy is dependent upon this fact. The proportion of anaplastic cells is counted and the following grades are defined:—

   Grade 1—Up to 25 per cent anaplastic cells (i.e., the least malignant).
   Grade 2—25–50 per cent.
   Grade 3—50–75 per cent.
   Grade 4—Over 75 per cent (i.e., the most malignant).

DEGENERATIVE CHANGES

Such changes occur as a result of:—

1. A neoplasm outgrowing its own blood-supply.
2. Growth beyond effective lymphatic drainage so that metabolites collect.

* Broders, A. C. (1926), 'Carcinoma, Grading and Practical Application', *Archs Path.*, **2**, 376.

Degenerative Changes, *continued.*

3. Tumour invasion of vessels, leading to haemorrhage, infarction, and/or ischaemia.

Degenerative changes occur mainly in the centrum of the tumour, and consist of one or more of the following:—

1. Haemorrhage. Usually due to vascular damage by invading tumour cells, but sometimes also due to flimsy and defective vessel walls and support, e.g., in bone sarcoma.
2. Necrosis. From ischaemia due to deprivation of blood-supply, and/or secondary infection. Necrosis may become manifest by ulceration on the surface of a tumour, or by cavitation within the substance of a tumour mass.
3. Cystic vacuolation. From accumulation of tumour secretions, from absorption of haematomas, or from autolysis of necrotic areas.
4. Fatty degeneration. Found particularly when an immature tumour is associated with a poor blood-supply, e.g., carcinoma of the kidney.
5. Mucoid degeneration. Occurs especially in cancers of the stomach and gall-bladder. Mucus is normally secreted in these organs, but mucoid degeneration also occurs in organs not normally mucin-producing, e.g., the breast.
6. Hyaline degeneration. Affects especially the stroma of slowly growing tumours with marked fibrous tissue formation.
7. Calcareous deposition, calcification, and ossification. Tend to appear in unabsorbed necrosed tissue.
8. Amyloid degeneration. Occurs, but is rare.

HARMFUL EFFECTS OF CANCER

Cancer is a great menace to human life; it is second to cardio-vascular conditions as a lethal disease. Its ill-effects arise from:—

1. Growth at the expense of host requirements, drawing upon nutriment that is ordinarily used by normal tissues.
2. Peripheral invasive growth, destroying host tissues, affecting their functions partially or completely with consequent deprivation to the general body economy.
3. Toxic products of tumours directly, or secondarily, following decomposition of tumour cells.
4. Tumour cell products which may upset normal physiological processes, e.g., as in hormone-producing tumours.
5. Metastases which affect wider areas of the host's tissues and extend the ill-effects of invasive destruction and deprivation of nutriment.
6. Invasion affecting blood-vessels, causing haemorrhage of varying degree.
7. The tumour outgrowing its own blood-supply, or invading adjacent vessels, so as to give rise to necrosis and degenerative changes. Such changes are commonly subject to secondary infection, which adds to the general and local effects.
8. The extent and situation of the tumour which may interfere with essential function, e.g., obstruction of the bowel, the urinary

tract, or a bronchus, or interference with circulation of the cerebrospinal fluid.

STRUCTURE

Tumours consist of two elements, parenchyma and stroma, in varying proportions in different tumours and in different parts of the one tumour. The parenchyma carries the malignant properties; the stroma appears to be derived from the normal surrounding connective tissue as a host reaction to the tumour cells. The stroma may act as a support and medium of nourishment to the parenchyma elements. Sometimes, the stroma formation is so extensive and dense as to appear to isolate and deprive cancer cells of nourishment; often, the stroma itself is invaded and destroyed by cancer cells spreading to more extensive areas.

TUMOUR-CELL MICRO-ANATOMY

Differentiation of tumour cells to mature forms becomes manifest in their individual shape, size, and internal architecture, and also in the composite arrangement of groups of cells.

The morphology of differentiated cells depends upon the tissue of origin of the tumour. In general terms, the cells are uniform in shape, size, cell-membrane character, disposition and density of nucleus, distribution and pattern of cytoplasm, and relative proportions of cytoplasm and nuclear material. Cells of a well-differentiated tumour arising from a particular gland present micro-anatomical features very like those of the normal gland. Lack of differentiation, i.e., immaturity, manifests in cells of varying and irregular shapes, usually polyhedral or rounded, irregular hyperchromatic nuclei, many of which undergo irregular mitotic division, and with an inconstant cytoplasmic content and arrangement. The anaplastic, or immature, cells may all be of a similar character, or they may exhibit considerable variation in their size, shape, and internal arrangement. This type of variation is known as pleomorphism.

Adult differentiation of cells almost invariably affects their composite arrangement, bringing about a structure and pattern similar to that of the adult organ. Contiguous cells are arranged in appropriate positions and proper relationship to stroma elements to form glandular elements like acini, alveoli, ducts, lobules, etc. By contrast, undifferentiated cells lack such a pattern of arrangement. Mature cancer arising from glandular epithelium is known as 'adenocarcinoma'. Anaplastic cancer cells often grow in sheets or cords, or their cells are deranged into small clumps, and are designated 'carcinoma simplex'.

While cancer cells do not present pathognomonic morphological features, a sum of the cytological characters in a number of cells, taken together with their relationship to one another and to the surrounding tissues, usually provides a high degree of accuracy in diagnosis. Refinements of technique and electron microscopy have not yet produced any unequivocal or constant diagnostic criteria, but they have added information on the intimate architecture of different malignant cells. The following descriptions of cancer-cell micro-anatomy should be set against the background of the reservation that there are no specific malignant alterations.

NUCLEUS

Size.—The nucleus of cancer cells is often larger than that in the benign cells of origin of the tumour. The increase in size is more noticeable relatively than absolutely, i.e., when measured against the size of the cell. Cowdry and Paletta* express this as an increase in the nucleo-cytoplasmic ratio. The variability of absolute and relative sizes of the nucleus is perhaps more significant, especially as it becomes more marked with increased malignancy and anaplasia.

Jacobj† considers that the volume of the nucleus is largely dependent upon the volume and number of chromosomes. Shultz‡ reports the importance of the amount of protein in the nucleus as a determinant of size.

Shape.—Nuclear shape varies with the type, and state or phase of nuclear division. Mitosis, in which a cell divides into two daughter cells, each containing a replica of the chromosome number and pattern, is characteristic of normal somatic tissues. Meiosis, a form of division in which the chromosome number is halved, i.e., reduction division, occurs normally in ova and spermatozoa. Amitosis, a third type of division, consists of simple division of the nucleus without polar rearrangement of chromosome material. Of the three modes of division, mitosis is the important one in the consideration of nuclear shape in malignancy, and it requires further elaboration.

Mitosis occurs in stages which are named (*Fig.* 1). During *prophase*, the centriole divides into two, each of which shifts towards an opposite pole of the cell. The chromosomes become more dense and defined. The *metaphase* follows, starting by progressive dissolution of the nuclear membrane. The chromosomes become more contracted and come to occupy an equatorial plate across the cell. The centrioles become connected by a spindle of fibres to, and across, the equatorial plate where they are attached to the chromosomes. The metaphase ends with the replication of chromosomes into daughter pairs. This is succeeded by the *anaphase*, in which each partner of the chromosome pairs migrates towards a polar centrosome (surrounding a centriole). When chromosomal separation is complete, the *telophase* is signified by the development of nuclear and nucleolar substance from chromosomal swelling and changes in which definition and intense staining are lost. The nuclear membrane is re-formed around each nuclear clump at the poles of the cell. The cell itself becomes increasingly narrowed at its equator, progressing to complete division into two separate cells, which come to rest in an *interphase*.

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* Cowdry, E. V., and Paletta, F. X. (1941), 'Changes in Cellular, Nuclear, and Nucleolar Sizes during Methylcholanthrene Epidermal Carcinogenesis', *J. natn. Cancer Inst.*, **1**, 745.

† Jacobj, W. (1942), *Wilhelm Roux Arch. Entwmech. Org.*, **106**, 124. Quoted by Oberling, Ch., and Bernhard, W. (1961), 'The Morphology of Cancer Cells', in *The Cell*, Vol. V (Ed. Brachet, J., and Mirsky, A. E.). New York: Academic Press.

‡ Shultz, J. (1952), 'Interrelation between Nucleus and Cytoplasm: Problems at the Biological Level', *Expl Cell Res.*, Suppl. **2**, 17.

The shape of the nucleus of a cancer cell during interphase is usually irregular, its outline being indented by deep fissures and bulged by lobulations. Shape during mitosis is closely related to abnormalities of cell division which are noted in a later section.

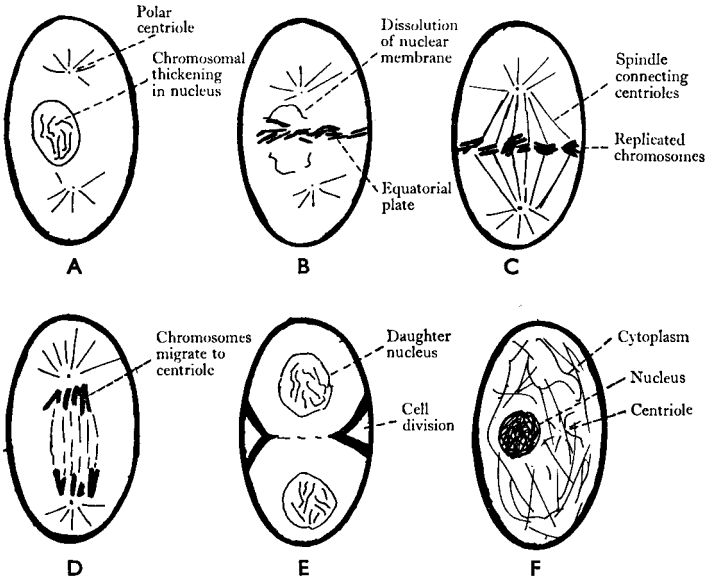

Fig. 1.—Diagram of phases of mitosis. A, Prophase; B, Metaphase I; C, Metaphase II; D, Anaphase; E, Telophase; F, Interphase.

Nuclear Membrane.—Details of irregularities of cancer cells have been recorded by Bernhard.* On electron microscopy, the deep indentations may present as thin canaliculi, limited by a double-walled nuclear membrane. Cytoplasmic structures within the invaginations may appear as inclusions within the nucleus. Often, nucleoli are situated at the bases of canaliculi, as if to provide a ready passage for nucleolar material into cytoplasm. A rich canalicular system is a marked feature in the large nuclei of Reed-Sternberg cells in Hodgkin's disease.

Nucleolus.—There may be one or several nucleoli, standing out as dark clumps of material within the nucleus. (*See Fig.* 2 which is a labelled diagram of the constituents of a cell.) They disappear during mitosis and reappear in the telophase. In some, but not all malignant cells, nucleoli are enlarged in relation to the nucleus

* Bernhard, W. (1963), 'Some Problems of Fine Structure in Tumor Cells', in *Progress in Experimental Tumor Research* (Ed. Homburger, F.). Basel: Karger.

Nucleolus, *continued.*

(Guttman and Halpern*). Page and others† remarked on the frequency of the nucleolar inclusions in malignant cells. These have been compared to inclusion bodies found in known viral neoplasms in experimental animals, e.g., the mouse polyoma, but proof of their viral nature in human and many animal tumours is lacking. The evidence is uncertain, as is well brought out in a report by Beaver.‡ He found comparable inclusion bodies in kidney-cell nuclei in lead poisoning. (However, lead is a carcinogen, and is discussed in Chapter XIII.) Viral inclusions have been suspected in cells of Hodgkin's disease, but Bernhard§ records their presence in only a few of a number of Hodgkin's affections examined by the electron microscope. This author emphasizes, 'the nucleolus of the cancer cell shows no specific characters associated with neoplastic transformation as such, and that all its changes and particularly its hypertrophy are merely a manifestation of metabolic disturbances to which the cell is subject'.

Heterochromatin.—This term is applied to the portion of chromosomal tissue which preserves its staining capacity during the interphase. Heterochromatin forms a part of the granular element of nucleoplasm. Its thread form is revealed on electron microscopy. It may undergo alteration in number, shape, and clumping, in malignancy, but there is, as yet, nothing that is peculiar to cancer.

Chromosomes.—Notes applicable to chromosomes are included in Chapter XXII. The discussion in the context of the present chapter relates to the possible bearing on characterization of the cancer cell.

The number of chromosomes in malignant tumours may be normal for the species, including man (Nowell and others‖); more rarely, the number is reduced (Koller¶). However, the common abnormality in cancer cells is an increase with aneuploidy, i.e., the increase is irregular and is not a multiple of the normal haploid number. This may occur in normal tissues, but is usually more marked and variegated in malignancy (Oberling and Bernhard**). The latter authors review the evidence for the concept that the seemingly complete irregularity of chromosomal number arises from the coincident presence of several stem lines, each producing a distinct chromosomal irregularity which is constant in that line, but when all the lines coexist in one tumour, marked diversity

* Guttman, P. H., and Halpern, S. (1935), 'Nuclear-nucleolar Volume Ratio in Rat Cancer', *Am. J. Cancer*, **25**, 802.

† Page, R. C., Reagan, J. F., and McCarty, W. C. (1938), 'Intranucleolar Bodies in Normal and Neoplastic Human Tissue', *Ibid.*, **32**, 383.

‡ Beaver, D. L. (1961), 'The Ultrastructure of the Kidney in Lead Intoxication with Particular Reference to Intranuclear Inclusions', *Am. J. Path.*, **39**, 195.

§ Bernhard, W. (1963), 'Some Problems of Fine Structure in Tumor Cells', in *Progress in Experimental Tumor Research* (Ed. Homburger, F.). Basel: Karger.

‖ Nowell, P. C., Hungerford, D. A., and Brooks, C. D. (1958), *Proc. Am. Ass. Cancer Res.*, **2**, 331. Quoted by Oberling, Ch., and Bernhard, W. (1961).

¶ Koller, P. C. (1947), 'Experimental Modification of Nucleic Acid System in Cells', *Symp. Soc. exp. Biol.*, No. 1.

** Oberling, Ch., and Bernhard, W. (1961), 'The Morphology of the Cancer Cells', in *The Cell*, Vol. V (Ed. Brachet, J., and Mirsky, A. E.). New York: Academic Press.

results. The different stem lines represent mutants and indicate the marked genetic instability of tumour cells; the mutations probably arise by reaction to altered environment, and may, during the course of tumour evolution, give rise to new lines with new properties.

The review by Oberling and Bernhard also includes descriptions of other changes found in cancer cells. Chromosomal morphology of neoplastic cells resembles other features of nuclear alteration, and presents in variable shapes and forms: from long filamentous threads to short, squat types. The anaphase may be disturbed by excessive cohesion between pairs, and striking irregularities are added to those affecting the form of the chromosomal material.

Mitotic aberrations are quite common in all the phases of cell division. Deviations may arise from abnormality of one or more intra- or extra-nuclear participating organelles. An example of abnormal chromosomal behaviour in anaphase has been mentioned above; during metaphase, formation of an equatorial plate may fail, i.e., the 'hollow metaphase', because the paired chromosomes congregate at the periphery; during telophase, more than two nuclei may form. Abnormalities may arise from irregular spindle development; absence of spindle fibres may result in failure of anaphase migration; and re-formation of the nucleus produces a cell with twice the normal chromosomal number, i.e., a form of polyploidy. Partial absence of spindle fibres leads to irregular aneuploidy. This may also arise from the formation of more than one spindle, giving rise to multipolar mitosis.

While deviations from normal mitotic division are common in malignant cells, none of them is characteristic and all of them may occur in non-cancerous conditions. Therefore, it is doubtful whether the abnormalities are part of the pathogenesis of malignancy; they may, in fact, be part of the effect upon the cell by some other agent.

CYTOPLASM

In the vast field of cancer research during the past 25 years, there has been little to alter the opinion by Bayne-Jones and others,* stated in a U.S. Public Health Report in 1938, to the effect that there are no fundamental differences and no striking variations in 'chemical make-up, enzyme content, metabolism or structure', between normal and malignant cells of the same tissue type. Nevertheless, the changes merit close study, for apart from significant quantitative alterations, hopeful anticipation of finding some crucial qualitative characteristic is justifiable. The structural features described in the text are included in *Fig.* 2.

As with malignant cell nuclei, so too with cytoplasm, the sum of the features in a field of cells is a valuable diagnostic element.

Size and Shape.—Normal cells maintain a fairly constant size and shape in a particular tissue or in the separate layers of one tissue;

* Bayne-Jones, S., Harrison, R. G., Little, C. C., Northrop, J., and Murphy, J. B. (1938), 'Fundamental Cancer Research', *Publ. Hlth Rep. Wash.*, **53**, 2121.

Cytoplasm—Size and Shape, *continued.*

whereas the size and shape of malignant cells derived from one tissue show marked variations. Whilst this generalization holds good for malignant cells from *one tissue*, it is important to note that there is a wider range of variation between normal cells of *different tissues* than is the case with malignant cells from *different tissues*. In the course of the evolution of malignant cells, their functions are reduced or eliminated, their environment is altered, and they come to assume structural organizations and forms more common to the different neoplastic cells than they are in their cells of origin. The result is that over the whole field of malignancy, there is a general tendency towards de-differentiation of cells to a more uniform primitive basic pattern.

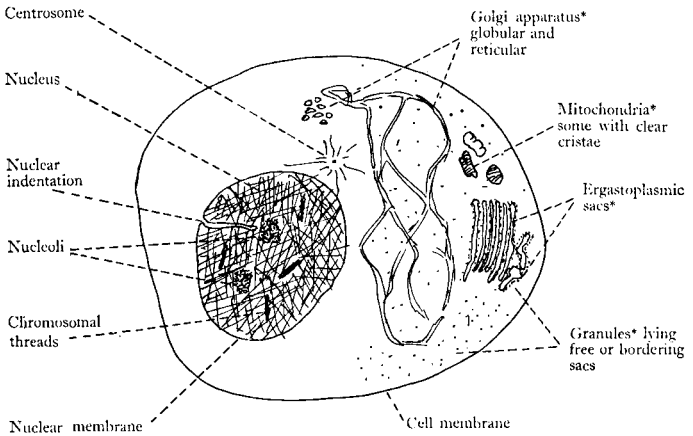

***Fig.* 2.—Diagram of cell constituents. * These constituents of cytoplasm are not confined to an area as suggested in the diagram.**

In most cancers, the cells are larger than those of the normal tissues from which they develop. In some, as shown by Cowdry and Paletta* in squamous-cell epitheliomas, the cancer cells are smaller; yet others show no notable differences in size.

Cowdry† records correlations between physiological activity and cellular change. Normal cells of many tissues respond to functional demands by an increase in size, and then become smaller with disuse; but malignant cells do not respond in like manner. Increase in normal cell volume follows storage of metabolites and foreign matter, but malignant cells exhibit only slight changes of this character.

* Cowdry, E. V., and Paletta, F. X. (1941), 'Changes in Cellular, Nuclear, and Nucleolar Sizes during Methylcholanthrene Epidermal Carcinogenesis', *J. natn. Cancer Inst.*, **1**, 745.

† Cowdry, E. V. (1955), *Cancer Cells*. Philadelphia: Saunders.

Cell Membrane.—Neither the light nor the electron microscope has revealed structural changes typically indicative of normal and malignant cell membranes.

There is a general tendency, most noticeable in anaplastic tumours, towards a rounded smoothness of membranous outline; differing from the irregularity due to microvilli present in varying numbers and sizes in normal cells. Normal cells in contact with one another often exhibit interlocking in jig-saw fashion of complementary surface villi (*Fig.* 3). It has been suggested that flattening of microvilli is one of the explanations for the diminution of mutual adhesiveness of malignant cells (*see* Chapter II). This attractive supposition must be tempered by several considerations. Not only are microvilli as rich in some tumour cells as those in the cells of their origin, but, as has been recorded by Mercer and Easty,* the villi may even be increased by comparison with their benign progenitors.

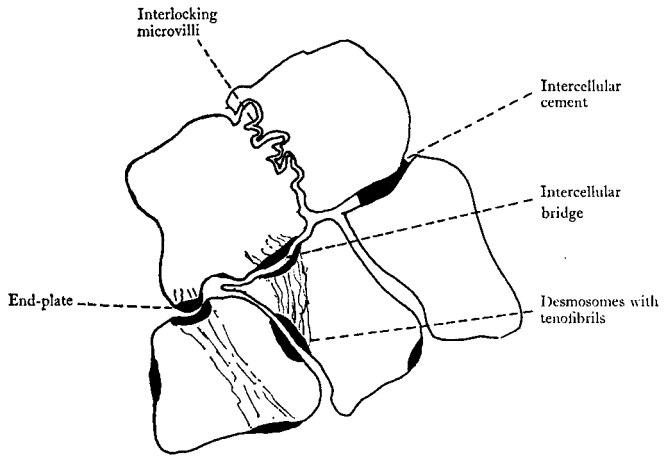

Fig. 3.—Diagram showing different forms of morphological cell cohesion.

Other elements of the ultrastructure of cell membranes may have a bearing on the maintenance of cell cohesion. Intercellular bridges, buttressed by desmosomes and terminal bars (*Fig.* 3) on the cell membrane, probably contribute to preserving cell contact. Oberling and Bernhard† review the evidence and refer to work showing disruptions of these connexions in a case of uterine cervical carcinoma, and in experimentally produced mouse skin tumours;

* Mercer, F. H., and Easty, G. C. (1961), 'The Cytology of the Walker Tumor', *Cancer Res.*, **21**, 52.

† Oberling, Ch., and Bernhard, W. (1961), 'The Morphology of the Cancer Cells', in *The Cell*, Vol. V (Ed. Brachet, J., and Mirsky, A. E.). New York: Academic Press.

Cytoplasm—Cell Membrane, *continued.*

and they note the absence of prickle cells in certain rabbit carcinomas. But here, too, finite deductions are subject to reservations, one of the most striking of which arises from the work of Bairati.* He reports that on purposive grouping together of Yoshida ascites (and therefore discrete) tumour cells, desmosomes are formed and appear to establish adhesion between the cells.

Other properties of cell membranes relative to cohesion and contact inhibition are noted in Chapter II.

Cowdry† reviews the various aspects of permeability of cell membranes: phagocytosis, cellular ingestion of microscopically visible particles, such as dyes and bacteria; pinocytosis, the drinking of fluids by cells; and clasmatosis, a reversal of pinocytosis, leading to pseudopodial protrusions which may become pinched off. Cancer cells do not exhibit any constant essential peculiarities of such processes. A similar conclusion is applicable to the viscosity and mineral content of cancer cells.

Mitochondria.—The search for specific changes in mitochondria was a part of the earliest micro-anatomical study of cancer cells (Cowdry‡). The early exciting promises have not been fulfilled, but the electron microscope has shown new features (Palade,§ Howatson and Ham,‖ and Bernhard¶), and although nothing conclusive has yet emerged, possibilities are still extant. Bernhard¶ reviews the findings in cancer cell mitochondria: alterations are more frequent than for other cytoplasmic organelles; their number and size are generally reduced and their shape is more variable than in normal cells. Irregularities of ultrastructure, such as that affecting their cristae and the swelling of the mitochondrial body, are related to cellular nutrition; a fact shown by restitution to normalcy when the cells are grown on an adequate medium.

Golgi Apparatus.—The original description, by Golgi,** of this cytoplasmic structure as a strand-like reticulum has been modified by later work. It is possibly a system of microminute canals, which may become fragmented and then return to canalicular form, in response to undetermined vital cellular activities which are not necessarily abnormal. As is the case with mitochondria, sophisticated methods are in use for the separation and analysis of the constituent chemical material of these elements of cytoplasm. The methods are not yet perfect and the conclusions are not yet finite.

* Bairati, A. (1961), 'Submicroscopic Structure of Yoshida Ascites Hepatoma', *Cancer Res.*, **21**, 989.

† Cowdry, E. V. (1955), *Cancer Cells.* Philadelphia: Saunders.

‡ Cowdry, E. V. (1918), 'The Mitochondrial Constituents of Protoplasm', *Contr. Embryol. Carneg. Instn*, **25**, 39.

§ Palade, G. E. (1953), 'An Electron Microscope Study of the Mitochondrial Structure', *J. Histochem. Cytochem.*, **1**, 188.

‖ Howatson, A. F., and Ham, A. W. (1955), 'Electron Microscope Study of Sections of Two Rat Tumors', *Cancer Res.*, **15**, 62.

¶ Bernhard, W. (1963), 'Some Problems of Fine Structure in Tumor Cells', in *Progress in Experimental Tumor Research* (Ed. Homburger, F.). Basel: Karger.

** Golgi, C. (1898), 'Sur la Structure des Cellules Nerveuses', (1) *Archs ital. Biol.*, **30**, 60.

Centriole.—Cellular polarity, i.e., the morphological arrangement of a cell reflecting its function and direction of action, is often indicated by an axis passing through the nucleus and a centrosome, within which is a centriole. The role of the centriole in mitosis has been noted earlier in this chapter, and it would appear justifiable to regard this microstructure as of basic importance in cell growth and multiplication. Centriole abnormalities may underlie mitotic aberrations. Cowdry* remarks on the loss of normal polarity of epithelial cells when cancer arises, and the fact that this may be reflected in variation of site of centrioles.

Ergastoplasm.—The term refers to basophilic-staining cytoplasmic material. It has been shown by Weiss† to be rich in ribonucleic acid (RNA). The material exists in organized saccular and also granular forms; according to Oberling and Bernhard,‡ the ergastoplasmic sacs tend to diminish in cancer cells, whereas the granules appear to increase.

Inclusions.—The cytoplasm of cancer cells often contains inclusions of different material, derived from phagocytosis, retained secretions and products of cellular activity, degenerative debris, and, in some proven animal virus tumours, virus particles. Apart from the last item, none of the inclusions is specific to malignancy.

PHYSIOLOGY OF CANCER CELLS

Most alterations of cancer-cell morphology are probably expressions of physiological changes and disorders. Tumour function, apart from a small number of exceptions, e.g., thyroid carcinoma may produce and secrete thyroxine and a liver cancer may produce bile, does not subserve any requirements of the general body economy. But functions and other physiological activities are deranged in malignant cells, and the nature of the derangements has been submitted to intensive study to gain a better understanding of the problem. Although much has been discovered, and light has been shed on many other biological phenomena, the dictum of Voegtlin§ that no distinctive property has been found to distinguish the malignant from the normal cell, and that the differences found are quantitative, not qualitative, still holds good today.

Le Breton and Moule‖ review the literature up to January, 1959, and state that there are still no answers to the questions—what is the initial and specific lesion that gives rise to the tumour? and what are the characteristics of a cell that is becoming cancerous?

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* Cowdry, E. V. (1955), *Cancer Cells*. Philadelphia: Saunders.

† Weiss, J. M. (1933), 'The Ergastoplasm. Its Fine Structure and Relation to Protein Synthesis as studied with the Electron Microscope in the Pancreas of the Swiss Albino Mouse', *J. exp. Med.*, **98**, 607.

‡ Oberling, Ch., and Bernhard, W. (1961), 'The Morphology of the Cancer Cells', in *The Cell*, Vol. V (Ed. Brachet, J., and Mirsky, A. E.). New York: Academic Press.

§ Voegtlin, C. (1938), 'Some Chemical Aspects of the Cancer Problem', *Science*, **88**, 41.

‖ Le Breton, E., and Moule, Y. (1961), 'Biochemistry and Physiology of the Cancer Cell', in *The Cell*, Vol. V (Ed. Brachet, J., and Mirsky, A. E.). New York: Academic Press.

Physiology of Cancer Cells, *continued.*

The known and postulated physiological and biochemical properties of cancer cells are noted in relevant sections of a number of later chapters. The changes have stimulated much theorizing to explain the origin and behaviour of cancer; and the number and variety of theories and hypotheses make the lack of factual proof very conspicuous.

SPREAD

Potential or actual spread of cancer cells from their place of origin to adjacent and remote sites in the body is a fundamental attribute of malignant tumours. The subject is reviewed in the next chapter.

CHAPTER II

CANCER SPREAD

The capacity for invasion of adjacent tissues and metastatic spread to more distant sites is fundamental to the concept and definition of malignant tumours.

DIRECT SPREAD

Tumour growth may take place by simple physical enlargement. Tissues in the proximity are pushed aside and compressed as the tumour occupies increasing space. This expansion characterizes the growth of benign tumours, and it plays a part in the enlargement and spread of malignant tumours. When it constitutes the dominant form of spread in malignant conditions, as happens in a minority of cases, it is usually classified as 'circumscribed cancer'. Even in such types of cancer, the additional form of spread by infiltration or invasion of contiguous tissues is present. This mode of direct spread, which is a special attribute of malignant neoplasia, involves actual migration of cancer cells into intercellular spaces and adjacent connective tissues. Apart from leucocytes and macrophages, adult cells do not normally migrate and invade other tissues. The correct and full explanation of the deviation of cancer-cell behaviour from normal would almost certainly provide considerable insight into the nature of the disease; but, although much is known, present knowledge falls short of completeness.

There are a number of theories explaining the invasive properties of cancer cells; more than one mechanism may operate, not only in different types of tumours but also in one tumour.

Persistent Multiplication.—Multiplication of cells, beyond normal restrictions and controls, and, for the most part, unrestrained and persistent, is so notable a characteristic of malignancy that it heads the list of neoplastic attributes. It is difficult to visualize this continuous increase of cells without concomitant space for it to occupy; and it is eminently reasonable to accept the proposition that increasing growth arising from cell multiplication demands room for its lodgement. A benign tumour grows larger within its capsule, which, in turn, pushes aside adjacent normal tissue to cater for increase in size. A malignant tumour is without a capsule; its growing edge, abutting on adjacent normal cells, pushes them aside and apart in the course of growth. Where there are zones or lines of reduced resistance, the force generated by increasing growth will act more readily.

This apparently self-evident mode of direct invasive spread is not universally accepted. It is increasingly common to find refutation of the thesis and denial of its existence. Willis,* who

* Willis, R. A. (1960), *Pathology of Tumours*. 3rd ed. London: Butterworths.

Persistent Multiplication, *continued.*

argues cogently in support of expanding growth creating a *vis a tergo* which thrusts cells at the growing edge into available crevices and spaces of contiguous structures, puts the mechanism in a balanced perspective. He writes: 'While proliferation is an important factor in invasive growth, it is certainly not the only or even the most essential one.' There is weighty evidence to sustain this opinion. Not all proliferating tumours invade surrounding tissues; many do so only after a period of purely expansive growth, e.g., circumscribed adenocarcinoma of the breast and kidney; and there are other tumours which do not build up a mass of cells capable of generating pressure extension into surrounding tissues, but which do exhibit infiltrative spread, e.g., there are relatively few cells in scirrhous cancer of the breast, yet invasive properties are marked. The same features characterize infiltrative cancers of the stomach. The rare type producing a leather-bottle or hose-pipe stomach, by its very extensive, diffuse infiltration, is particularly illustrative of this aspect, as cells are so sparse as to be very difficult to find. A further indication of malignant cell invasive behaviour comes from tissue-culture experiments. Wolff and colleagues*† have shown that when malignant tumour is laid against an organ in a culture medium, malignant cells invade the organ.

All this evidence points to the importance of the biological properties of cancer cells themselves and those of host tissues in determining the existence and manner of spread. Work on cellular adhesiveness offers part, if not substantial, explanation for some of the infiltrative characters of cancer.

Cellular Adhesiveness.—During microscopical examination of specimens from cancer of the lip, Coman‡ noted that the cells could be moved apart from one another much more readily than was possible with normal cells. He conducted experiments to measure the degrees of resistance to separation offered by normal, benign, and malignant cells. Using microneedles physically to pull cells apart, he measured the extent of bend in the needles, and, at the same time, observed morphological changes during separation of cells. The results of the experiments are summarized as follows:—

1. Normal cells of the lip and uterine cervix possessed high grades of mutual adhesiveness. *Fig.* 4 A illustrates that adhesion between cells was such that, during physical separation, they were pulled out into elongated shapes before parting with a sudden snap-like effect.
2. Benign tumours from lip and cervix presented squamous cells with the same degree of mutual adhesiveness as for normal cells.

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* Wolff, E., and Schneider, N. (1957), 'La Culture d'un Sarcome de Souris sur des Organes de Poulet Explantées *in Vitro*', *Archs Anat. microsc. Morph. exp.*, **46**, 173.

† Wolff, E., and Wolff, E. (1958), 'Les Résultats d'une Nouvelles Méthode de Culture de Cellules Cancéreuses *in Vitro*', *Revue fr. Étud. clin. biol.*, **3**, 945.

‡ Coman, D. R. (1944), 'Decreased Mutual Adhesiveness, a Property of Cells from Squamous Cell Carcinoma', *Cancer Res.*, **4**, 625.

3. Malignant squamous cells from lip and cervix had markedly reduced measures of adhesiveness, well below those found in normal and benign neoplastic cells. The physical separation of malignant cells (*Fig.* 4 B) was attended by only slight distortion, and ultimate divorce was not accompanied by an elastic-like recoil.

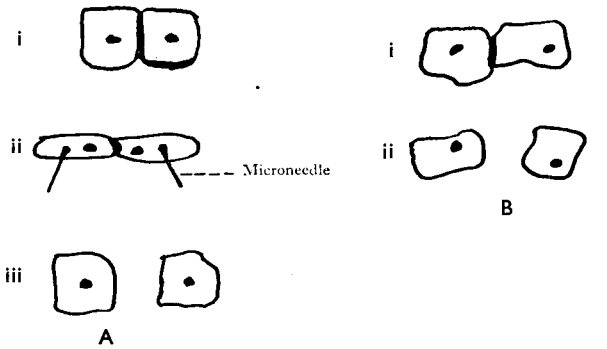

Fig. 4.—Illustrating Coman's experiments. A, Separation of normal cells accompanied by distortion and recoil effect. (i) Normal cells; (ii) Elongation of cells during physical separation; (iii) Separate normal cells with normal shape regained. B, Separation of malignant cells accompanied by slight distortion and without recoil. (i) Malignant cells; (ii) Readily separated; cells with irregular shapes and architecture.

Coman postulated the possibility that diminution of adhesiveness constituted a physical basis for malignancy, and he thought that it might explain both local invasion and distant metastases. He further suggested that the reduced calcium content of malignant cells was correlated with the diminished adhesive properties.

This original discovery stimulated many other investigations which have produced a considerable body of facts and much theoretical reasoning. McCutcheon and others* found the same decrease of adhesiveness in cells of adenocarcinomas. Coman and Anderson† reported a difference in ultrastructure of the cell membrane of normal and cancer cells: the regular particles, measuring from 30 Å to 60 Å in diameter, covering the surface of a normal cell, were replaced by irregular particles, varying from 30 Å to 300 Å in cancerous epithelial cells. A significant correlation between adhesive properties and malignancy was brought out by

* McCutcheon, M., Coman, D. R., and Moore, F. B. (1948), 'Studies on Invasiveness of Cancer: Adhesiveness of Malignant Cells in Various Human Adenocarcinomas', *Cancer*, **1**, 460.

† Coman, D. R., and Anderson, T. F. (1955), 'A Structural Difference between the Surfaces of Normal and of Carcinomatous Epidermal Cells', *Cancer Res.*, **15**, 541.

Cellular Adhesiveness, *continued.*

Coman in 1960.* It was shown that within an hour of application of the carcinogen 7,12-dimethyl-benzanthracene to a rabbit's ear, membranes of cells so exposed exhibited lowered adhesiveness; in the control experiment of applying a non-carcinogenic anthracene in the same medium, there was no such effect upon the epithelial cells.

PHYSICAL AND BIOCHEMICAL ASPECTS OF CELLULAR ADHESIVENESS.—A recent review by Abercrombie and Ambrose† includes facts and ideas culled from a wide spread of publications. Adhesiveness may be regarded as the resultant of opposing attraction and repulsion forces. Repulsion is electrostatic; e.g., red blood-corpuscles repel one another by their negative surface charges. Mutual attraction of cells appears to be an expression of electrostatic and chemical forces in which calcium ions have a predominant role. Decrease of calcium is accompanied by reduction of attraction and mutual adhesion. The place of immunological activity, particularly agglutination of cells by antigen-antibody reactions, is strongly suggested as a mechanism of intercellular adhesion, but as the property under review is adhesiveness between cells of identical type, it is difficult to place immunological factors in the picture.

Work on biophysical and biochemical bonds established between mutually adhering cells is another line of promising research. Cell membranes permit the passage and interchange of certain cellular elements; in the course of contact between like cells, bonds of protein and chemical structure are established and are visible morphologically under an electron microscope. Bonds between membranes vary from relatively long continuous contacts to those limited to one or more pseudopodial-like projections; the cellular cytoplasm adjacent to the site of contact often shows increased density. Luibel and others‡ report that attachment plates between cancer cells are fewer in number and less regular than in normal cells; and the dense plaque abutting on the site of intercellular cohesion is often absent or poorly defined in cancer cells.

Which of the mechanisms of maintaining or inhibiting cell contact alters in malignancy, and whether such alteration is a consequence, cause, or coincident feature of cancer, are unsettled questions. What is material in this chapter is the fact, now widely confirmed, that cohesiveness between cancer cells themselves and between cancer and normal cells is reduced, and that it is highly probable that this transformed property plays an important role in tumour infiltrative spread. It appears likely

* Coman, D. R. (1960), 'Reduction in Cellular Adhesiveness upon Contact with a Carcinogen', *Cancer Res.*, **20**, 1202.

† Abercrombie, M., and Ambrose, E. J. (1962), 'Surface Properties of Cancer Cells: A Review', *Ibid.*, **22**, 525.

‡ Luibel, F. J., Sanders, E., and Ashworth, C. T. (1960), 'An Electron Microscopic Study of Carcinoma in situ and Invasive Carcinoma of the Cervix Uteri', *Ibid.*, **20**, 357.

that diminution of cohesive power is a major mechanism of the escape of malignant cells from what has been described as 'contact inhibition'.

Contact Inhibition.—Following a wound of epithelium, the normal process of repair consists of proliferation of cells in the neighbourhood of the traumatically created edges, effecting regeneration of epithelium and closure or cover of the wound by the meeting of advancing margins of epithelium. The junction of one advancing edge of epithelium with another brings growth of epithelium in this direction to a stop, so that one layer does not grow into or overlap the other, suggesting that contact between the reparative cells inhibits further growth and spread. This mechanism was also demonstrated by Abercrombie and Heaysman* in fibroblasts, and it was named 'contact inhibition'.

Organization of normal cells into regular patterns of tissue is probably a manifestation, in part at least, of contact inhibition; and whatever may prove to be the ultimate explanation of this form of local control, the suggestion that it arises from some adhesive property of cell membranes is acceptable in the light of current knowledge. Such a hypothetical premise forms a background to the theory that malignant cells escape from the inhibitions of cell contact by reason of the loss of cohesive properties on their surfaces.

Lwoff† draws attention to the occasional occurrence of compatibility between the proteins of viruses and host cells; the viral membrane enters and becomes part of the cell surface, as is proved by the acquisition by the cell of haemagglutinating properties passed on by the virus. The author suggests that viral action of this sort may be responsible for freeing the cell from contact inhibition and conferring malignant invasive characters upon it.

Cell Motility.—The relative absence of adhesive powers of malignant cells has been linked with the motile properties of cells to explain malignant invasion.

Virchow, in 1863,‡ recorded the powers of movement of malignant cells. Many subsequent studies, including tissue cultures in more recent years, corroborate the early report; both mesodermal and epithelial tumour cells, as single entities or in small aggregations, possess the property of motility.

Amoeboid movement, details of which have been described by Enterline and Coman,§ appears to be the main mechanism by which the cells migrate. Coman|| puts the case that normal cells possess the same powers, but that mutual adhesiveness restricts

* Abercrombie, M., and Heaysman, J. E. M. (1954), 'Social Behaviour of Cells in Tissue Culture. II. Monolayering of Fibroblasts', *Expl Cell Res.*, **6**, 293.

† Lwoff, A. (1960), 'Tumor Viruses and the Cancer Problem', in a Symposium on the 'Possible Role of Viruses in Cancer', *Cancer Res.*, **20**, 820.

‡ Virchow, R. (1863), 'Ueber Bewegliche Thierische Zellen', *Virchows Arch. path. Anat. Physiol.*, **28**, 237.

§ Enterline, H. T., and Coman, D. R. (1950), 'The Amoeboid Motility of Human and Animal Neoplastic Cells', *Cancer*, **3**, 1033.

|| Coman, D. R. (1953), 'Mechanisms Responsible for the Origin and Distribution of Blood-borne Tumor Metastases: A Review', *Cancer Res.*, **13**, 397.

Cell Motility, *continued.*

their movements. Abercrombie and Ambrose* add membrane undulations as another possible mechanism of tumour-cell migration.

Lytic Substances of Cancer Cells.—Malmgrén and others† found that marked amounts of proteolytic enzymes were liberated from ascitic tumour cells into ascitic fluid. Several investigations, reviewed by Abercrombie and Ambrose, provide corroborative evidence of leakage of enzymes from other kinds of growing tumours as a result of increased permeability of tumour-cell membrane.

The released enzymes may affect surrounding tissues: cytolysis or a hyaluronidase-like effect may establish pathways for penetration by growing malignant cells.

Direct Invasion and Lines of Spread.—There are thus a number of biological characters of malignant cells, each of which, or more probably combinations of which, may account for their unique behaviour with major deviations from the pattern and set organization of normal tissues.

Of the factors possibly concerned in promoting disorganization and invasive spread of cancer cells—namely multiplication, reduced surface adhesiveness, cell motility, altered metabolic and electrostatic qualities, increased permeability of membranes with release of cytolytic enzymes—the most fundamental at the present stage of knowledge appear to be diminished adhesiveness and the capacity for cellular movement.

Direct invasion by malignant cells involves extension along intercellular tissue spaces, which may be predetermined by anatomical configuration, or they may be created by migrating tumour cells. Soft tissues with potential tracks are more liable to invasive growth than are dense, solid structures; so that, e.g., bone-marrow is much more susceptible than bone cortex; muscles more than ligaments; and visceral parenchyma more than its capsule. A nerve with its sheaths often provides a suitable track for extension.

A much less common direction of spread is intracellular. Tumour cells penetrate the membrane of a normal cell and grow within it, e.g., Willis‡ records cancer cells eroding through sarcolemma and growing within a muscle cell; other cells occasionally involved are hepatic, renal, and adrenal epithelium.

LYMPHATIC SPREAD

Lymphatic vessels offer pathways of low resistance to the spread of neoplastic cells; their importance and frequent involvement have been recognized for over a hundred years. Among others, it was reported by

* Abercrombie, M., and Ambrose, E. J. (1962), 'Surface Properties of Cancer Cells: A Review', *Cancer Res.*, **22**, 525.

† Malmgrén, H., Sylven, B., and Révész, L. (1955), 'Catheptic and Dipeptidase Activities of Ascites Tumour Cells', *Br. J. Cancer*, **9**, 473.

‡ Willis, R. A. (1952), *The Spread of Tumours in the Human Body.* London: Butterworths.

Waldeyer in 1867;* and in English literature, Stiles presented an early account in 1899.†

Cancer cells readily transgress the permeable and relatively wide-meshed walls of lymphatic capillaries. Within the channel, growth and extension of malignant cells are unimpeded for varying distances. Extension may advance by propagation of a column of malignant cells, persistent multiplication driving the leading cells progressively farther along the lymph-vessel: a form of extension known as permeation. At one time, particularly following the classic publication on breast cancer by Handley in 1922,‡ permeation was held to be the usual and more important mode of lymphatic extension. It is now considered unusual except in capillaries. A picture of cancerous plugging of small lymphatic channels may occasionally be seen with the naked eye on pleural and peritoneal surfaces as a criss-cross of irregular mosaic pattern, and, more rarely, at other sites. Any lymph-vessel may be invaded: interstitial, perineural, and perivascular channels. At histological examination, the latter two are apt to catch the eye because the major structural feature (i.e., the vessel or nerve) tends to take a prominent place in the section.

The more common form of dissemination of tumour along lymphatic channels is embolization: single cells, or small aggregations, separate off and travel with the lymph-stream. The direction of flow is usually central, but retrograde or side-channel courses may follow a block to the stream farther ahead, so that flow from larger to smaller lymph-vessels occurs. In this manner, for example, when major lymph-vessels are blocked by breast cancer cells, reverse flow may cause metastatic nodular infiltration of the skin of the breast. Another well-recognized example arises when efferent vessels from a lymph-node become blocked; then spread along communicating channels may lead to involvement of a whole group of lymph-nodes, which become matted together by adhesions. Lateral flow may arise because of special normal anastomotic communications, e.g., thoracic duct communications (*vide infra*). An embolus of malignant cells may be held up within a lymph-vessel and implant in its wall to establish a metastatic growth at that site. The hold-up of an embolus, however, is more usual in a lymph-node.

Among their other functions, lymph-nodes act as filter stations along the course of the lymphatic stream. Lymph enters a node via its afferent vessels, a number of which pass through the peripheral capsule of the node, taking lymph into its subcapsular sinus; thence lymph percolates slowly through the substance of the node, being mechanically filtered and exposed to cellular phagocytic and other biological action. Towards the hilum of the node, the lymph is again channelled and it leaves the node as an efferent vessel which flows farther centrally.

Zeidman and Buss,§ in experiments on rabbits, found that some time following injection of tumour cells into popliteal afferent lymphatic

* Waldeyer, W. (1867), 'Die Entwicklung der Carcinome', *Virchows Arch. path. Anat. Physiol.*, **41**, 47.

† Stiles, H. J. (1899), 'On the Dissemination of Cancer of the Breast', *Br. med. J.*, **1**, 1452.

‡ Handley, W. S. (1922), *Cancer of the Breast.* London: Middlesex Hosp. Press.

§ Zeidman, I., and Buss, J. M. (1954), 'Experimental Studies on the Spread of Cancer in the Lymphatic System. I. Effectiveness of the Lymph Node as a Barrier to the Passage of Embolic Tumor Cells', *Cancer Res.*, **14**, 403.

Lymphatic Spread, *continued.*

vessels, cancer growth began in the subcapsular sinus of a popliteal node; it continued to grow, but remained trapped for an appreciable period before the next node was affected; it was also shown that nodal involvement progressed from node to node without skipping and leaving an intervening node free of cancer. These authors concluded that lymph-nodes are effective barriers to cancerous spread for a limited period, and that the same circumstances probably apply to man. This view is not accepted by all authorities, some of whom hold that a lymph-node may provide suitable soil in which cancer flourishes.

The thoracic duct may be invaded, especially from upper abdominal carcinoma and from metastatic affection of the upper abdominal, or coeliac, group of pre-aortic lymph-nodes. The duct may be permeated or carry emboli. From the thoracic duct, lymph carrying malignant cells flows into the left subclavian veins; but there are a number of lymphatic connexions on the way that explain certain nodal metastases. Zeidman* studied the fate of tumour emboli in the rabbit's thoracic duct. He found that injection of tumour cells into the thoracic duct gave rise to metastatic tumours in mediastinal lymph-glands. Many of these tumours began in the subcapsular sinus of a node, indicating that communications from the thoracic duct to mediastinal nodes in these cases was afferent to the nodes. In some metastatic tumours, growth began in the hilar region of a node, indicating that the cancer cell had travelled from the thoracic duct to the node via an efferent vessel from the node, i.e., against the normal current for this node. These experiments demonstrated a pathway for mediastinal node involvement; and also suggested that in man, where lymph-channel communications are similar to those of the rabbit and include communications with intercostal and supraclavicular nodes, the explanation for involvement of these three sets of glands (including Virchow's or the left supraclavicular node) was to be found.

VENOUS SPREAD

Apart from the route just described, namely via the thoracic duct or other lymph-vessels emptying their content directly into veins, malignant cells reach the blood-stream in other ways.

Tumours may infiltrate directly into veins of various sizes, and grow within the lumen as a permeating plug, or with release of emboli which are carried centrally in the blood-stream. Whether the pattern of spread is permeation or embolization does not seem to depend upon the calibre of the vein, as is the case in lymph-vessels, but rather upon specific properties of particular tumours. Hypernephroma and chondrosarcoma are two outstanding examples of tumours extending by vein permeation. They erode into veins and propagate by continuous growth even to the extent of entry into the right side of the heart, and occasionally into pulmonary arteries. The degree of cohesiveness of the cancer cells may play a major role in determining the mode of spread. Small veins and

* Zeidman, I. (1955), 'Experimental Studies on the Spread of Cancer in the Lymphatic System. III. Tumor Emboli in Thoracic Duct. The Pathogenesis of Virchow's Node', *Cancer Res.*, **15**, 719.

capillaries are more frequently invaded than larger vessels; they are more intimately associated with tumours and their thin walls offer less resistance to penetration.

In general, venous involvement is common: Willis,* in a series of 64 autopsy examinations of cases with head and neck cancer, found veins affected in 30. Both portal and systemic venous systems are often affected, but arterial invasion is exceptionally rare.

The factors determining whether an intravenous cancerous embolus will survive to take seed at some distant site, and what influences occasion particular sites of metastatic deposits, provide problems which are not all finally settled.

The 'seed and soil' concept furnishes some of the answers. Cancer cells represent the seeds; they vary in their capacity for implanting and growing. The more undifferentiated the tumour, the greater the incidence of blood-borne metastases. This correlation has been shown clearly in rectal carcinoma. Dukes,† using the grading of tumours proposed by Broders,‡ i.e., a grading based upon histological enumeration of the proportion of anaplastic cells, found the incidence of venous spread as recorded in *Table II.*

Table II.—VENOUS SPREAD IN DIFFERENT GRADES OF CANCER OF THE RECTUM

Grade of Cancer	*Incidence of Venous Spread*
I (up to 25 per cent of anaplastic cells)	Less than 5 per cent
II (25 to 50 per cent anaplastic cells)	11·8 per cent
III (50 to 75 per cent anaplastic cells)	More than 25 per cent
IV (over 75 per cent anaplastic cells)	31 per cent

The increased powers of invasion of veins by anaplastic tumours is parallel to their powers for direct invasion of surrounding tissues, and may well prove to be related to a combination of increased mitosis plus diminution of adhesiveness. The organ in which metastases occur represents the 'soil'. Willis,§ among many others, has recorded that microscopical examination of the lungs in deaths from malignant tumours often reveals tumour emboli undergoing degeneration; the lung thus appears as an unfavourable soil for many blood-borne seeds. The liver, on the contrary, seldom exhibits such failure to give root to cancer emboli. Here, almost invariably, the soil appears amenable. The reasons for the take or rejection of transported cancerous tissues may reside in the respiratory (especially the degree of oxygenation of the tissues) and metabolic activities of the 'soil'; and much current research is being directed to these aspects of the problem.

* Willis, R. A. (1941), 'A Review of 500 Consecutive Cancer Necropsies', *Med. J. Aust.*, 2, 258.

† Dukes, C. E. (1944), 'The Surgical Pathology of Rectal Cancer', *Proc. R. Soc. Med.*, 37, 131.

‡ Broders, A. C. (1926), 'Carcinoma, Grading and Practical Application', *Archs Path.*, 2, 376.

§ Willis, R. A. (1952), *The Spread of Tumours in the Human Body.* London: Butterworths.

Venous Spread, *continued.*

Portal circulatory spread takes malignant cells to the liver. A special pattern of distribution depending upon the site of the primary tumour is often noticeable. Primary tumours in the field drained by the superior mesenteric vein tend to metastasize to the right lobe of the liver; portal drainage via the inferior mesenteric and splenic veins carries most metastases to the left lobe. This distribution has been ascribed to the general maintenance of separation of venous streams derived from superior mesenteric and splenic tributaries in the main portal vein until it branches right and left at the porta hepatis. Within the liver, a metastatic or primary tumour may give rise to further metastases distributed by the portal system in more peripheral zones of the liver. Tumour cells may also invade hepatic veins and so be transported on a systemic route.

Spread by Caval System.—Systemic venous spread commonly produces metastases in the lungs and in certain bones. The caval system brings venous blood containing tumour cells to the right heart, whence it is pumped into the lungs via pulmonary arteries. Aggregations of cells are held up in larger pulmonary branches, whereas small clumps or single cells lodge in or near the capillary zone. Although the lungs do not particularly favour implantation and growth of metastases, emboli to it are so plentiful that the small percentage that does take root adds up to make the lungs the commonest site of blood-borne metastases in the human body.

Spread by Vertebral System.—Appreciation of the mechanism of venous metastases to the axial part of the skeleton, namely the vertebral column, skull, and pelvis, has been facilitated by the investigations of Batson.*† He reports the demonstration of a 'vast intercommunicating system of veins which . . . is constantly and physiologically the site of reversals of flow'.

In the vertebral column, a segmental pattern of veins at each vertebral level presents (*Fig.* 5) perivertebral and epidural venous plexuses, which receive tributaries from bone, spinal cord, and other soft tissues. At intervertebral spaces, there are intervertebral veins which communicate with perivertebral and segmental veins of the thoraco-abdominal wall (i.e., intercostal, lumbar, and lateral sacral) and with veins of the pelvis and the head and neck. There are also connexions with azygos and pulmonary veins, and occasionally with renal and portal veins. Vertebral veins at segmental levels are linked together to produce a longitudinal course, from the sacrococcygeal level to the base of the skull.

At the base of the skull, the longitudinally directed vertebral plexus ends in free communications with the great dural sinuses, which themselves drain the brain and meninges and the diploic veins of the skull. Free anastomoses, via emissary veins, with extracranial veins and plexuses, add to the area of a great lake of

* Batson, O. V. (1940), 'The Function of Vertebral Veins and their Role in the Spread of Metastases', *Ann. Surg.*, **112**, 138.

† Batson, O. V. (1942), 'The Role of the Vertebral Veins in Metastatic Processes', *Ann. intern. Med.*, **16**, 38.

venous blood. Most of these veins drain into the internal jugulars, but their many intercommunications and sluggish course provide an extensive by-pass system.

At the lower end of the vertebral system, a trellis work of veins drapes the visceral surfaces of the sacrum, lumbar spine, and iliac wings. This plexus drains the related bones and their segmental groups of veins and also has numerous connexions with veins of the

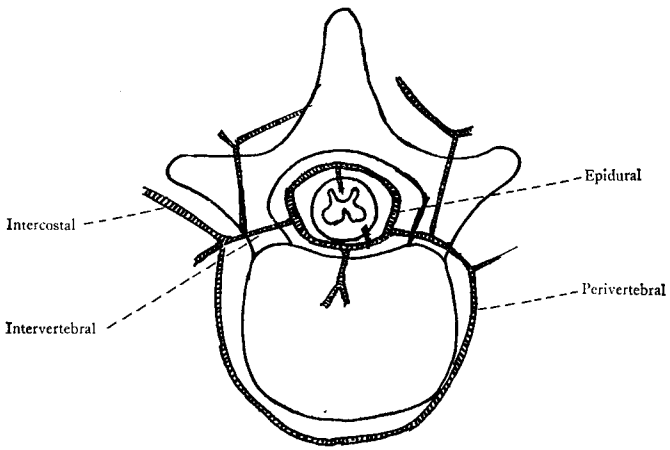

Fig. 5.—Vertebral segmental pattern of veins.

pelvic viscera. Batson found that, in the cadaver, injection of the dorsal vein of the penis (which freely inosculates with retropubic and prostatic venous plexuses) filled the pelvic end of the vertebral system (*Fig.* 6) as well as draining into the internal iliacs and inferior vena cava. Between the vertebral and caval veins there were rich and free anastomoses.

Changes in the calibre of caval veins cause pressure changes which are directly transmitted to vertebral veins, so that the latter may act as collateral channels when caval drainage is reduced. This happens in many normal circumstances: inspiration creates negative intrathoracic pressure so inducing increased flow from the venae cavae into the heart and consequent flow from vertebral into caval veins; expiration, and more particularly forced expiration as in coughing, increases intrathoracic pressure leading to compression of the great caval veins and thus brings about a reversal of venous flow into vertebral veins. Increased intra-abdominal pressure, as by deep inspiration, coughing, and straining, similarly leads to this direction of flow. Therefore, malignant cells that have invaded tributaries of the vertebral system, as well as those emboli within the caval system that have been shunted into the vertebral system

Spread by Vertebral System, *continued.*

by forces regulating venous flow, may readily give rise to metastases in skull and brain, and in vertebral and pelvic bones, without passing through the lungs into the arterial system.

Clinical practice offers several examples supporting the idea of this type of venous spread. Mammary veins are tributary to intercostal and subclavian veins which have communications with thoracic vertebral veins; it is suggested that the frequent axial

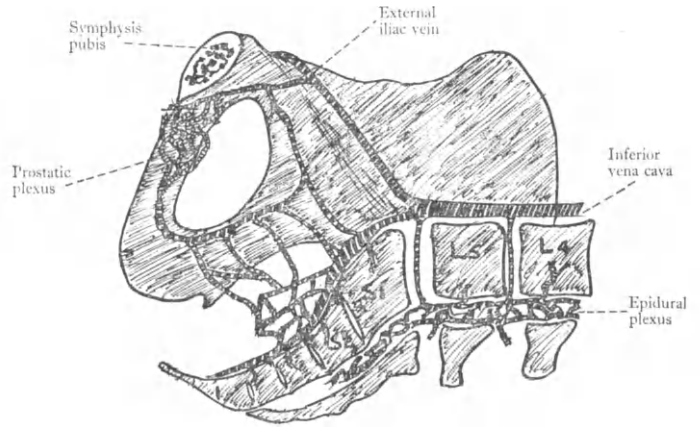

Fig. 6.—The pelvic part of the vertebral plexus of veins.

skeletal metastases from breast cancer travel by this route. Thyroid veins drain mainly into the jugulars, but also anastomose with veins of the cervical vertebral region; and bone secondaries may well arise in this manner. Cancer of the prostate frequently gives skeletal metastases in pelvic and vertebral bones, probably by direct drainage to the vertebral systems as well as by veins between caval and vertebral systems. In like manner, cerebral metastases (often the only ones) of bronchogenic carcinoma may be explained.

SYSTEMIC ARTERIAL SPREAD

The not uncommon presence of multiple metastatic growths in kidneys, adrenals, limb bones, and other organs is explicable only on the basis of systemic arterial transportation of cancer cells. As direct invasion of arteries is very exceptional, it is a reasonable presumption that cancer cells reach the left side of the heart by passing through lung vessels, from the pulmonary arterial side to pulmonary veins. Zeidman and Buss* demonstrated experimentally that isolated malignant cells

* Zeidman, I., and Buss, J. M. (1952), 'Transpulmonary Passage of Tumor Cell Emboli', *Cancer Res.*, 12, 731.

are capable of passing through the lungs to reach the aorta. This does not necessarily imply that malignant cells have traversed the capillary bed; they may pass via arteriovenous shunt vessels which by-pass capillary networks, a route described by Prinzemetal and others.*

A second plausible explanation is that a metastatic tumour forms in the lung; it grows and invades pulmonary vein capillaries, and then its cells reach the left side of the heart and the arterial system.

NON-VASCULAR IMPLANTATION SPREAD

Cancer cells may be released into serous and cerebrospinal spaces and into duct structures. At each of these sites, the cells may take seed on a surface and start an implantation metastasis.

This is not infrequent on the peritoneum, e.g., a cancer of the stomach or ovary, especially colloid or mucinous tumours, reaches and erodes through the serosal surface by directex tension, and then spreads over an increasing area of the surface by continuity at the site of break-through; and, by implantation, cancer affects more distant sites on the peritoneum, mainly in the pelvic pouch and along mesenteric attachments. Bronchogenic carcinoma may similarly break through the pleural surface, and cells released into pleural fluid may then seed, particularly in the costophrenic sinuses, diaphragmatic surface, and paravertebral recesses. Resultant peritoneal and pleural effusions are usually marked; they are blood-stained and contain cells which may be recognized with Papanicolaou techniques.

Cerebrospinal fluid may act as a vehicle for transporting malignant cells, e.g., from medulloblastoma, which implant on arachnoid and ventricular linings, or along perivascular subarachnoid prolongations (Virchow-Robin spaces). Such implantations are most common near the basal regions of the brain and at the lower end of the spinal canal.

Paths for cell transfer may be offered by tubes and ducts of viscera, e.g., from uterus along Fallopian tubes and from nasopharynx along Eustachian tubes. It is doubtful whether the ureter transfers cells from the renal pelvis to implant in the lower reaches of the urinary tract; it seems more likely that the so-called implantation secondaries are, in fact, primaries appearing at different times at multifocal sites.

A form of contact or implantation growth occurs in epithelial cavities. This arises from constant or repeated physical contact of unaffected epithelial lining against a cancer. Examples are contact cancers in the mouth and the vulva.

IATROGENIC SPREAD

Cancer has been known to extend along the track of a needle used for diagnostic aspiration of a 'lump'. Cole and others† report that spread may affect not only a skin-graft at its recipient site but also at the donor site. Stab wounds for operative drainage, and suture lines following surgery, may also become involved in cancer extension. The evidence is strong and points to the promotion of tumour spread by a diagnostic

* Prinzemetal, M., Ornitz, E. M. jun., Simkin, B., and Bergman, H. C. (1948), 'Arteriovenous Anastomoses in Liver, Spleen and Lungs', *Am. J. Physiol.*, **152**, 48.

† Cole, W. H., McDonald, G. O., and Roberts, S. S. (1959), 'Dissemination of Cancer and its Prevention', *Jl R. Coll. Surg. Edinb.*, **4**, 218.

Iatrogenic Spread, *continued.*

or therapeutic procedure: either a track is made available for direct invasion, or cancer cells are dislocated and transplanted to a new place.

Dissemination along veins may also be promoted and/or accelerated by handling and manipulation during an operation, and possibly during rough examination.

Knowledge of this iatrogenic danger has led to the adoption of special operative techniques; early control of vessels; early control of epithelial tubes and ducts; the use of cytotoxic agents before, during, and after operation, systemically and locally; the avoidance of massaging or traumatizing tumour tissues; and scrupulous prevention of cell transference. Smithers* recommends preoperative radiotherapy as well as chemotherapeutic cover at operation as prophylactic measures against spread during surgery.

* Smithers, D. W. (1962), 'Reflections on the Spread of Tumours by the Blood Stream and the Prevention and Control of Metastases', *Br. J. Radiol.*, **38**, 581.

CHAPTER III

AETIOLOGY—INCIDENCE

In the whole field of many different neoplastic diseases, there are sectors in which cause and biological properties are deducible with a reasonable degree of certainty. However, the unknown areas of aetiology and biology exceed the known, and there are, at present, no acceptable all-inclusive generalizations to explain cause or pattern of neoplasia in general.

There are authoritative opinions that the very diversity of neoplasms makes it unlikely that there exists a single or common underlying basis for all tumours; but that, on the contrary, the answer to the problem of the 'cause of cancer' is more likely to be found in a multiplicity of pathogenic factors for a variety of pathological entities.

There are many facets to the aetiology of cancer. Some provide good evidence of pathogenic agencies; others are in the stage of theory and speculation; all are of importance as their correlation might furnish far-reaching appreciations of cancer problems. In this respect, the incidence of cancer is of basic importance.

INCIDENCE

Gross Mortality.—Cancer is second on the list of lethal diseases in man, causing about one of every six deaths. The overall absolute incidence is increasing. This is due to a number of factors: people are living to older ages when the risks of cancer are higher; improved clinical knowledge and better diagnostic measures account for part of the higher figures; and there are indications of exposure on a wider scale to carcinogenic agents.

The magnitude of the increase is well demonstrated by a comparison of the causes of death in England and Wales reported by the Registrar-General in 1910* and 1959.† In 1910, tuberculosis, diseases of the heart, pneumonia, and respiratory diseases, preceded cancer in the list; in 1959, cancer was cause number three, following heart and central nervous system diseases. In 1910, cancer caused 7·2 per cent of deaths; in 1959, it had risen to 18·6 per cent. The comparison is represented diagrammatically in *Fig.* 7.

The gross statistical records are as stark in other parts of the world. An example from the records of New South Wales, Australia, is depicted in *Fig.* 8.

In the United States of America, cancer also comes second to diseases of the heart as a cause of death. *Fig.* 9 is a diagram of

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* Registrar-General, *Annual Report of Births, Deaths, and Marriages in England and Wales* (1910). Vol. 73. London: H.M.S.O.

† Registrar-General, *Statistical Review of England and Wales for* 1959. *Tables, Part* 1, *Medical.* London: H.M.S.O.

Gross Mortality, *continued.*

comparative mortality-rates constructed from the tables of mortality per hundred thousand living per year in the U.S.A., as reported by White and Geschickter.*

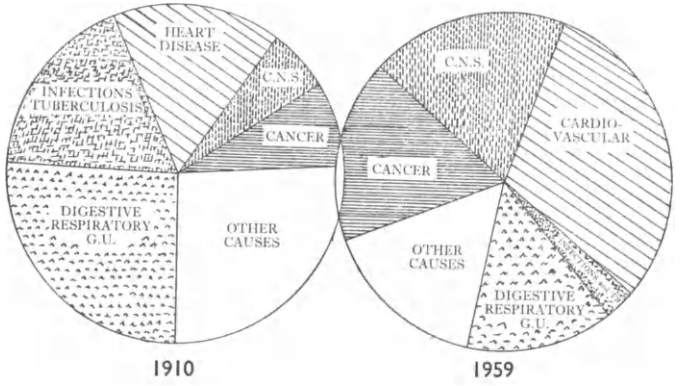

Fig. 7.—Change in pattern of causes of death in England and Wales from 1910 to 1959. (Adapted from Reports of Registrar-General, England and Wales.)

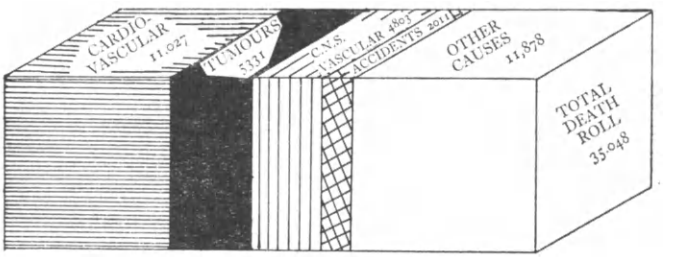

Fig. 8.—The 'N.S.W. 1961 Coffin'. Causes of death. (Adapted from Records of *New South Wales Year Book*, 1963.)

RESERVATIONS REGARDING STATISTICS.—These mortality-rates, derived from analyses of death certificates, are subject to reservations on account of inaccuracies arising from the recorded information. Some of the important sources of inaccuracy are:—

1. DEATHS AMONG THE ELDERLY.—There is little doubt that many errors creep into entries in certificates for deaths of old people.

* White, B. V., and Geschickter, C. F. (1947), *Diagnosis in Daily Practice.* Philadelphia: Lippincott.

The cause of death and the associated pathological conditions are not infrequently hurriedly and incompletely certified in such deaths, as reasons for precision are not regarded as very stringent, by contrast with the attitude adopted towards deaths at younger ages. As the older age-groups bear a greater incidence of tumours, this error is an appreciable quantity. Sample studies of the nature of such inaccuracies have pointed to a 'negative', rather than a 'positive', influence upon the statistical picture. That is, the tumour aspect is more often omitted from the certificate, and 'heart' and 'lung' diseases are certified as 'primary' causes more frequently than is in fact the case.

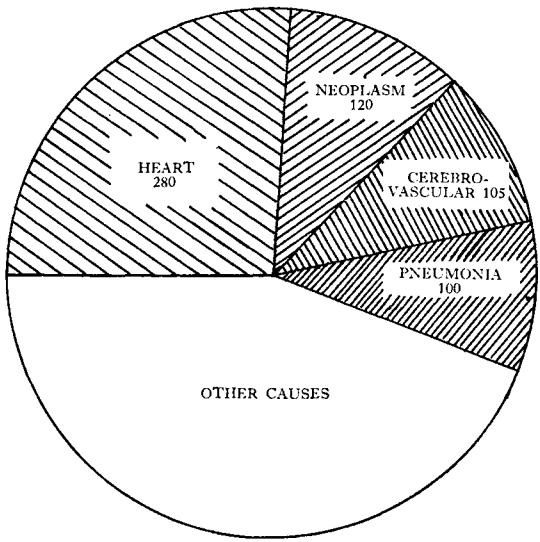

Fig. 9.—Mortality per 100,000 living per year (U.S.A.). (Adapted from tables from White, B. V., and Geschickter, C. F. (1947), *Diagnosis in Daily Practice.* Philadelphia: Lippincott.)

The precise extent of the error is difficult to calculate, but it seems clear that the correction for this older age-group would add to the figures describing tumours as primary and contributory mortal diseases.

2. DIAGNOSIS OF 'DEEP' TUMOURS.—At any age, the diagnosis of 'deep' tumours, e.g., lung, stomach and intestinal tract, kidney, male and female internal genito-urinary organs, and brain, may prove to be difficult; the difficulty is more pronounced in centres not specially equipped for complete

Gross Mortality, *continued.*

investigation. Kennaway and Kennaway* record a significant instance. They noted a diagnostic error of 42 per cent in a group of 84 lung cancers proven at autopsy. The extent of error varies from place to place: at centres specially designed and administered for the treatment of cancer, errors are few; in more peripheral areas, mistakes are common. All the death certificates are taken together to extract 'mortality statistics', which must therefore be viewed in the light of the different standards of diagnostic exactitude.

3. AGE COMPOSITION.—This is an important consideration, especially when mortality-rates either of different periods of time or of different communities are under comparison. It is obvious that if, in a particular community, there is a high proportion of older aged people, the tumour rate will be high simply because more tumours occur at such ages. In a similar-sized group of people with a smaller ratio of old to young age composition, and if all other factors are equal, the tumour rate will be lower. This consideration applies to other forms of statistical record as well as mortality-rates; and all figures of rates of occurrence of tumours should be corrected for age to eliminate this source of error.

Morbidity Rates.—The substantial qualifications applicable to mortality statistics emphasize the importance of other methods of assessing and describing the occurrence of cancer. The cancer *incidence rate* is a record of the new cases of neoplasm per hundred thousand population per year. The cancer *prevalence rate* refers to the number of cancers, whether new or already existing from previous years, per hundred thousand living population per year. Both these rates provide a better measure of the occurrence of cancer than does mortality statistics. They are also subject to margins of error, in that they are dependent upon reports and notifications, which may be inaccurate; but such inaccuracies are comparatively small and the degree of vitiation of deduction is generally low. When different forms of statistical studies, and different approaches in the study of one disease, lead to a similar set of deductions, the validity of the deductions assumes a greater weight and significance. The importance of the summation of the evidence is well illustrated in the evaluation of different statistical studies of cancer of the lung and the reasonable conclusion that its main cause is cigarette smoking (*see* Chapter IX).

Another meaningful illustration of the usefulness of statistical methods may be seen in records of epidermoid cancer of the skin. The role of sunlight as a cause in fair-skinned people has been confirmed by many statistical studies (*see* Chapter VI). Among dark-skinned peoples, the incidence of squamous-cell and basal-cell carcinomas is very much less; and when these cancers do occur, their localization is notably different from that in people of light

* Kennaway, E. L., and Kennaway, N. M. (1947), 'A Further Study of the Incidence of Cancer of the Lung and Larynx', *Br. J. Cancer*, **1**, 260.

Table III.—PROBABILITY OF DEVELOPING CANCER FROM BIRTH ONWARDS BY SEX AND SITE BASED ON CANCER MORBIDITY REPORTS, NEW YORK STATE, EXCLUSIVE OF NEW YORK CITY, 1949–51 (*Goldberg and others*, 1956)

MALE		FEMALE	
Site	Probability	Site	Probability
	per cent		per cent
Skin	2·988	Breast	5·544
Prostate	2·613	Intestines	2·691
Lung and bronchus	2·114	Large	2·650
Stomach	1·994	Small	0·041
Intestines	1·894	Skin	2·507
Large	1·851	Cervix	2·319
Small	0·043	Fundus uteri	1·600
Rectum	1·224	Stomach	1·348
Bladder	1·140	Rectum	1·016
Leukaemia and aleukaemia	0·622	Ovary	0·993
Pancreas	0·618	Pancreas	0·530
Lip	0·486	Bladder	0·528
Oesophagus	0·476	Leukaemia and aleukaemia	0·508
Larynx	0·402	Lung and bronchus	0·369
Kidney	0·324	Biliary passages	0·360
Liver	0·267	Liver	0·302
Mouth	0·260	Kidney	0·236
Brain	0·243	Rectosigmoid	0·224
Tongue	0·239	Lymphosarcoma	0·206
Rectosigmoid	0·222	Vulva	0·199
Lymphosarcoma	0·218	Hodgkin's disease	0·173
Hodgkin's disease	0·210	Thyroid gland	0·166
Testis	0·159	Brain	0·163
Biliary passages	0·142	Oesophagus	0·146
Pharynx	0·127	Salivary gland	0·113
Bone	0·110	Bone	0·093
Salivary gland	0·084	Mouth	0·080
Thyroid gland	0·068	Tongue	0·070
Breast	0·049	Lip	0·040
		Larynx	0·034
		Pharynx	0·032
All others	2·278	All others	2·399
All sites	21·572	All sites	24·990

complexion. In the latter, the lesions occur mainly on parts exposed to ultra-violet light, viz., face, ears, and dorsum of hands; in dark people, the lesions are most numerous on the legs (Higginson and Oettlé*), especially in the immediate neighbourhood of old scars. The inference emerges that, in the dark-skinned, trauma is a major causative agent, and sunlight plays little or no part at all.

* Higginson, J., and Oettlé, A. G. (1960), 'Cancer Incidence in the Bantu and "Cape Coloured" Races of South Africa. Report of a Cancer Survey in the Transvaal (1953–1955)', *J. natn. Cancer Inst.*, **24**, 589.

Morbidity Rates, *continued.*

Table IV.—Primary Cases. Anatomical Site and Sex (*Harnett*, 1952)

	Males		Females	
	No.	Per cent	No.	Per cent
Nasal cavity	6	0·09	2	0·03
Paranasal sinuses	57	0·81	45	0·63
Nasopharynx	27	0·38	17	0·24
Tonsil	114	1·62	12	0·17
Pharynx	284	4·05	101	1·41
Intrinsic larynx	114	1·62	10	0·14
Trachea	7	0·10	—	—
Lung	842	12·00	182	2·54
Mediastinum	20	0·29	10	0·14
Thymus	4	0·06	—	—
Thyroid	21	0·30	78	1·09
Lip	155	2·21	8	0·11
Mouth	277	3·95	37	0·52
Tongue	247	3·52	40	0·56
Salivary glands	24	0·34	27	0·38
Oesophagus	395	5·63	75	1·05
Stomach	856	12·20	549	7·66
Small intestine	12	0·17	11	0·15
Colon	408	5·81	484	6·76
Rectum	850	12·11	540	7·54
Anus	15	0·21	17	0·24
Liver	11	0·16	11	0·15
Gall-bladder and bile-ducts	33	0·47	54	0·75
Pancreas	134	1·91	111	1·55
Kidney	81	1·15	33	0·46
Adrenal	11	0·16	9	0·13
Bladder	315	4·49	108	1·51
Prostate	383	5·46	—	—
Testis	63	0·90	—	—
Penis	67	0·95	—	—
Breast	23	0·33	2129	29·71
Ovary and Fallopian tubes	—	—	361	5·04
Cervix uteri	—	—	859	11·99
Corpus	—	—	288	4·02
Vagina	—	—	35	0·49
Vulva	—	—	110	1·54
Skin	662	9·43	418	5·83
Sarcoma of soft tissue	112	1·60	93	1·30
Sarcoma of bone	65	0·93	58	0·81
Brain	132	1·88	76	1·06
Spinal cord	9	0·13	10	0·14
Eye	29	0·41	32	0·45
Ear	—	—	1	0·01
Metastatic cancer	152	2·17	124	1·73
Totals	7017	100·00	7165	100·03

Despite the heavy critical attacks launched by a number of authorities against statistical methods, there is considerable value in such studies; many have pointed the road to discovering a cause of a particular neoplasm; others have added weight to inferences derived from other different forms of study. Provided that statistics are viewed with discrimination and treated with appropriate reservation, they contribute a helpful addition to the study of tumours.

As has been noted, rates of incidence and prevalence contain relatively few errors. Two examples of tables of these less fallible figure will serve to indicate their usefulness.

On the basis of reports of cancer morbidity in New York State, Goldberg and others* have deduced the probability of developing cancer. Their figures are reproduced in *Table III*. The table is of importance, not only for its picture of the probable incidence of cancer generally, but also for the particular organs affected. It shows that of all males, 1 of 5 will probably develop neoplasia; and that 1 of every 4 females will suffer from the disease. Calculations of this sort require corroboration from other sources before confident acceptance, but they are an indication of the measure of the problem for population groups comparable to that in New York State.

Sex Distribution.—*Table III* also shows the probable incidence of neoplasia in both sexes. It is instructive to compare these figures for New York State with the second table exemplifying incidence rates (*Table IV*) as reported by Harnett.† This is based upon a survey of 15,201 cases reported in London over a period of some 18 months during 1938–9. The two tables are not strictly comparable in view of the fact that the figures for New York State are expressed as a percentage of all living people, whereas those for London give the actual numbers and the relative incidence of cancers expressed as a percentage of the total number of cancers reported. Notwithstanding this consideration, the figures in both centres show many remarkable similarities of relative occurrence of different tumours and their distribution in the two sexes.

A simplified and approximate table extracted from the figures given by Harnett serves to accentuate and clarify the inequalities of incidence between the sexes (*Table V*).

The margin of error arising from diagnostic inaccuracies is appreciably reduced in comparisons of mortality-rates between the sexes at different ages, as it is a reasonable assumption that the faults fall equally on both sides of the comparison, and so tend to cancel one another.

Age.—Cancer affects all ages. *Table VI* gives the age distribution reported in the London Survey (Harnett, 1952) parallel with the figures of the Registrar-General for mortality-rates in England and Wales during the same years.

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* Goldberg, I. D., Levin, M. L., Gerhardt, P. R., Handy, V. H., and Cashman, R. E. (1956), 'The Probability of Developing Cancer', *J. natn. Cancer Inst.*, **17**, 155.

† Harnett, W. L. (1952), *Survey of Cancer in London*. London: British Empire Cancer Campaign.

Age, *continued.*

The table shows that the peak decade of incidence in males was from 60 to 69 years, which accounted for more than 35 per cent; in females the peak was from 55 to 64 years. From the age of 25 to 59 years, the rate in females was higher for every period of 5 years; this is shown in the figures for incidence as well as for mortality. From the age of 60 to 80, the reverse was the case; males accounting for a higher incidence and a greater mortality.

Table V.—Sex Ratio for Cancer at Certain Sites

Site	Male : Female Ratio
Stomach	3 : 2
Rectum	3 : 2
Lungs	4·5 : 1
Skin	3 : 2
Lip	19 : 1
Mouth	7·5 : 1
Tongue	6 : 1
Tonsil	9·5 : 1
Pharynx	3 : 1
Intrinsic larynx	11·5 : 1
Oesophagus	6 : 1
Colon	1 : 1
Thyroid	1 : 4
Kidney	2·5 : 1
Breast	1 : 100

Table VI.—Age and Sex Distribution

Age-groups	London Survey, 1938–9		Registrar-General, 1938–9	
	Males	Females	Males	Females
Years				
0–4	0·27	0·17	0·18	0·15
5–14	0·51	0·36	0·19	0·15
15–24	1·45	0·94	0·54	0·38
25–34	2·47	3·64	1·43	1·53
35–39	2·61	4·55	1·55	2·21
40–44	4·15	7·29	2·48	4·07
45–49	6·03	10·36	4·39	6·45
50–54	9·68	12·67	7·42	9·14
55–59	14·07	14·12	11·61	11·71
60–64	17·97	14·38	16·25	13·87
65–69	17·67	13·01	18·82	14·88
70–74	12·65	9·64	16·83	14·40
75–79	7·51	5·92	11·54	11·54
80–84	2·35	2·25	5·08	6·33
85–	0·61	0·71	1·69	3·18

In both sexes, below the age of 65 years the mortality-rates were lower than the incidence figures; but above 65 years, the ratio was reversed, becoming increasingly marked with advancing age.

In females, the ratio of mortality : incidence in the age-groups 25 to 54 is particularly notable. During the first decade of this period, mortality-rates amount to less than half the incidence rates; thereafter the divergence narrows progressively. In view of the known relatively short survival time of untreated malignant disease in younger age-groups among females, it would seem possible that the magnitude of the ratio might reflect a measure of the success of therapy. An idea such as this would stimulate studies of statistical records over longer periods of time, to compare trends of incidence, therapy, and mortality; and it would also stimulate comparative studies of the records in different communities and different countries, to see whether there were variations and to seek clues to their interpretation. Such statistical information does not, on its own, provide a complete picture or give final answers; but, taken together with other findings, it can add substantial evidence to reinforce, or bring into question, interpretations and theories.

Incidence in Children.—Malignant disease in children is rare. Paterson* gives the figure of about 1 malignant case in 200 admissions to children's hospitals. Notwithstanding its absolute rarity, it is a major lethal disease relative to other causes of death in children. The Manchester Children's Tumour Registry shows that malignant disease is the commonest natural cause of death in children from 1 to 15 years of age. In the U.S.A., cancer causes 12 per cent of deaths from 1 to 14 years of age.

The causes of death of children in North America, Europe, and Australia, in order of frequency at different age-groups, are given by Cohen and Lee† as:—

From 1 to 5 years:—

1. Accidents.
2. Influenza and pneumonia.
3. Congenital malformations.
4. Malignancy.

From 5 to 14 years:—

1. Accidents.
2. Malignancy.

The highest incidence of malignancy in children is during the first 5 years of life, with its peak in the first and second years, suggesting an embryonal character of neoplasia.

SEX AND AGE IN CHILDREN.—There is an overall male preponderance in a ratio of 3 : 2. The distribution of the five common neoplastic conditions, accounting for 80–90 per cent of all cases, are, according to Cohen and Lee, as follows:—

1. Leukaemia: Mostly of acute lymphocytic type and representing the commonest condition in children. Under the age of 1 year,

* Paterson, E. (1955), 'Malignant Disease in Children', *S. Afr. med. J.*, **2**, 1199.

† Cohen, D., and Lee, C. W. G. (1961), *An Investigation of Malignant Diseases in Childhood.* New South Wales Cancer Council, Publ. No. 5.

Incidence in Children, *continued.*

the male to female ratio is 2 : 1; at later ages, the incidence is more equally distributed in the sexes.

2. Cerebral tumours: Are next in frequency; with a peak incidence at 2 years of age.
3. Lymphosarcoma: The general ratio in the sexes is 3 males : 2 females. Eighty per cent occur under the age of 8 years.
   a. Hodgkin's disease: Affects males in 90 per cent.
   b. Reticulin-cell sarcoma is also more common in males in a ratio of 3 : 2.
4. Wilms's tumour: The sexes are equally affected. It presents mainly during the first 2 years of life: it is rare after 8 years.
5. Neuroblastoma: The sex ratio is 3 males: 2 females. The condition occurs mainly during the first and second years of life; two-thirds of all cases occur in children under 3 years.

Cancer Trends.—The study of cancer trends, its progressive variations of occurrence over a period of time, in different age-groups, among different communities and social classes, under various environmental influences, and in all sorts of conditions of life, may be of value in aiding elucidation of causative agencies and biological behaviour of neoplasia.

Cohorts.—Comparisons of mortality-rates at different periods of time and in different circumstances, made on the basis of the deaths during one year, or a bracket of years, for a range of age-groups, is not the most satisfactory indication of trends in mortality. It records the susceptibilities of *different groups* of people of various ages at the *same period* of time. The method does not bring into account environmental and endogenous influences during the lives of the individuals prior to their deaths from cancer. As these influences constitute a fundamental part of the very problems which statistical studies may help to solve, any method of taking cognizance of them assumes a singular importance. The interpretations of trends are more realistic when comparisons of age-adjusted mortality-rates refer to groups of people born during a particular period and living out their lives in similar circumstances and surroundings.

To meet this requirement, the system of cohorts was devised. A group of people, born say during a five-year period 65 years previously, is analysed in terms of cause and age at death. Such a cohort is then compared to others for similarities and differences in death-rates generally and from affections of particular organs.

The cohort method is subject to certain qualifications. Apart from the imperfections already noted in regard to death certification, the abstraction of standard information from records 65 to 70 years old is made difficult by changes in terminology and reclassification of diseases over this long period of time; the discovery of new diseases, and the improvements by reason of new knowledge and techniques, also change the pattern and accuracy of the records. However, with advances in accuracy and uniformity of registration of causes of death, and the ever-wider recording of morbidity in addition to mortality, the cohort method holds much

promise for the provision of less fallible comparisons and more soundly based interpretation of trends.

Although the cohort method is a relatively recent innovation and its full value will emerge only with the passage of time, its merit is already becoming apparent. An aspect of its application is illustrated by an example from the work of Stocks.* He compares the mortality-rates for breast and uterine cancers in cohorts of women born about 1865, 1875, 1885, 1895, 1900, and 1905. The rates for uterine cancer decreased at every age-group in each cohort with the advance of time; in breast cancer, there was no notable alteration of trend. The probability that advances in management were much the same for both conditions led to the idea that some factors not related to therapy accounted for the progressive diminution of fatality from cancer of the uterus. Study of statistical records of age of marriage and commencement of regular sexual intercourse, and parity, suggests that changes in social practices might be responsible for the reduced mortality. The subject is discussed in Chapter VII.

Some of the more outstanding findings in studies of cancer trends in England and Wales and the U.S.A. are: A general increase among males and decrease among females over the first half of the century has brought about a reversal of the sex cancer-mortality ratio. During the earlier years of the century, female deaths exceeded male deaths; now the relative rates are transposed. A marked drop in mortality-rates since 1911 for cancer of the lips and oropharynx in men has been accompanied by a considerable and progressive increase for cancer of the lung. Little or no change in the rates for stomach cancer in males in England and Wales offers an unexplained contrast with the decreasing fatality in the U.S.A. A final example is that pertaining to Hodgkin's disease and lymphosarcoma generally, where the trends show progressive increases in both countries.

* Stocks, P. (1953), 'Studies of Cancer Death Rates at Different Ages in England and Wales in 1921 to 1950: Uterus, Breast and Lung', *Br. J. Cancer*, **7**, 283.

CHAPTER IV

GEOGRAPHICAL PATHOLOGY. GENERAL CONSIDERATIONS AND GASTRIC CANCER

GENERAL CONSIDERATIONS

Geographical pathology involves the comparative study of the occurrence and distribution of disease in peoples belonging to different communities throughout the world and the correlation of these data with social and geographical environment. Geographical in this definition has come to have a connotation wider than that pertaining to regional distribution; it includes the study of various ethnic, national, social, and occupational groups in different regions as well as in one area, and demography and epidemiology have come to occupy basic disciplines within its ambit.

Geographical pathology as a technique for investigating cause and effect of disease has been used for many years. August Hirsch* wrote the first textbook on the subject. It appeared in 1861, and was regarded as so important that it was translated into English by Charles Creighton† in 1883. The importance of epidemiological aspects is manifest in many notable contributions that form milestones in the history of medicine. Oettlé‡ quotes the researches of John Snow into the transmission of cholera. From a study of the occurrence and distribution of cholera in one London suburban area which drew its water-supplies from two different companies, he was able to deduce that the disease was conveyed by contaminated water. In 1775, the renowned Percivall Pott recognized that 'chimney-sweep's disease', a cancer of the scrotum, was caused by soot: one of the first defined occupational cancers.

The prevalence of cancer, especially its disparities in the heterogeneous patterns of its distribution, the variety of forms in distinct population groups, the peculiarities of type and organ affection in diverse occupational groups, the relationship to social habits and customs and to categories of social classes, together with the effect of other environmental agencies, all provide valuable information in the appraisal of their place in the scheme of the aetiology of cancer. Research related to these subjects and the interpretation of the findings are of fundamental importance, providing promising contributions to the understanding of the pathology of cancer as well as to its prevention and treatment.

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* Hirsch, A. (1861), *Handbuch der Historisch-geographischen Patologie.* Enke: Erlangen.

† Creighton, C. (1883), Translation of German text by A. Hirsch (1861), *Handbook of Geographical and Historical Pathology.* London: The New Sydenham Society.

‡ Oettlé, A. G. (1962), 'Cancer and Environmental Influences. Some Observations on its Geographical Pathology', *S. Afr. Cancer Bull.*, 6, 110.

Occupational and industrial cancers and those malignant lesions associated with social customs and modes of life are indeed intimately part of geographical pathology; they form circumscribed entities and are dealt with in separate chapters.

Tables of 'High' and 'Low' Mortality-rates.—High and low mortality-rates set side by side for comparison are invariably intriguing, often puzzling, and sometimes illuminating. A number of examples have been abstracted from World Health Organization reports and analyses of comparative statistics by Segi* and Segi and Kurihara.† They are recorded in *Table VII* which illustrates the problems brought to light. What are the reasons for the contrasts? Why does Japan present such a high mortality-rate for cancer of digestive organs as compared with the much lower rate in the United States? Have the Chilians some special resistance to skin and breast cancer, or are certain cancerigenic agents absent in Chile, although present in New Zealand? Berman‡ records the

Table VII.—'HIGH' AND 'LOW' MORTALITY-RATES PER 100,000 POPULATION—AGE-ADJUSTED

(*Examples from W.H.O. reports and analyses by Segi*)

CANCER	HIGH	LOW
Buccal cavity and pharynx	Ireland, 5·07	Chile, 0·8
Digestive organs and peritoneum	Japan, 71·3	U.S.A., 39·87
Respiratory system	U.K. and Wales, 15·36	Japan, 3·12
Uterus	Japan, 13·99	Ireland, 3·87
Breast	New Zealand, 10·35	Chile, 2·31
Urinary organs	Canada, 5·25	Japan, 1·03
Skin	Ireland, 3·25	Chile, 0·68
Stomach	Japan, 69·9	U.S.A. White, 13·7
Leukaemia	Denmark, 7·6	Japan, 2·9

remarkable contrast in incidence of primary carcinoma of the liver: among Western peoples the rate relative to all other cancers varies from 1·2 per cent to 2·5 per cent; among Africans, Indonesians, Japanese, and Chinese, the relative rate climbs to between 13 and 50 per cent. In Javanese males, the relative rate is 79·3 per cent. The explanation is there, it awaits discovery; it may resolve not only the riddle of primary cancer of the liver, but also point to a more general rationale in a wider area of the cancer problem.

The data are amenable to a number of explanations of different degrees of validity, varying from those fully proven to others still

* Segi, M. (1960), *Cancer Mortality for Selected Sites in 24 Countries* (1950–1957). Dept. Publ. Health, Tohoku University School of Medicine. Japan: Sendai.

† Segi, M., and Kurihara (1962), *Cancer Mortality for Selected Sites in 24 Countries* (1958–1959). *Ibid.*

‡ Berman, C. (1955), 'The Aetiology of Primary Carcinoma of the Liver with Special Reference to the Bantu Races of Southern Africa', *S. Afr. med. J.*, **29**, 1195.

Tables of 'High' and 'Low' Mortality-rates, *continued.*

in the realm of pure speculation. A consideration of cancers of particular organs provides an appreciation of the value of geographical pathology, an insight into methods of study, and clues to its obvious potential in terms of pathogenesis and prophylaxis. In this chapter, the geographical pathology of cancer of the stomach is reviewed.

GASTRIC CANCER

Epidemiology.—Many investigations attest to the contrasting epidemiological statistics of gastric cancer among various peoples and in different regions.

Dungal* writes of the remarkable prevalence in Iceland. It also figures high in incidence in Norway and Finland, where Saxen† records that of all cancers, 28·6 per cent are gastric cancers in the male, and 22·9 per cent in females. A high peak is reached in Japan where Segi and others‡ and Takeda§ have conducted extensive studies. The incidence is increasing in Japan in both males and females, and, in 1956–7, it was more than 5 times that in the United States of America (*see Table VII*). By contrast, carcinoma of the stomach is rare in certain other countries. Bonne‖ notes the low incidence among Javanese; and there are comparable statistical records for some other tropical countries, as in Indo-China, Nigeria, and other parts of central Africa.

The search for an understanding of the reasons for these demographic contrasts has led to researches and explorations into widely diverse aetiological fields.

Dietary and Environmental Factors.—Dietary habits may offer a possible explanation. Icelandic peoples have a diet high in fish and mutton protein, much of which is cured by 'smoking' and 'salting'. Dungal¶ analyses the peculiarities of Icelandic diet; he finds little positive or conclusive, but suggests that certain hydrocarbons produced by special forms of preparation of food may act as inducers of cancer.

Cold to temperate climates may affect food and dietary habit. Saxen ascribes part of the high rate in Finland to a lack of fresh vegetables in the diet which leads to an iron-deficiency anaemia and thus may be a factor. He also finds tapeworm infestation common; this absorbs vitamin B_{12}, so causing pernicious anaemia, which is

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* Dungal, N. (1958), 'Cancer in Iceland', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

† Saxen, E. (1961), 'Gastro-intestinal Cancer in Finland', in Symposium on 'Geographical Pathology of Gastrointestinal Cancer', *Acta Un. int. Cancr.*, **17**, 367.

‡ Segi, M., Fukushima, I., Fujisaku, S., Kurihara, M., Saito, S., Asano, K., and Kamoi, M. (1957), 'An Epidemiological Study of Cancer in Japan', *Gann*, **48**, Suppl., Apr.

§ Takeda, K. (1961), 'Geographical Pathology of Cancer of the Stomach in Japan', in Symposium on 'Geographical Pathology of Gastrointestinal Cancer', *Acta Un. int. Cancr.*, **17**, 316.

‖ Bonne, C. (1937), 'Cancer and Human Races', *Am. J. Cancer*, **30**, 435.

¶ Dungal, N. (1961), 'Can Smoked Food be Carcinogenic?' in Symposium on 'Geographical Pathology of Gastrointestinal Cancer', *Acta Un. int. Cancr.*, **17**, 365; and (1961), 'The Special Problem of Stomach Cancer in Iceland', *J. Am. med. Ass.*, **17**, 789.

variously estimated as having a 6 to 10 times higher incidence of gastric cancer.

Doll* has compared diets in Japan and Iceland; they appear to be different. In contrast to the rich animal protein and fat in Iceland, Japanese diet, consisting mainly of boiled rice and vegetables with little fish, is poor in fats and protein. The absence of an obvious common cancerigenic factor in the diet introduces the consideration of food habits and methods of preparation, trace elements of possible carcinogenic chemicals derived from soil and/or water-supplies, and other possible pathogenic agents. Investigation of a number of these has been made practicable by the fact that there are marked variations of incidence in localized areas of one geographic region.

In the United States of America, Haenszel† reports a lower incidence than in other countries, with general diminution in the figures since 1928. The rate is higher in northern than in southern states; and foreign-born citizens are more liable than native born. No relationship to diet has been established, but measurement of the cancer risk in terms of social class reveals figures of interest. Gastric cancer rates are higher in the lowest social class, with an excess of over 50 per cent as compared with the highest class. Similar contrasts have been reported in gastric cancer mortality-rates in England and Wales by Logan,‡ and in Copenhagen by Clemmesen and Neilsen.§

The fact that for many years the mortality-rate from gastric carcinoma in North Wales was twice that in South-east England led to careful analyses of the disease distribution and its correlation with environmental conditions. Stocks|| and Stocks and Davies¶ record such investigations. In North Wales and parts of Cheshire, soils from the gardens of homes where stomach cancer had caused death showed a high organic content; whereas organic content was low in other gardens (viz. 'non-cancer' and 'other-organ-cancer' deaths). This soil correlation was also discovered in parts of Devon. Trace elements of chromium, cobalt, and zinc were also found to be high in the more northern regions. These findings raise the possibility of the presence of a carcinogen in highly organic soil, which might be ingested in vegetables or water, or indeed with actual soil when unhygienic eating habits are practised. The question of carcinogenic activity of trace elements, such as zinc, chromium, and cobalt, also arises. In this regard a highly edifying observation was

* Doll, R. (1956), 'Environmental Factors in the Aetiology of Cancer of the Stomach', *Gastroenterology*, **86**, 320.

† Haenszel, W. (1961), 'Incidence and Mortality from Stomach Cancer in the United States', *Acta Un. int. Cancr.*, **17**, 347.

‡ Logan, W. P. D. (1954), 'Social Class Variations in Mortality', *Publ. Hlth Rep., Wash.*, **62**, 1217.

§ Clemmesen, J., and Neilsen, A. (1951), 'The Social Distribution of Cancer in Copenhagen. 1943 to 1947', *Br. J. Cancer*, **5**, 159.

|| Stocks, P. (1958), 'Statistical Investigation concerning the Causation of Various Forms of Human Cancer', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

¶ Stocks, P., and Davies, R. I. (1960), 'Epidemiological Evidence from Chemical and Spectrographic Analysis that Soil is concerned in the Causation of Cancer', *Br. J. Cancer*, **14**, 8; and (1960), 'Investigation of a Localised High Incidence of Gastric Cancer', *Publ. Hlth, Lond.*, **74**, 408.

Dietary and Environmental Factors, *continued.*

recorded by Nicholson.* In one area of a town in Devon, where there were two tanneries using chrome for processing and the soil content of zinc, chromium, cobalt, and organic carbon was high, the mortality-rate from cancer of the stomach was excessive, whereas in another area of the same town as well as in a nearby village, where there were no tanneries, the death-rate was not raised.

The findings in Wales that gastric cancer is more common where the soil is peaty, and where domestic water-supplies come from surface sources, e.g., shallow wells, rivers, and lakes, and are therefore more likely to be polluted, are significantly comparable with the findings in the Netherlands as reported by Tromp.† Here, too, those living on reclaimed peat soil were much more vulnerable than those on sea-clay, cover-sand, river-clay, and loess soils. Tromp found that in towns where water-supplies came from wells, the mortality was lowest, by contrast with the highest rate in towns where river water was used.

Stocks‡ points to the correspondence between soil character and naturally occurring radioactivity. Peat-soil areas have a high radioactivity, whereas with chalky and limestone formations, activity is low. Perhaps this accounts for divergencies in cancer incidence. A discovery calling for hesitation in coming to any final conclusion is that in North Wales the greater portion of the excessive gastric cancer death-rate falls among quarry and farm labourers. This different rate does not occur among the wives of such labourers as compared with other married women. It is a matter for conjecture whether some occupational factor involving close contact with a particular soil is responsible.

There are many suggestive ingredients in these research pursuits. As already indicated, statistical analyses are subject to reservations; and much wider inquiries and further investigations are necessary before finite deductions may be made. Whatever the answers are, it does seem that multiple agencies operate in various combinations and that the so-called poly-aetiological theory has much to commend it.

Immigrant Populations.—Some of the problems of cancer causation related to geological characters, water and food supplies, indigenous dietary and culinary habits, and regional parasitic infestations, are amenable to examination among emigrant peoples and mixed ethnic populations. Such a study was carried out in Hawaii and

* Nicholson, G. (1960), 'The Medical Officer of Health and Cancer', *Publ. Hlth, Lond.*, 74, 403.

† Tromp, S. W. (1954), 'Statistical Study of the Possible Relationship between Mineral Constituents in Drinking Water and Cancer Mortality in the Netherlands. (Period 1900–1940)', *Br. J. Cancer*, 8, 585; and (1954), 'Possible Influence of Soil on Cancer. A Statistical Study of Geological Factors in the Netherlands', *Am. J. clin. Path.*, Suppl. 33.34.

‡ Stocks, P. (1958), 'Statistical Investigations concerning the Causation of Various Forms of Human Cancer', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

is reported by Quisenberry.* The population of more than half a million comprises 35 per cent Japanese, 23 per cent Caucasian, 19 per cent Hawaiian, and 23 per cent other groups. Digestive system neoplasia is the most prevalent form and constitutes 36 per cent of all cancers; in this system, gastric cancer accounts for 47 per cent and is followed by colonic affection in 20 per cent. The contrast between these figures and those of the U.S.A. is evident (*see Table VIII*).

Table VIII.—CANCER DEATH-RATE FOR U.S.A. AND HAWAII PER 100,000 POPULATION—AGE-ADJUSTED

DEATH-RATE (BY SITE)	U.S.A. 1955	HAWAII 1954–6
All sites	146·5	159·1
Digestive system	52·3	71·8
Stomach	13·5	36·0
Large intestine	15·3	7·6

The stomach cancer rate is more than two and a half times as high in Hawaii, and conversely, the colon rate is twice as high in the U.S.A.

An almost identical relative proportion pertains in comparisons between the Japanese and Caucasian elements in Hawaii, viz. gastric cancer is more prevalent in Japanese residents by two and a half times that in Caucasians, and colonic cancer is twice as common in Caucasians than it is among Japanese.

These contrasts and comparisons cannot be fortuitous, and, over wide areas of the world, active studies are in progress for their interpretation. Whilst this is still elusive, the scope of the investigations are indicated by the following examples. Japanese in Hawaii eat a large amount of white rice, which has a low vitamin B_1 content; they also eat raw fish which inactivates this vitamin. Is the lack of vitamin B_1 causally related to gastric cancer? Plummer-Vinson's disease, probably originating from nutritional deficiency, is known to be causally related to postcricoid carcinoma; Wynder† puts the case that it is also connected with neoplasms of the remainder of the alimentary tract. The Japanese drink very hot tea, hot rice wine (sake), and also eat pickles. Do these cause injury? Do they contain carcinogens? Do they add to the nutritional deficiency? Oral hygiene, use of tobacco, occupational exposure to carcinogenic agents, and the place of heredity and genetic factors, are among the possible aetiological circumstances that are under attention.

* Quisenberry, W. B. (1961), 'Gastro-intestinal Cancer in Hawaii', *Acta Un. int. Cancr.*, **17**, 324.

† Wynder, E. L. (1957), 'Non-occupational Aspects of Environmental Cancer', *Cancer (Am. Cancer Soc.)*, **7**, 14.

Peptic-ulcer Cancer.—Segi and Kurihara* consider that there is a close aetiological correlation between peptic ulcer and gastric carcinoma in Japanese men; they report that 25 per cent of gastric cancers arise in gastric ulcers.

This is against the trend of opinion in Western countries, where most authors agree with Dible,† Newcombe,‡ and Willis§ in the view that the incidence of peptic-ulcer cancer is a very restricted one of between 1 and 6 or 7 per cent of all gastric cancer. Whilst errors of clinical, radiological, and histopathological differential diagnosis between non-cancerous and cancerous lesions are well documented, they are, in the main, subject to the test of time. A carcinoma of the stomach may masquerade as a benign ulcer, but time, in terms of 6–12 months, will correct any misdiagnosis. Similar rectification is almost inevitable when the cytological appearances have been wrongly interpreted as between benign and malignant lesions. The main difficulty arises, however, in appreciating whether a proven cancer is neoplastic *de novo* or is superimposed upon a preceding peptic ulcer.

The criteria for deciding this issue have been described by Kark‖ under the following heads:—

HISTOLOGICAL CRITERIA.—

1. In benign ulcer, there is complete destruction of muscle in the floor; and the muscularis mucosae is fused with the muscular layer at the edge of the ulcer. In malignancy arising *de novo*, muscle remnants are left in the base, and there is no fusion at the margins; if a malignant ulcer has no muscle in its base, it may have been preceded by a peptic ulcer.
2. In benign ulcer, there is dense fibrosis underlying granulation tissue in the floor. This is absent in malignancy *de novo*, and malignant cells infiltrate both the floor and the margin of the ulcer. If malignancy supervenes upon a benign ulcer, the fibrous floor and the walls offer a considerable bar to infiltration of cancer cells.
3. In benign ulcer, endarteritis is present, but is absent in a malignant ulcer unless the malignancy occurred on a previously benign ulcer.

These criteria cannot be firmly held, as a malignant ulcer arising *de novo* may be secondarily eroded by gastric juices.

CLINICAL CRITERIA.—

1. A long-term follow-up of benign ulcers suggests a low incidence of malignant degeneration.

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* Segi, M., and Kurihara, M. (1960), 'Cancer in Japan from the Viewpoint of Geographical Pathology', *Tohoku J. exp. Med.*, **72**, 169.

† Dible, J. H. (1925), 'Gastric Ulcer and Gastric Carcinoma: an Enquiry into their Relationship', *Br. J. Surg.*, **12**, 666.

‡ Newcombe, W. D. (1933), 'The Relationship between Peptic Ulceration and Gastric Carcinoma', *Ibid.*, **20**, 279.

§ Willis, R. A. (1960), *Pathology of Tumours*. 3rd ed. London: Butterworths.

‖ Kark, W. (1964), *A Synopsis of Surgical Pathology*. Bristol: Wright.

2. The previous history of proven gastric malignancy does not suggest an unusually high incidence of preceding benign ulceration.

MORBID ANATOMICAL CRITERIA.—

1. The different sites of the main occurrence of benign and malignant ulcers in the stomach also point to the infrequency of malignant degeneration.
2. Benign ulcers are common in the duodenum, whereas carcinoma is extremely rare.

A consideration of the combined evidence—histological, clinical, and morbid anatomical—indicates that malignant degeneration is a rare complication, occurring in less than 5 per cent of benign ulcers.

It is patent that the diagnosis of peptic-ulcer cancer is a matter of considerable complexity and therefore uncertainty. In this light, the statistical place of peptic ulcer as a cause of cancer cannot be regarded as settled, and the solution to the discrepancy raised by the deductions in Japan, where gastric ulcer is accorded a high place as a precursor, and in England, where it is regarded as uncommon, must await further evidence and close study.

Gastritis and Cancer.—Strode* found that hypertrophic gastritis was more than twice as prevalent in Japanese than in other males in Hawaii; hypoprotinaemia and achlorhydria are common associates. The supposition is that these are precancerous conditions.

Ringertz† advances the view that one, or more, of the changes in chronic gastritis is precancerous. Mucosal irregularities produced by chronic gastritis usually show mixed hypertrophic and atrophic appearances: hypertrophy is common in the superficial epithelium, with resultant granularity or polypoid heaping. Atrophy commonly affects basal glandular layers, but may also dominate the picture with the production of a smooth, thin, flattened mucosal surface. Hyperplastic elements may undergo de-differentiation or metaplasia to intestinal type epithelium. Atrophic patches may undergo repeated surface breakdown alternating with repair. All of these changes may precede the advent of frank malignant change.

In pernicious anaemia, chronic gastritis is a common feature. It differs from other forms of chronic gastritis in its distribution. In pernicious anaemia, gastritis affects mainly the fundus; in 75 per cent of the other forms, it is mainly antral. A suggestive parallelism exists in regard to the regional distribution of gastric cancer: in cancer not associated with pernicious anaemia, 75 per cent occur in the antral segment; in pernicious anaemia, cancer is located in the fundus in an excessive number, amounting to 2 to 3 times the expected ratio.

The correlation between gastritis and cancer, bearing the implication that the causes of gastritis (mainly of environmental source)

* Strode, J. E. (1957), 'Giant Hypertrophy of Gastric Mucosa (Hypertrophic Gastritis)', *Surgery*, **41**, 236.

† Ringertz, N. (1961), 'The Pathology of Gastric Cancer and its Relationship to Gastritis, Polyps and Ulcer', *Acta Un. int. Cancr.*, **17**, 289.

Gastritis and Cancer, *continued.*

and therefore also causes of cancer, has a factual background but it is not by any measure established nor is it universally accepted.

A relevant reference to this is a report by Videbaek and Mosbech* of a higher incidence of gastric carcinoma in relatives of pernicious-anaemia patients; a finding which provokes scepticism about conclusions based solely on statistical surveys, and which also raises the possibility of genetic factors as part of the aetiology. Some support for this aspect derives from the association of gastric cancer and blood group A.

Gastric Polyps and Cancer.—Benign tumours of the stomach are rare; in a number of instances their occurrence is associated with familial affection.

Spriggs and Marxer† have described the pathology and proneness to malignancy of benign adenomas and papillomas. Usually situated in the antral region, they are multiple in about half the cases, and are more common in males. In about 25 per cent, carcinomatous change supervenes. Despite this marked malignant propensity, their relative infrequency leads Boles‡ to write that they account for few gastric cancers, amounting to about 2 per cent.

Most polyps of the stomach probably represent extensions of hypertrophied epithelium in chronic gastritis, and its relation to cancer has already been referred to.

Achlorhydria and Cancer.—An association is suspected for several reasons. Achlorhydria is almost invariably found with stomach cancers. Pernicious anaemia, widely considered as a precursor of malignancy to the extent of 6–10 times an excess incidence, is accompanied by low gastric secretion and atrophic gastritis. Chronic gastritis, which may have a place as a premalignant condition, is commonly associated with achlorhydria.

Hollander§ considers that the several different mucinous secretions of the stomach act as a defensive mechanism against chemical and physical irritants, e.g., hydrochloric acid and pepsin, various potentially harmful foods, drinks, and condiments, and physical and thermal injuries. Mucus forms the first line of defence by acting as a diluent and vehicle washing away irritants, by mechanically coating surface epithelium and by neutralization of acids. The second line of defence is the remarkably rapid regenerating power of mucinous epithelium on the necks and bodies of glandular tubular structures.

This mucous barrier may be disturbed by reduction in blood-supply, repeated and dominating trauma, chronic gastritis, etc. The theory is that achlorhydria, which includes diminution of

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* Videbaek, Aa., and Mosbech, J. (1954), 'The Etiology of Gastric Carcinoma elucidated by a Study of 802 Pedigrees', *Acta med. scand.*, **149**, 137.

† Spriggs, E. I., and Marxer, O. A. (1943), 'Polyps of the Stomach and Polypoid Gastritis', *Q. Jl Med.*, **12**, 1.

‡ Boles, R. S. (1960), 'Precancerous Lesions of the Stomach: How to treat them', *Post-grad. Med.*, **27**, 359.

§ Hollander, F. (1961), 'Gastric Mucus Secretion in Relation to Cancer of the Stomach', *Acta Un. int. Cancr.*, **17**, 307.

mucus, means lowered resistance followed by repeated cellular damage with ultimate defective healing, possibly combined with hypertrophy, eventually leads to cancer.

The concept may explain an excess incidence of carcinoma in pernicious anaemia and achlorhydria (an incidence which has not yet been proved conclusively), but there are several considerations calling for reservation of judgement. It is reasonable to anticipate that the prominent and common result of failure of the mucous barrier would be peptic ulceration. The weight of authoritative opinion is against peptic ulcers being premalignant. Therefore the breakdown of mucous defences does not seem to be correlated with gastric cancer. Perhaps even more pertinent are the geographical surveys done to test the influences of gastric secretions. Willis* refers to test-meal analyses performed on people with known differences of cancer incidence: there was no significant difference in the tests on English and Dutch people, or between upper and lower social classes. Similarly, despite marked contrasts in prevalence of gastric cancer among Chinese and Malays in Java, neither gastric secretion nor morphology of gastric mucosa showed suggestive variation.

Incidental to this discussion on *pros* and *cons* of a particular theory, is an illustration of the value of geographical pathology in co-ordination and evaluation of evidence on a problem in cancer.

The discussion on the place of various aspects of cancer of the stomach bears witness to the many gaps in our knowledge and to the complexity of the subject. It is most unlikely that a simple, single causative element will be found. The indications are that multiple factors, in different combinations with interrelated biological balance, are more likely to provide the answers to the problems.

* Willis, R. A. (1960), *Pathology of Tumours*. 3rd ed. London: Butterworths.

CHAPTER V

GEOGRAPHICAL PATHOLOGY OF CANCER IN AFRICA

There are a number of examples of neoplastic disease occurring in peculiar distributional patterns in Africa. The rationale has not been elucidated in most instances, but the theories and postulates are edifying and interesting; and investigations stimulated by them are full of promise and hope.

AFRICAN LYMPHOSARCOMA OF CHILDREN
(*Burkitt Tumour*)

The characters of this tumour were first co-ordinated and brought to clinical prominence by Burkitt in 1958,* since when he has added further detailed studies.†‡

Clinical Features.—A jaw tumour is the common herald (50–60 per cent); it often affects 2 quadrants; and in about 11 per cent, all 4, i.e., both maxillae and both mandibles. In 1 of 3 cases, the tumour extends to, and distorts, the orbit. Abdominal tumours are invariably associated; they may be retroperitoneal, or cause hepatomegaly, or affect both ovaries, and less frequently other abdominal sites. Extra-abdominal foci, as in the thyroid, spine, testis, and large bones, are less common. Peripheral lymph-node enlargement is not usual, and the lungs are only exceptionally involved. Death is usual within 6 months, but life may be prolonged by methotrexate.

Incidence.—The peak age is 5–6 years; 96 per cent occur from 2 to 14 years. The sexes are equally affected. It constitutes more than half the cancers of children in Uganda. It is independent of race and tribe, Indians and Africans being equally susceptible. Similar findings are reported by Edington and Maclean§ in Nigeria, where it is second to cancer of the cervix in terms of all malignant disease diagnosed in Ibadan.

Pathological Features.—There are several characters indicative of a multicentric origin. Its effect on bone is osteolytic. Histologically there are sheets or clumps of undifferentiated blast cells with varying numbers of histiocytes. Mitoses are numerous and often atypical. Degeneration, haemorrhage, and necrosis are common.

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* Burkitt, D. (1958), 'A Sarcoma involving the Jaws in African Children', *Br. J. Surg.*, **46**, 218.

† Burkitt, D. (1962), 'A Lymphoma Syndrome in African Children', *Ann. R. Coll. Surg.*, **30**, 211.

‡ Burkitt, D. (1963), in *Cancer Progress Volume* (Ed. Raven, R. W.). London: Butterworths.

§ Edington, G. M., and Maclean, C. M. N. (1964), 'Incidence of the Burkitt Tumour in Ibadan, Western Nigeria', *Br. med. J.*, **1**, 264.

Geographical Distribution.—Burkitt* has conducted 'tumour safaris' and has called attention to a remarkable distribution across a wide belt of tropical Africa with an extension down the east coastal plain (*Figs.* 10 and 11), and has correlated this with climatic and physical geographic features.

Fig. 10.—Map of Africa showing roughly a shaded area in which the altitude is mainly below 5000 ft.; the temperature is above 60 °F.; and the annual rainfall is above 20 in.

Fig. 11.—Map of Africa showing roughly a shaded area from which Burkitt's tumour has been reported. Not all cases fall into '*the geographical area*'; * indicates centres (Johannesburg and Cape Town) from which a few cases have been reported and which lie outside the area with special physical and climatic conditions.

In Uganda and Kenya, an altitude ceiling to the occurrence of the tumour seems to exist: it does not affect residents at altitudes above 5000 ft. (1524 m.). The altitude barrier above which the condition does not arise diminishes progressively with distance from the equator. This critical height above sea-level appears to be linked to temperature, which, in the zone concerned, did not fall below 60° F. (16° C.). A further finding was that the annual rainfall was above 20 in. (51 cm.).

The areas involved, the altitude limits, and the local temperatures, closely correspond to the known present and past distribution of the tsetse fly.

Zanzibar and Pemba, islands off the east coast of Africa, within the latitude of the geographic zone affected, have not provided any cases of the lymphosarcoma. It is surmised that the 30-mile stretch of sea, separating the islands from the mainland, isolates them from one or other element of the causative chain.

It is known (Haddow quoted by Burkitt†) that the coincident topographical distribution with altitude, temperature, and humidity

* Burkitt, D. (1962), 'Determining the Climatic Limitations of a Children's Cancer Common in Africa', *Br. med. J.*, **2**, 1019.

† Burkitt, D. (1962), 'A Tumour Syndrome affecting Children in Tropical Africa', *Post-grad. med. J.*, **38**, 71.

Burkitt Tumour—Geographical Distribution, *continued.*

factors are related to the existence of certain mosquitoes. Burkitt* quotes Davies as the first to suggest the possibility of a virus as the cause of the tumour. Thus a theory of an arthropod vector of a virus carcinogen is propounded. The age incidence is thought to substantiate the idea of virus induction. The absence of cases in Zanzibar and Pemba is also adduced as supporting evidence: the vector is assumed to be incapable of crossing the 30-mile expanse of sea.

The stimulating character of the observations and theories about Burkitt's tumour are obvious. Much new thought has been provoked and many investigators are giving the condition close and detailed attention. Several recent reports tend to cast some doubt on the theory of vector-borne virus-induced lymphosarcoma. Chapman and Jenkins† record 5 cases from Natal; the topographical and other environmental circumstances conform to those described by Burkitt, thus extending the geographical boundary of incidence, but not undermining the theory. Gluckman‡ reports 3 cases in the Transvaal in Caucasian children. Bennett and Anstey§ describe 6 cases arising in and about Cape Town. The climatological surroundings of these cases differ from the criteria used to develop the theory of an arthropod vector. On the other hand, a fillip has been given to the virus theory by the isolation of a reovirus from one case of Burkitt's lymphoma by Bell and others.|| Serological tests suggest its relationship to the lymphoma. It is too early to form conclusions, and much work is still required before all the facts are reconciled by an acceptable explanation.

PRIMARY CARCINOMA OF THE LIVER

Berman¶** has recorded the high prevalence of primary cancer of the liver in Central, East, and South Africa. The tumour, which is rare or uncommon in most other parts of the world, accounts for a proportion varying from 17 to 53 per cent of all cancers in certain ethnic groups in these geographical areas. The figures are comparable with the excessive rates in Indonesia, where Kouwenaar†† records it in Javanese, Chinese, and Batak males.

In Africa, the condition is also more common in males, the ratio to females being 10 : 1. The peak age of incidence is from 20 to 40 years.

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* Burkitt, D. (1962), 'A Tumour Syndrome affecting Children in Tropical Africa', *Post-grad. med. J.*, **38**, 71.

† Chapman, D. S., and Jenkins, T. (1963), 'The Burkitt Lymphoma in Natal', *Med. Proc.*, **9**, 320.

‡ Gluckman, J. (1963), 'Multifocal Lymphoma in South Africa', *S. Afr. Cancer Bull.*, **7**, 7.

§ Bennett, M. B., and Anstey, L. (1963), 'Burkitt-Type Lymphosarcoma (Correspondence)', *S. Afr. med. J.*, **37**, 476.

|| Bell, T. M., Massie, A., Ross, M. G. R., and Williams, M. C. (1964), 'Isolation of a Reovirus from a Case of Burkitt's Lymphoma', *Br. med. J.*, **1**, 1212.

¶ Berman, C. (1951), *Primary Carcinoma of the Liver. A Study in Incidence. Clinical Manifestations, Pathology and Aetiology.* London: Lewis.

** Berman, C. (1958), 'Primary Cancer of the Liver in Africa' ,in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

†† Kouwenaar, W. (1950), 'Cancer Incidence in Indonesia', *J. natn. Cancer Inst.* ,**11**, 642.

Pathology.—The liver is invariably the seat of Laennec's 'hobnail' cirrhosis. The tumours present in one of two gross forms: massive, or small, diffuse, multiple lesions. Microscopically there are two types: hepatoma being more common than cholangioma.

An outstanding feature is the well-being of the patient until a short time before death.

Theories of Causation.—

1. Heredity. The absence of an increased prevalence among American Negroes points to factors other than heredity.
2. Parasitic Infection. Hou* reported that 15 per cent of primary carcinomas of the liver in Hong Kong were of cholangiocellular type and were invariably associated with *Clonorchis sinensis*; this, in turn, was associated with intrahepatic calculi in more than 60 per cent of cases. The parasite causes marked bile-duct hyperplasia, which undergoes multicentric malignant transformation. There is no evidence of this or other parasitic carcinogen in the African type.
3. Topography and Climate. The absence of common features tends to rule out these conditions as part of the aetiology.
4. Cirrhosis of Viral Origin. This theory of pathogenesis has been argued by Steiner,† but the evidence is not yet solidly based and the theory is still *sub judice*.
5. The usual association with cirrhosis of postnecrotic type raises the possibility of a common cause for cirrhosis and cancer, or an agent causing cirrhosis which then advances to malignancy. Many possible lines of pathogenicity have been investigated: intestinal parasites, liver flukes, schistosoma, hydatid, spiced foods, syphilitic infection, hepatitis of different origins, haemochromatosis, and siderosis. As causative elements, they all remain insubstantial. More recently *A. flavus* has become suspect (*see* Chapter XX), and is under investigation.
6. The particular types of alcohol imbibed by the African Bantu is held by many as the important cause. It is possible that the alcohol, often brewed and kept in 'petrol-tin' containers, carries a metallic solute which is the basis of the genesis of cirrhosis and cancer. Neither of these theories has been proved.
7. Chronic malnutrition is widespread. The staple diet of maize in South Africa and the diet of plantains, yams, and potatoes in Central Africa, gives rise to deficiencies in animal protein, fats, calcium, and vitamin B. Gillman and others‡ consider that dietary elements are causally implicated. This theory is plausible as malnutrition commonly causes liver damage which has a high incidence in the population at risk.

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* Hou, P. C. (1956), 'Relationship between Primary Carcinoma of the Liver and Infestation with *Clonorchis sinensis*', *J. Path. Bact.*, **72**, 239.

† Steiner, P. E. (1960), 'Cancer of the Liver and Cirrhosis in Trans-Sahara Africa and the United States of America', *Cancer*, **13**, 1085.

‡ Gillman, J., Gillman, T., and Gilbert, C. (1950), 'Observations on the Aetiology of Cancer of the Liver', *J. natn. Cancer Inst.*, **11**, 653.

Primary Carcinoma of the Liver—Theories of Causation, *continued.*

Higginson* discusses the various theories but does not give any one firm or final support. The question is still 'open'.

CARCINOMA OF URINARY BLADDER

Haematuria of the Egyptian fellah has been a common clinical entity since the dawn of recorded history. In more recent years, the cause was discovered to be a trematode parasite, schistosoma (or bilharzia), mainly *S. haematobium*, but also *S. mansoni.*

Infestation is prevalent in Egypt, Sudan, Morocco, West Africa, Somaliland, Equatorial Africa, Madagascar, Rhodesia, and Transvaal. Outside of Africa, it occurs particularly in the Middle East. The geographical distribution is closely linked with requirements in the life-cycle of the parasite.

Life-cycle.—The ovum has a chitinous shell; it hatches on reaching water, releasing an embryo or miracidium, which is ciliated and free-swimming. To continue life for more than about 24 hours, it requires a suitable host, which is a species of fresh-water snail. On reaching the liver of this intermediate host, a sporocyst is formed; daughter sporocysts are then budded. These produce vast numbers of bifid-tailed cercariae which are discharged by the snail into water; they are able to swim about for a day or two and continue the cycle if they reach their definitive host, man. They are able to penetrate unbroken skin and mucous membranes if swallowed with water; after a course via systemic veins to heart and lungs, they reach the portal system of the liver and there mature into adult male and female worms, which mate, and then the female deposits her eggs in viscera differing with the type of worm: *S. mansoni* in the colon and rectum, and *S. haematobium* mainly in the urinary bladder. Many ova pass into the lumen of the viscus affected, are excreted, and, if they find suitable water, start the cycle over again.

Incidence and Epidemiological Background.—In Africa, infestation reaches its highest peaks in Egypt. Ferguson† estimated that at least 61 per cent of all fellaheen were affected. Shaw and Ghareeb‡ put it at 60–70 per cent of all Egyptians.

The environmental conditions of fresh water accompanied by the existence of a snail as an intermediate host in the Nile basin and delta, provide essential elements of the source of bilharzial infection in Egypt. The means of economic subsistence, the mode of work, religious, social, and sanitary customs, all favour the very wide-spread prevalence of the disease.

Considerable numbers of the population earn their livelihood by farming; most of the farmers work near an economic margin of poverty, using primitive methods, characterized by lack of

* Higginson, J. (1956), 'Primary Carcinoma of the Liver in Africa', *Br. J. Cancer*, **10**, 609.

† Ferguson, A. R. (1913), *Glasg. med. J.*, **29**, 13. Quoted by Gelfand, M. (1950), *Schistosomiasis in South Central Africa.* Cape Town: Juta.

‡ Shaw, A. F., and Ghareeb, A. A. (1938), *J. Path. Bact.*, **46**, 401. Quoted by Gelfand, M. (1950), *Schistosomiasis in South Central Africa.* Cape Town: Juta.

personal protection. They work barefooted, up to the ankles in cercarial-infested water, thus providing a common portal of entry. The severe climatic conditions result in the fellaheen drinking infected water, so exposing another portal for invasion.

Religious observance calls for thrice daily pre-prayer rinsing of mouth and nostrils with available water. Ablutions are required after defaecation and urination. Not only does this enhance the possibility of infection during washing, but it also prompts the individual to excrete at the water's edge, leading to reinfestation of the water by more ova and miracidiae. Bathing in canals and riverside shallows, for relief from the harsh climate and by younger people as a sport, is a further mode of infection.

Topographical characters, occupation, agricultural methods, customs and habits, all contribute to the determination and location of bilharzial infection. The incidence is greater in lower than in upper Egypt; it affects particularly the rural and agricultural population.

Elsewhere in Africa, the sources of infection are not so heavily involved or perpetually reinfected, but the conditions are similar on a reduced scale. In places, the habits or religious traditional observances vary, and occupations may differ, but the fundamental cycle of infection remains constant. In South Africa, interesting telluric aspects enter the picture. Nearly all rivers of Transvaal and Natal flowing east have infected or potentially infectable snails, and are therefore dangerous. Rivers flowing west appear to be innocuous.

Pathology of Vesical Schistosomiasis.—The studies of Prates and Gillman* and Aboul Nasr and others† have provided detailed descriptions of the lesions of bilharzial cystitis from the earliest stages of infestation. The initial reaction to the subepithelially-deposited egg usually occurs near the trigone and consists of acute hyperaemia, local mild necrosis associated with intense cellular infiltration by lymphocytes, plasma cells, and eosinophils, apparently out of proportion to the number of eggs. Around this is a zone of proliferating fibroblasts. The reaction is focal when eggs are sparse and separated from one another; but it is diffuse when the eggs are more numerous.

Focal reactions produce tubercles; more extensive affections give rise to sessile nodules and pedunculated papillomata. The granulomatous reaction matures, accompanied by the appearance of giant cells, calcification of eggs, disappearance of histiocytes, and fibrosis. Tubercles then present an appearance of wet sandy patches; larger lesions show atrophy or disappearance of the overlying mucosa, and some may exhibit squamous metaplasia of transitional epithelium. The mucosal covering may undergo

* Prates, M. D., and Gillman, J. (1959), 'Carcinoma of the Urinary Bladder in the Portuguese East African with Special Reference to Bilharzial Cystitis and Preneoplastic Reactions', *S. Afr. med. J.*, **24**, 13.

† Aboul Nasr, A. L., Gazayerli, M. E., Fawzi, R. M., and El-Sibai, I. (1962), 'Epidemiology and Pathology of Cancer of the Bladder in Egypt', *Acta Un. int. Cancr.*, **18**, 528.

Pathology of Vesical Schistosomiasis, *continued.*

necrosis, leaving ulcers; or healing and fibrosis may lead to fibrous and/or calcified plaques. Haemorrhage and secondary infection are common.

Eggs may be shed into the lumen of the bladder to be voided with urine, or they may be released into surrounding tissues of the submucosal, muscular, and serosal layers. Female worms also exercise their effect in the vessels of these layers, with the combined result of extensive fibrosis.

Epithelial reactions of several kinds may proceed in association with one another in different parts of the bladder. Reparative proliferation may restore the transitional epithelial cover; distortion and/or downgrowth may form peg-like or pseudoglandular elements, which raise the surface into polyps, and, more deeply, present a picture of cystitis glandularis. Squamous metaplasia and leucoplakia, changes which are regarded as neoplastic, occur in about 11 per cent of bilharzial cystitis in Portuguese East Africans.

Neoplastic Change.—Although there are no records of unequivocal histological changes from benign to malignant cells, many authorities believe that neoplasia does arise by progressive change from bilharzial epithelial reactions or their complications.

Aboul Nasr and others describe squamous metaplasia, with and without keratin formation, in more than half their cases of bladder cancer; squamous metaplasia affected surface transitional epithelium and von Brunn's subepithelial nests. Columnar-cell metaplasia also occurs in von Brunn's nests and gives rise to cystitis glandularis with which adenocarcinoma was found to be associated. The commonest type of bladder cancer in Egypt was squamous epithelioma, accounting for about 60 per cent; next was transitional-cell cancer, comprising just over 33 per cent; and adenocarcinoma formed less than 5 per cent of the total. Gillman and Prates* analyse 100 cases of bladder cancer in Portuguese East Africans. Using a slightly modified form of the classification proposed by Dukes and Masina,† they record papillary transitional as 21 per cent; solid transitional as 4 per cent; transitional with metaplasia as 3 per cent (i.e., transitional-cell cancers total 28 per cent); anaplastic, 13 per cent; and squamous cell as 59 per cent. Adenocarcinoma was not found in their series. The high relative incidence of squamous-cell cancers in this report accords with that found in Egypt, and differs markedly from that found in England.

This fact is taken in association with other findings: the earlier age of onset of bladder cancer in the two African states as compared with that in the U.S.A.; the frequency of associated infestation with schistosomes; and it is vigorously argued that bilharzia plays a causative role, either directly or indirectly, in bladder carcinoma.

* Gillman, J., and Prates, M. D. (1962), 'Histological Types and Histogenesis of Bladder Cancer in the Portuguese East African. With Special Reference to Bilharzial Cystitis', *Acta Un. int. Cancr.*, **18**, 560.

† Dukes, C. E., and Masina, F. (1949), 'Classification of Epithelial Tumours of the Bladder', *Br. J. Urol.*, **21**, 273.

Gelfand* challenges this point of view. He claims that the characteristic histological composition of bilharzial lesions is chronic granulation tissue lined by transitional epithelium. His personal studies of Mashonaland Africans, where schistosomiasis is endemic, did not reveal a raised incidence of bladder cancer.

There is support on both sides of the debate, and the issue is not yet settled.

Perhaps the strongest support for the thesis that bilharzia is causally related to cancer derives from statistical analyses of epidemiological data. Hueper† quotes findings by Kartulis and Goebel indicating an excessive incidence of cancer in autopsies on bilharzial cases. Other works quoted show that from 7·6 to 20 per cent of the total cancer in Egypt comprised tumours of the bladder, whereas the related figures in Europe and the U.S.A. were 2–3 per cent. According to Aboul Nasr and others‡ the incidence of bladder cancer relative to all cancer in males in Egypt is 11 per cent, in females 3·9 per cent, and for both sexes it totals 7·5 per cent. These authors quote figures from Alexandria University Hospitals where 5·4 per cent of admissions for malignant disease and 8 per cent of autopsy material comprised bladder cancers.

Not all authorities are willing to accept the statistical evidence. Willis§ advises caution, and considers that there is need for further careful studies.

KAPOSI'S SARCOMA

Over 90 years ago, Moricz Kaposi‖ described and established as an independent entity the condition which he named 'idiopathic multiple pigment sarcoma of the skin'. Several case reports and a review of 12 cases did not contribute materially to the knowledge of the disease which remained obscured in the background of rarity for 75 years. Then it was discovered to be relatively common in parts of the African continent. Reports on various aspects increased to the extent that there are now about 1000 publications, including an extensive monograph by Bluefarb.¶

Clinical Features.—The features differ in white and Negro patients. The differences are mainly of degree and do not amount to different types of one disease.

SKIN.—Rothman** describes the initial lesions in white patients as macules with a dark blue to violaceous colour, usually showing a superadded tinge of brown. These macules generally evolve to

* Gelfand, M. (1950), *Schistosomiasis in South Central Africa*. Cape Town: Juta.

† Hueper, W. C. (1942), *Occupational Tumours and Allied Diseases*. Springfield, Ill.: Thomas.

‡ Aboul Nasr, A. L., Gazayerli, M. E., Fawzi, R. M., and El-Sibai, I. (1962), 'Epidemiology and Pathology of Cancer of the Bladder in Egypt', *Acta Un. int. Cancr.*, **18**, 528.

§ Willis, R. A. (1960), *Pathology of Tumours*. 3rd ed. London: Butterworths.

‖ Kaposi, M. (1872), 'Idiopathisches Multiples Pigmentsarkom der Haut', *Arch. Derm. Syph.*, **4**, 265.

¶ Bluefarb, S. M. (1957), *Kaposi's Sarcoma. Multiple Idiopathic Haemorrhagic Sarcoma*. Springfield, Ill.: Thomas.

** Rothman, S. (1962), 'Some Clinical Aspects of Kaposi's Sarcoma in the European and North American', *Acta Un. int. Cancr.*, **18**, 364.

Kaposi's Sarcoma—Clinical Features, *continued.*

flat plaques of infiltration and then to nodular projections of variable shape, ranging from 0·5 to about 3 cm. in diameter. The characteristic colour together with the location, which is at first almost invariably on the feet or hands, the former being more frequent, and later presenting lesions progressively more centripetally (e.g., from soles to dorsa of feet, to legs, to thighs), make up a combination of clinical phenomena strongly suggesting a diagnosis of Kaposi's sarcoma.

Some skin lesions may involute while others are progressing. Ulceration is not common in the European, but it is in the Negro. Coalescence of neighbouring lesions produces large, florid, and often fungating tumorous deformities. Tumefaction and verrucous changes are more common in the African. Much of the pleomorphism of skin manifestations is explained by Keen* on the basis of the rate and intensity of development of secondary changes. Advance may be very slow and appear stationary for years; subcutaneous thickenings may remain separate from epidermis for long periods, or infiltrate into skin, producing a nodule, which becomes spongy and haemorrhagic and soon ulcerates if the process is rapid. The surface involved may extend quite quickly and lesions may be found on the hard palate, penis, or ear pinna.

Oedema, of solid type, occurring in about two-thirds of patients, affects particularly the lower limbs. It is common even when skin lesions are sparse.

INTERNAL ORGANS.—It has become abundantly clear that Kaposi's sarcoma is only partly, and probably not predominantly, a dermatological condition. Internal organs often provide the initial seats of the disease, which may not affect skin at all, as is the common presentation in children. While the skin may be the most frequently and intensely involved organ, it is, more often than not, associated with affection of other organs. The gastro-intestinal tract, abdominal solid viscera, lymph-glands, urogenital organs, respiratory organs, pericardium and heart, and bone, all figure in the lists of involved organs.

Radiological Features.—A number of publications referring to one or more aspects of bone pathology in Kaposi's sarcoma preceded the first systematic analysis by Davies in 1956.† Subsequently, Palmer‡ presented a review of radiological findings in approximately 200 cases. Bone involvement may be clinically silent but it signifies advanced and widespread pathology. Whilst any bone may be the seat of tumour tissue, the phalanges, metacarpals, metatarsals, and tarsus are the most commonly affected, followed by the leg and forearm bones. Decalcification of affected bones is almost invariable; the loss of density is accompanied by 'rubbing

* Keen, P. (1962), 'The Clinical Features of Kaposi's Sarcoma in the South African Bantu', *Acta Un. int. Cancr.*, **18**, 380.

† Davies, A. G. M. (1956), 'Bone Changes in Kaposi's Sarcoma. An Analysis of 15 Cases occurring in Bantu Africans', *J. Fac. Radiol.*, **8**, 32.

‡ Palmer, P. E. S. (1962), 'The Radiological Changes of Kaposi's Sarcoma', *Acta Un. int. Cancr.*, **18**, 400.

out' of trabecular patterns, and increase in medullary component at the expense of cortical shadow, which may show an expansion of bone diameter. Clear-cut cysts and erosions, without adjacent reaction, are striking features. The erosions appear like bites taken out of the margin of the bone; occasionally, the larger cysts and erosions have a reactive sclerotic surround.

Radiographic techniques have been devised to demonstrate subcutaneous and soft-tissue tumours; they are usually clearly defined, and, when multiple, may have a 'cluster of grapes' outline. 'Soft' films also show small focal calcifications in the lower limbs. Palatal tumours have been shown as not uncommon. In children, lymph-node enlargement can be demonstrated.

Kaposi's Sarcoma in Children.—Davies and Lothe* report a 4 per cent incidence in children. There were more cases in the first decade of life, with a peak from 2 to 4 years, than in the second decade. All their cases were males. although other reports include female cases. All differed clinically from adult cases; most had bilaterally enlarged cervical, axillary, and inguinal nodes; some had eyelid lesions with lacrimal and salivary gland involvement. Skin lesions were present in more than half the cases, but were unusual on the extremities. Biopsy and histological examination established the diagnosis in all cases.

Age and Sex Incidence.—After the second decade, there is an increasing incidence in each decade with peak figures from 30 to 50 years in Negroes and 15 to 20 years later in Europeans. Males preponderate to the extent of about 10 : 1.

Clinical Progress and Mortality.—The disease itself can cause death by tumour extension. There is a considerable range in speed of progress, some cases dying within a year of diagnosis, others surviving for 20 years and more. Recently established systems of treatment, by intra-arterial perfusion of nitrogen mustard, and by irradiation, have both claimed marked alleviation with eradication of many skin lesions and soft-tissue tumours. Both forms of therapy promise to prolong life.

Histopathology.—Extensive studies by Murray and Lothe† have revealed that wherever the lesion, and whatever the age, the basic histological pattern is uniformly one of proliferation of vasoformative tissue and spindle cells. The condition begins in perivascular cells, especially along lines of veins. In the skin, the lesion starts mainly in the mid corium. Angiomatous tissue, or spaces filled with erythrocytes, give the lesions their bluish colour, and haemosiderin deposits add the brownish hue. Proliferating spindle cells, with pleomorphic nuclei, are arranged in irregular fascicles of whorled, interweaving, sweeping streams. Networks of reticulin and collagen fibres divide the bundles of spindle cells in diverse

* Davies, J. N. P., and Lothe, F. (1962), 'Kaposi's Sarcoma in African Children', *Acta Un. int. Cancr.*, **18**, 394.

† Murray, J. F., and Lothe, F. (1962), 'The Histopathology of Kaposi's Sarcoma', *Ibid.*, **18**, 413.

Kaposi's Sarcoma—Histopathology, *continued.*

patterns. At the periphery of a lesion, there are dilated lymphatic vessels, giving rise to the idea that they are responsible for the oedema so often part of the picture.

Histogenesis.—The cell of origin is a controversial subject. There are theories of origin from the reticulo-endothelial system, from lymphatic vascular tissue, from endothelial or perithelial cells of blood-vessels, from perivascular Schwann cells, and from other sources.

Relationship to Lymphomas.—This is quite remarkable and is too frequent to be fortuitous. Bluefarb* records an association with mycosis fungoides in 3 cases; a similar number of instances with Hodgkin's disease and with lymphosarcoma; 5 cases with lymphatic leukaemia and 2 with myeloid leukaemia. The distinctive histopathology of Kaposi's sarcoma labels it a distinct entity, and the association with the lymphoma group remains unexplained.

Relationship to Oedema.—Stewart and Treves† reported the advent of lymphangiosarcoma in swollen, oedematous arms following mastectomy for breast cancer. Bluefarb raises the question of whether Kaposi's sarcoma is comparable and is superimposed upon a limb oedematous from some other cause. The evidence in African cases negates this suggestion. There are many types of swollen limbs and elephantiasis due to obstructive lymphatic disease in Africa; the occurrence of Kaposi's sarcoma in them has not been noted. Early Kaposi lesions often arise before oedema begins; and the subsequent advance of the tumour and the oedema run together. A final and apparently conclusive argument is that the histopathology of Kaposi's sarcoma is unique and differs from that reported in postmastectomy sarcoma.

Geographical and Racial Distribution.—Demographic and epidemiological data in Europeans and Africans appear to point to different interpretations. Rothman‡ describes a racial distribution among Europeans, affecting particularly Central and Eastern European Jews and certain Mediterranean peoples. McCarthy and Pack§ report in similar vein. Of 36 patients, 30 were Jews or Italians; some were immigrants, others were born in the U.S.A. These authors also consider the condition to be racial in distribution. In Africa, environmental factors are strongly suggested. The highest incidence in Central Africa is in the North-east Congo Basin, from

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* Bluefarb, S. M. (1957), *Kaposi's Sarcoma. Multiple Idiopathic Haemorrhagic Sarcoma.* Springfield, Ill.: Thomas.

† Stewart, F. W., and Treves, N. (1948), 'Lymphangiosarcoma in the Postmastectomy Lymphoedema. A Report of Six Cases in Elephantiasis Chirurgica', *Cancer*, **1**, 64.

‡ Rothman, S. (1962), 'Remarks on Sex, Age and Racial Distribution of Kaposi's Sarcoma and on Possible Pathogenic Factors', *Acta Un. int. Cancr.*, **18**, 326.

§ McCarthy, W. D., and Pack, G. T. (1950), 'Malignant Blood Vessel Tumours; a Report of 56 Cases of Angiosarcoma and Kaposi's Sarcoma', *Surgery Gynec. Obstet.*, **91**, 465.

where it fades progressively with increasing distance, until in sandy zones there are no case reports. It does not affect particular races or tribes. In Transvaal, Oettlé* found that it was 10 times as frequent in Bantu as compared with Whites; it did not occur in Coloureds or Indians; and in some of the Bantu cases, members of three successive generations were affected. There is evidence, therefore, that both racial and environmental conditions may affect the incidence. They are not necessarily mutually exclusive aetiological factors.

Relative Incidence.—Among the first to report the excessively high incidence of Kaposi's sarcoma in Africa were Quenum and Camain.† They recorded that in certain parts of Equatorial Africa, Kaposi's sarcoma accounted for 10 per cent of all malignancies: an incidence which has been computed to represent 200 times that found in Chicago. The incidence reaches its highest peak in parts of what was Belgian Congo, at a level of 12·8 per cent of all cancer; in Uganda it is about 4 per cent; in Transvaal it is 1·4 per cent and is the commonest tumour of the limbs in Bantu.

* Oettlé, A. G. (1962), 'Geographical and Racial Differences in the Frequency of Kaposi's Sarcoma as Evidence of Environmental or Genetic Causes', *Acta Un. int. Cancr.*, **18**, 330.

† Quenum, A., and Camain, R. (1956), 'Les Aspects Africains de la Maladie de Kaposi, Reticulopathic Maligne Systematisée', *Annls Anat. path.*, **3**, 837.

CHAPTER VI

GEOGRAPHICAL AND OCCUPATIONAL PATHOLOGY OF SKIN CANCER

INCIDENCE

Although cancer of the skin is common, its representation in many statistical tables is falsified by the fact that it is not often fatal; this impression gains reinforcement because not more than a minor proportion requires hospitalization for treatment. Its prevalence is similarly not widely documented because it is not notifiable except in a few very restricted localities and circumstances. *Table III*, p. 33, by Goldberg and others* giving the probability of developing cancer, based on Cancer Morbidity Reports of New York State in 1949–51, puts skin cancer in the male at the head of the list, and it is not much farther down in the female column. Harnett† records (*see Table IV*, p. 34) the numbers of skin neoplasms among 15,201 reported cancers in London 1938–9. In males, they accounted for 9·43 per cent, being placed fourth after stomach, rectum, and lung, as the site affected. In females, the ratio of skin to all-site cancers was less than in males, accounting for 5·83 per cent and being topped by cancers of the breast, uterus, stomach, rectum, and colon. O'Donnell and others‡ put the incidence in the United States of America at a much higher level: 41,000 new male cases of skin cancer per annum amounts to 15·9 per cent of the total male cancer incidence; in females, 25,000 new cases per year amounts to 9·9 per cent of all cancer.

In both sexes, skin cancers comprise two main types: about 95 per cent are epidermoid, and about 5 per cent melanomas.

GEOGRAPHICAL PATHOLOGY

These figures provide an idea of the commonness of skin cancer. Comparative figures of epidermoid cancers on a geographical basis, as well as among different peoples of one region, are revealing of pathogenic agencies and of the practicability of prophylactic preventive measures of a highly successful order. Examples illustrative of statistical contrasts are those by Khanolkar,§ who records that carcinoma of the skin forms 3 per cent of all cancers in Bombay, 26 per cent in Habana, and 97 per

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* Goldberg, I. D., Levin, M. L., Gerhardt, P. R., Handy, V. H., and Cashman, R. E. (1956), 'The Probability of Developing Cancer', *J. natn. Cancer Inst.*, **17**, 155.

† Harnett, W. L. (1952), *Survey of Cancer in London*. London: British Empire Cancer Campaign.

‡ O'Donnell, W. E., Day, E., and Venet, L. (1962), *Early Detection and Diagnosis of Cancer*. St. Louis: Mosby.

§ Khanolkar, V. R. (1950), 'Cancer in India in Relation to Race, Nutrition and Customs', *J. natn. Cancer Inst.*, **11**, 640.

cent in white people in Habana. Hueper* quotes the prevalence rate for three northern urban areas in the United States, which varies from 12·3 to 16 per cent, by contrast with rates in three southern urban areas, which vary from 26 to 43·3 per cent. Comparable figures have been reported by many other observers. By and large, the range of figures indicative of prevalence runs parallel to the hours of sunshine to which the diverse peoples and communities are exposed; with breaks in the parallelism caused by varying sensitivity among different people. Ultra-violet light from the sun is the main cause of skin cancer. Other radiations are also capable of inducing skin changes leading to cancer: artificially produced ultra-violet light, X-rays, radium, and related emanations. Other skin carcinogens, e.g., arsenic and polycyclic hydrocarbons, are also considered in this chapter.

SUNSHINE

Epidemiological studies have for many years indicated the important causative agency of solar radiation. Seventy years ago, in 1894, Unna† correlated chronic skin changes and their cancerous propensities with exposure to sunlight. He found the skin changes in outdoor workers, especially among sailors. Subsequent surveys have shown an excess prevalence in Australia, South Africa, Argentine, and among distinct occupational groups, namely, farmers (e.g., in Australia and South Africa), sailors (particularly Scandinavian), fishermen, other outdoor workers, and among those who practise exposure to acquire what they regard as socially desirable sun-tan.

Exposure to irradiation is thus closely related to geographical habitat: with long periods of sunlight; warm to hot weather leading to light clothing and thus more exposed skin; higher altitudes where actinic rays are stronger; and drier areas, because penetration by the sun's rays are relatively unhindered. It is strongly influenced by occupation, outdoor employment being the common factor. Finally, social custom, including outdoor sport and sun-tanning, contributes its share of victims.

COMPLEXION

A second set of circumstances with an equally potent bearing on the incidence of skin cancer consists of host susceptibilities and resistances. Susceptible types are fair-skinned, light, blond or ginger-haired, blue-eyed, and with the kind of complexion that freckles and burns rather than tans on exposure to the sun. An extreme, and rare, variety of this type is the inheritor of xeroderma pigmentosum, whose skin is so sensitive to actinic light that skin cancers invariably occur at a young age. Resistant people have the opposite characters: they are brunette or dark-haired, brown or dark-eyed, and have pigmented, dark skins that either tan readily or are so dark that sunshine causes no visible change of colour. Negroes and Bantu races are only exceptionally affected; in these races, most of the recorded cases of actinic epidermoid tumours occur in albinos.

* Hueper, W. C. (1952), 'Environmental Factors in the Production of Human Cancer', in *Cancer*, Vol. 1 (Ed. Raven, R. W.). London: Butterworths.

† Unna, P. G. (1894), *Die Histopatologie der Hautkrankheiten*. Berlin: Hirschwald.

Complexion, *continued.*

The higher incidence in males, by 1·5–4 times as much as in females, is due to the increased exposure to the sun in male occupations, and to their shorter hair (longer hair in females gives considerable protection to ears and forehead).

PATHOLOGY OF ACTINIC CANCER

Epidermoid cancers are of two main varieties: squamous cell and basal cell. Each type has a predilection for particular areas of skin, which are illustrated in *Fig.* 12. Whilst there are many exceptions to this pattern of distribution, the mucocutaneous junction of the lips is a favoured site for squamous-cell cancer, but is rarely the seat of

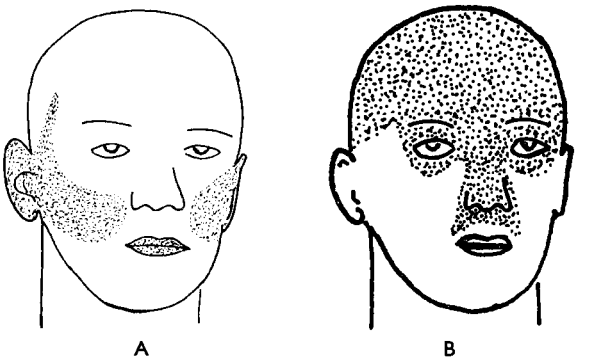

Fig. 12.—Common pattern of distribution of carcinoma of the face. A, Squamous-cell carcinoma. Mainly lips, cheeks, and ears. B, Basal-cell carcinoma. Mainly nose, nasal and nasolabial folds, eyelids, and forehead.

rodent ulcers. Multiple lesions are common, and sometimes both basal and squamous varieties occur in one case; not infrequently, cytological differentiation between the two varieties is vague and indefinite in a single lesion.

Premalignant solar dermatitis is usual. The changes are more characteristic in middle and older ages, but it also occurs in younger patients. The earliest changes affect skin colour and texture: red, grey, and/or yellow-brown discolorations occur in patchy, irregular areas of different sizes, from a pin-head to a half centimetre or so; early atrophy is evidenced by loss of elasticity; it is often associated with irregular scaling and the appearance of flattened, plaque-like nodules. Progress is usually slow, and the typical 'senile' type of keratosis set in dry atrophic skin may take several years to develop. The typical features consist of pigmentary changes which produce a motley of multiform hyperpigmented, brown freckle-like areas mixed with leucodermic spots; many of the 'freckles' are associated with smooth, thin, inelastic skin; epithelial heaping creates hyperkeratotic warts, sometimes large

enough to form 'horns'. The advent of malignant change is common but not inevitable. It takes a variable time and the latent period between exposure and cancer is given a wide range of 15–40 years.

Squamous-cell Epithelioma.—The tumours are often multiple, each one arising from a zone of cells rather than from a single or small clump of cells. Variation from highly differentiated to rapidly growing anaplastic types occurs in one case as well as in different patients. Differentiated cancers exhibit well-defined prickle cells, and mature cornifying cells with the production of 'pearls' or 'cell nests'. Anaplastic tumours are characterized by primitive, undifferentiated cells, infiltrating rapidly by cords and strands of spreading malignant cells. Metastasization to lymph-nodes varies with the grade of cellular malignancy and with the site of the cancer: from the head and face, lymph-node involvement of the neck is common; from the hand and forearm, the more central the tumour, the higher the rate of nodal spread.

The cancer often begins as a thickening or nodule; an edge of raised, hard, rolled, irregular tissue develops; the surface ulcerates and remains unhealed, eventually becoming crateriform; infiltration spreads through the base and floor to involve deeper tissues.

Basal-cell Carcinoma.—As with squamous-cell tumours, this is also often multiple, indicating that the carcinogen provokes similar reactions over a wide area of its application. Ideas on histogenesis are still subject to differences of opinion. It probably arises from deeper layers of epidermis, and also from cells of hair follicles and other dermal appendages; the former giving the superficial, ulcerative, 'rodent' lesions, and the latter producing sub-epithelial types such as 'Brooke's tumour', 'turban tumours', and some sweat-gland tumours.

Microscopically, solid masses of darkly pigmented cells extend into the dermis in columns, ending in expanded, club-like bases, or in irregular angular shapes, at a fairly uniform level below epidermis. Cornification is exceptional, and so is 'pearl' formation.

Its growth is usually extremely gradual, but it is destructive of all tissues in its path. Lymphatic and blood spread are extremely rare.

IONIZING RADIATION AND SKIN CANCER

Exposure to artificially produced ionizing radiations gives rise to reactions which are well recognized. The effects from natural sources of ionizing radiation are largely a matter of theory, based upon deductions of the known sequels arising from the former source. Radiations manufactured for medical diagnostic and therapeutic purposes, for use in industry in various ways, or for war, are classifiable among the causes of occupational cancers. Natural radiations belong, more strictly speaking, to the geographical pathology of cancer.

The first case of cancer arising from industrial contact with ionizing radiation was described by Frieben in 1902.* This case in a man

* Frieben, K. (1902), 'Cancroid des Rechten Handrükens', *Dtsch. med. Wschr.*, 28. Verens-Beilage.

Ionizing Radiation and Skin Cancer, *continued.*

employed on the manufacture of X-ray tubes appeared 6 years after the discovery of X-rays by Röntgen. Oberling* quotes Cluneto as having demonstrated in 1910 that X-rays were in fact carcinogenic. Since that time a voluminous literature has followed concerning the physical and biological effects of ionizing radiations. A review of basic knowledge on aspects related to cancer is the subject of Chapter XI. Radiations affect blood-forming organs and other tissues as well as skin, but only the latter pertains to this chapter.

Effects on Skin.—The effects may be delayed for several days or even longer. A dose of 500 to 1000 rad of X- or gamma-radiation causes skin erythema. Somewhat lower doses produce epilation. At these levels, recovery is usual; with higher dosages hair-loss may be permanent. At about 1500 rad, sebaceous glands are affected and the skin loses its normal greasy texture. Sweat-glands are affected at somewhat higher levels. At 1500 rad and above, skin damage is permanent: at about 3000 rad, necrosis with moist desquamation is the rule.

Recovery and healing are tardy and incomplete: the skin becomes thin and atrophic with loss of elastic tissue; areas of change of pigmentation, both hyperpigmentation and depigmentation, occur together with irregular patches of telangiectases. Other vascular changes, endarteritis obliterans, periphlebitis, and areas of hyalinization, are constant. The skin is liable to ulcerate following mild trauma and infection. Foci of hyperkeratosis and acanthosis are typical. Radiation dermatitis is less frequent than it used to be as a result of the protective measures now widely adopted.

Cancer, of squamous-cell variety in about 95 per cent, and often multicentric in origin, is superimposed upon a high proportion of cases of radiation dermatitis. Cancers have occurred at young ages, in the late teens and early twenties, when basal-cell types are less uncommon than among older people (Glucksmann and others†). Such early occurrence has been noted especially following X-ray and radium therapy to skin conditions in childhood, e.g., haemangiomas, excess hair, and skin rashes.

According to an M.R.C. report,‡ the latent period before the advent of cancer after exposure to irradiation varies from 12 to 56 years, with an average of 33 years. The hands, arms, and chest are the areas mostly affected.

CARCINOGENIC POLYCYCLIC HYDROCARBONS

These carcinogens have provided a most extensive field for laboratory cancer research. Since the report by Yamagiwa and Ichikawa§ in 1918 that painting with tar produces cancer of the skin of rabbit ears,

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* Oberling, C. (1952), *The Riddle of Cancer.* Revised ed. New Haven: Yale Univ. Press.

† Glucksmann, A., Lamerton, L. F., and Mayneord, W. V. (1952), 'Carcinogenic Effects of Radiation', in *Cancer,* Vol. 1 (Ed. Raven, R. W.). London: Butterworths.

‡ Medical Research Council (1956), *The Hazards to Man of Nuclear and Allied Radiations.* London: H.M.S.O.

§ Yamagiwa, K., and Ichikawa, K. (1918), 'Experimental Study of the Pathogenesis of Carcinoma', *J. Cancer Res.,* 3, 1.

there has been intensive work in research laboratories throughout the world, and today several hundred hydrocarbon substances are known tumorigenic agents. Innumerable lessons and valuable pointers to biological behaviour have been derived from these studies. They have also led to the solution of many aspects of causation of certain types of skin cancer in man; types which belong, in the main, to the occupational segment of the geographical pathology of cancer.

Percivall Pott,* almost two hundred years ago, observed and deduced that chimney sweep's 'wart' was a cancer arising after a latent interval of about 20 years of exposure to soot. This probably represents the first discovery of a carcinogenic hydrocarbon affecting man; and also the first description of an occupational cancer. Since then, the list of hydrocarbon occupational cancers of the skin has grown to appreciable lengths as is indicated in *Table IX*.

Table IX.—DISCOVERIES OF HYDROCARBON CARCINOGENESIS AFFECTING THE SKIN

(*Abstracted and modified from Hueper*†‡)

AGENT	DISCOVERER	DATE
Soot	Pott	1775
Paraffin oil	Bell	1876
Coal tar, lignite	Volkmann	1876
Petroleum still coke	Derville and Guermonprez	1890
Coal tar, bituminous	Butlin	1892
Anthracene oil	Oliver	1908
Coal tar, anthracite	Zweig	1910
Petroleum paraffin oil	Schamberg	1910
Shale lubricating oil	Wilson	1910
Creosote oil	O'Donovan	1920
Petroleum lubricating oil	Heller	1930

Pathology of Hydrocarbon-induced Lesions.—Erythema, folliculitis, and eczematoid lesions are common early manifestations. Pigmentary changes follow, and may accompany the more acute skin reactions; both hyperpigmentation and leucoderma occur. Benign hyperplastic lesions, keratoses, scaly papules and warts, cutaneous papillomata and horns, follow after a variable time, and become the multicentric seats of epidermoid carcinomas after a further 12–20 years or longer.

In an effort to solve the problem of which element of the skin is primarily concerned in the transformation from normal to cancer

* Pott, P. (1775), *Chirurgical Observations relative to the Cataract, the Polypus of the Nose, the Cancer of the Scrotum, the Different Kinds of Ruptures, and the Mortification of the Toes and Feet.* London: Hower Clarke & Pollins.

† Hueper, W. C. (1942), *Occupational Tumours and Allied Diseases.* Springfield: Ill.: Thomas.

‡ Hueper, W. C. (1952), 'Environmental Factors in the Production of Human Cancer', in *Cancer*, Vol. 1 (Ed. Raven, R. W.). London: Butterworths.

Pathology of Hydrocarbon-induced Lesions, *continued.*

tissues, Cowdry* has analysed a large number of skin components: different proteins, minerals, cells, vitamins, etc., but without coming to any conclusive theory as to the natural course of the disease.

Soot.—In addition to the classic example of chimney sweeps, other occupations are exposed, e.g., printing, paint and shoe-polish manufacturing, rubber mixing, fire-stoking, fireworks, etc.

Soot becomes lodged in the perineoscrotal fold and in the corrugations of scrotal skin; persistence of lodgement is promoted by sebum secreted in quantity and by unhygienic washing habits. Soot also tends to collect in the axilla, but here skin secretions are not tacky and thick as in the perineal region, they are watery and so tend to wash away soot and prevent its accumulation and contact with skin.

The employment of children as chimney sweeps has long been abolished, and hygiene has improved, so that the high incidence of scrotal-skin cancer has accordingly fallen; but that it still affects men in this occupation is shown in a study of 1487 fatal scrotal cancers in England and Wales, where Henry† found 103 cases in chimney sweeps.

Oils and Paraffins.—Considerable variation in carcinogenic potency exists. Highly refined oils have low activity, and medicinal liquid paraffin is regarded as entirely non-carcinogenic. Shale oils are more dangerous than petroleum oils. The techniques of distilling and refining play a part in carcinogenicity, so that no general rule as to crudeness of the oil is universally applicable, e.g., it has been found that certain light spindle oils used in textile spinning machines are more active than the more crude heavier oils used for motor engine lubrications. The geographical origin of the oil has a notable influence: Venezuela and Borneo provide highly potent varieties, whereas petroleum oils from Russia and Texas have a low carcinogenic power.

Murray§ quotes Twort and Lyth in listing the following as indicative of potency:—

Specific gravity—the higher, the more potent; but certain mixtures of low specific gravity do not fit this rule.

Refractive index—an increase (which arises from an increase in the amount of heterocyclic compounds present) implies greater power. Exceptions to this rule also occur.

Fluorescence—intensity and blueness indicate greater danger.

Colour—colourless oils are harmless; those with colour are suspect.

Saturation—well-hydrogenated oils are less active than those not saturated.

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* Cowdry, E. V. (1953), 'Epidermal Carcinogenesis', in *Recent Advances in Cancer*, **1**, 57. New York: Academic Press.

† Henry, S. A. (1946), *Cancer of the Scrotum in Relation to Occupation*. London: Oxford Univ. Press.

§ Murray, R. (1958), 'Occupational Cancer of the Skin', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

Iodine content—in unprocessed oil, the higher the iodine, the greater the carcinogenicity.

The occupations at risk are plentiful and widespread: employees at oil-production fields, in refineries and distributive branches; textile workers and cotton mule spinners; operators and repair and maintenance personnel concerned with a wide variety of apparatus and machines requiring oil lubrication; these form an indicative but not exhaustive list.

Mule spinners were shown by Henry* to contribute the greatest proportion of occupational skin cancers in England and Wales; about one-third of 4632 notified cases of skin cancer came from this industrial group. The mule is a machine for spinning cotton; spindles on it are oil-lubricated to maintain a high revolution speed; excess oil is thrown off, contaminating the workers' clothing. Much of the workers' task involves bending across the machine to catch up and repair broken threads, so that clothing at the level of the groins becomes heavily impregnated with oil; this is transferred to skin and tends to remain on the scrotum. The initial lesion is often one or more small warty nodules, which, after a variable, but usually long latent period averaging 50 years, becomes a squamous-cell carcinoma. Prophylactic measures, especially changing the type or mixture of lubricating oil to a much lower carcinogenic potency, and converting from mule spinning to ring-frame machines, have reduced the incidence of this skin cancer to much lower numbers.

Scrotal-skin cancer illustrates and typifies a malignant reaction to carcinogens used in industry. Other oil-induced cancers arise from exposure of different areas of skin in various occupations. Examples are squamous epithelioma on the hands and forearms of shale miners; and on these areas as well as the chest, face, and neck in certain engineering trades where oil droplets are sprayed from the machinery used.

Coal Tars and Derivatives.—A remarkable number and variety of occupations are exposed to the risks of the cancerigenic properties of tars and related products. Some of the more generally known uses of these substances are road-surfacing, damp courses and water-proofing of mastic, jute, and felt, electrical equipment and batteries, brush and broom making, caulking, creosote preservation of wood, sealing of cables, in many different chemical industries using tar as a primary source of different products, and as a fuel.

The pathological course is not very different from that caused by mineral oils, but the latent period tends to be shorter, with an average of 20–24 years.

ARSENIC

Arsenic is probably one of the main inorganic chemical carcinogenic agents. In addition to neoplasms of the lung, liver, and urinary bladder, it is a potent cause of cancer of the skin. Arsenic is widely used in

* Henry, S. A. (1946), *Cancer of the Scrotum in Relation to Occupation.* London: Oxford Univ. Press.

Arsenic, *continued.*

industry, as in the making of paints, inks, and dyes, in electroplating and enamelling, in rubber products, in photography, and as a pest poison in gardening and agriculture. It is a constituent of many medicinal mixtures and of a number of cosmetics.

The arsenic may be applied locally where it is absorbed, or it is ingested when it acts as a systemic toxin. Arsenical dermatitis, exhibiting hyper- and de-pigmentation, with keratoses, often affects unexposed parts of skin, e.g., axilla, groin, and perineum, and commonly also the palms and soles. Cancerous ulceration, of squamous-cell type in two-thirds, is not uncommon as a sequel of chronic arsenical dermatitis.

CHAPTER VII

GEOGRAPHICAL PATHOLOGY. RELIGIOUS CUSTOMS, TRADITIONAL PRACTICES, AND SOCIAL HABITS

The geographical distribution of a number of cancers has led to the discovery of causative agents arising from the customs and habits of the people affected. In many of these cancers, knowledge gleaned from geographical aspects lightens but a segment of an otherwise dark aetiological prospect; in others, the information is so pertinent as to make possible the elimination of the disease by abolition of the relevant practice. This is, however, seldom an immediately practicable proposition because people cling to customary practices as essential features of their daily lives. It is not enough to know the cause; patience, sympathy, and understanding by those carrying out a policy of long-term general and special educative programmes, and often betterment of social conditions and improvement in economic circumstances, are the basic necessities for success in the prevention of these cancers.

Several such cancers arise from customs and habits in different parts of India. Khanolkar* has studied them and written surveys and reviews, from which much of the following information on cancer in India is culled.

Kangri Cancer is one of the best known of the cancers arising from a customary practice. People of Kashmir, in North-Western India, keep warm in winter by using an earthenware pot, covered by a woven-reed basket, and kept hot by its content of burning and smouldering leaves and other vegetation. This heated pot, known as a kangri, is held against the body, under the outer garments and often against bare skin. In the upright position, it rests against the abdomen, and in the sitting or squatting posture, the hot jar is in contact with the abdomen, parts of the anterior surfaces of the upper halves of the thighs, and, in females, with the lower inner quadrants of the breasts. Erythema or more severe burns are often distributed on these skin surfaces. Squamous-cell carcinoma is a sequel in a notable proportion (*Fig.* 13).

Whether it is chronic irritation and repeated burn injuries, accompanied by cycles of successive repair and breakdown over years, or the presence of chemical carcinogens in the smoke and gas

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* Khanolkar, V. R. (1944), 'Oral Cancer in Bombay, India. A Review of 1000 Consecutive Cases', *Cancer Res.*, 4, 313; (1945), 'The Susceptibility of Indians to Cancer', *Indian J. med. Res.*, 33, 249; (1950), 'Cancer in India', *Acta Un. int. Cancr.*, 6, 881; and (1950), 'Cancer in India in Relation to Race, Nutrition, and Customs', *J. natn. Cancer Inst.*, 11, 640.

Kangri Cancer, *continued.*

given off by the smouldering vegetation in the kangri jar, or a combination of both, that act as the inducers of cancer, are still debatable issues. The burns themselves seem to play an important role, for cancer often starts near the edge of a burn ulcer.

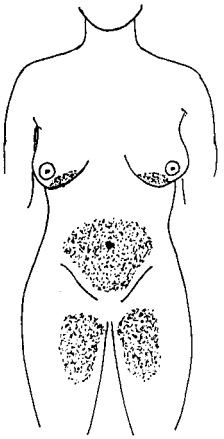

Fig. 13.—Common distribution of kangri burns and cancer in females.

Dhoti Cancer.—The dhoti is a length of material fashioned into a garment by tying it tightly around the waist; one end is then passed under the perineum and fixed to the waist portion at the back. Skin-pressure injuries affect areas over the iliac crests, groins, and perineum. Squamous-cell carcinoma in these areas has an excessively high incidence, which is most marked in the lower social classes. It is probable that pressure sores plus accumulation of dirt due to lack of adequate washing and cleaning are the underlying causes of the cancers.

Chutta Cancer.—In Andhra, a state in India, the habit of smoking a cigar (i.e., chutta) with the burning end inside the mouth is widespread. Carcinoma of the palate, at the site of impingement of hot smoke, is a common condition. Repeated thermal trauma and/or tobacco smoke provide the carcinogenic agents. *See Fig.* 14 for the 'chutta' and other oral cancers associated with customs in India in the use of tobacco and other substances.

Carcinoma of the Base of the Tongue and the Tonsils.—Epidemiological surveys showed that Hindus from Gujerati had a twofold incidence of these cancers as compared with similar people in the Deccan; furthermore, this type and location of cancer in Gujeratis greatly outnumbered all other buccopharyngeal tumours among them.

Two probable causes, originating in special smoking and tobacco habits, were discovered to explain these statistical findings. The Gujerati smokes a 'home-made' cigarette composed of tobacco flakes loosely rolled in a dried leaf, and he also chews tobacco; whereas his Deccani relative does not engage in such practices.

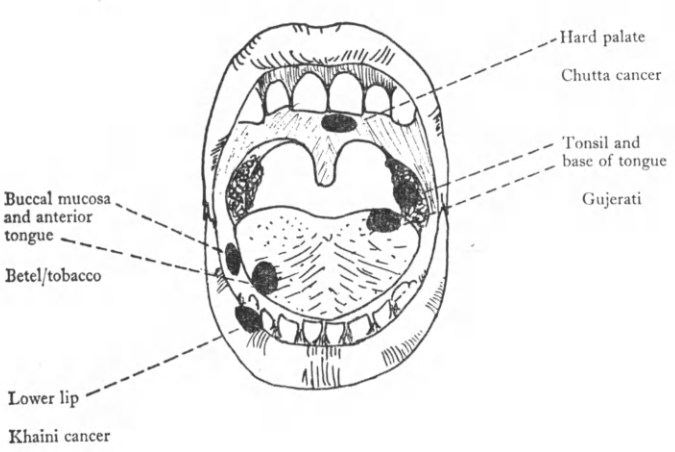

Fig. 14.—Oral cancer and habits in India.

Khaini Cancer occurs particularly in Uttar Pradesh and Bihar. Khaini, the name given to a mixture of tobacco and slaked lime, is habitually sucked by many men in the districts; the quid is kept in the lower alveolar-labial fornix for this purpose for many hours during the day. It leads to a high incidence of carcinoma of the lower lip.

Betel-chewing Oral Cancer.—The betel palm bears a nut-like drupe with an outer fibrous husk. The nut is astringent, mainly due to its content of tannin, and it is also peppery to the taste. In addition to tannin and other substances, it contains at least 6 alkaloids which are pyridine derivatives. Usually the nut is chipped or ground, mixed with slaked lime, commonly made from powdered shells, further spiced with kateku, or ginger, or pepper, or a mixture of a number of such tasteful additives; all of which are wrapped in a betel leaf and chewed. Many habitués add tobacco to the mixture; others do not. The tobacco reinforcement may be the custom in certain geographical areas, or it may be a matter of taste and preference among a section of people in one area. The variation has given rise to interesting findings on cancer incidence and to a debate on the precise pathogenic factor.

Betel-chewing Oral Cancer, *continued.*

According to Khanolkar,*† tobacco is the crucial agent causing cancer among betel chewers. He notes that among Javanese who chew betel without tobacco, the incidence of oral cancer is low. Farago‡ quotes Marsden's (1958) report that in Malaya, the incidence of oral tumours is high in Indians, rare in Malays, and unknown in Chinese; and that this is explained by the fact that only Indians add tobacco to their betel preparations. Muir and Kirk§ raise some doubt as to whether it is solely tobacco that is cancerigenic. They report the presence of a mild carcinogen in betel leaf. Farago adds yet another interesting and provoking facet to the subject. All except 2 of his 110 patients suffering from oral cancer were inveterate betel chewers, but none chewed tobacco with the betel. However, most of them smoked, usually a local tobacco or an imported 'twist' rolled into newspaper or tobacco leaf to make a 'cigarette'. Sanghvi and others|| emphasize both smoking and chewing in tumour induction in the oropharynx. The reconciliation of apparently divergent observations probably boils down to oral sepsis (occasioned by betel chewing, lack of oral hygiene, and nutritional avitaminosis) plus the superimposed tobacco factor to make a combined, or 'two-stage' or 'multi-stage', carcinogenic mechanism.

Whichever theory ultimately proves valid, the epidemiological and geographical data provide very substantial evidence of this oral cancer being caused by an agent associated with the custom of betel chewing together with the use of tobacco. The relative prevalence of oral cancer among those who practise the habit is highly significant. Khanolkar† puts oral cancer as comprising 35·9 per cent of all cancers among them. Krishnamurthi and Shanta¶ state that at the Cancer Institute, Madras, oral cancer with a frequency of 34 per cent is the most common of all forms of malignancy. The authors ascribe the reason for this excessive rate to the common habit of 'tobacco, betel, and nut chewing'.

Nasopharyngeal Cancer.—Whereas the cancers hitherto described in this chapter have causes which are now recognized with a reasonable degree of certainty, there are other cancers with a distinctive geographical distribution among Far Eastern peoples, suggestive of an origin from some regular and particular practice, but whose cause is still elusive. One of these is nasopharyngeal cancer among

* Khanolkar, V. R. (1950), 'Cancer in India in Relation to Race, Nutrition, and Customs', *J. natn. Cancer Inst.*, **11**, 640.

† Khanolkar, V. R. (1951), 'Cancer in Relation to Race, Nutrition, and Customs', *Acta Un. int. Cancr.*, **7**, 51.

‡ Farago, C. (1963), 'Review of 110 Cases of Cancer of Oral Cavity in Papua and New Guinea', *Br. med. J.*, **1**, 1264.

§ Muir, C. S., and Kirk, R. (1960), 'Betel, Tobacco and Cancer of the Mouth', *Br. J. Cancer*, **14**, 597.

|| Sanghvi, L. D., Rao, K. C. M., and Khanolkar, V. R. (1955), 'Smoking and Chewing of Tobacco in Relation to Cancer of the Upper Alimentary Tract', *Br. med. J.*, **1**, 1111.

¶ Krishnamurthi, S., and Shanta, V. (1963), 'Evaluation of Treatment of Advanced Primary and Secondary Gingival Carcinoma', *Ibid.*, **1**, 1261.

Chinese, Malays, Dayaks, and Filipinos. Marsden* records the incidence among these races as 10 per cent or more, as against an incidence of less than 1 per cent in other peoples. Yeh and Cowdry† report that it accounts for 30·42 per cent of cancers in males in Formosa as against 0·2 per cent in the United States of America. Although many pathogenic elements contained in social customs (e.g., smoke from kerosene lamps or candles, or special habits in smoking tobacco) have been suggested, they remain, at the moment, conjectures. Further study is required to elucidate this puzzle.

Cancer of the Penis.—Among religious observations, that involving circumcision has had a definite influence upon the prevalence of cancer of the penis.

Racial demographic data are highly significant. Penile cancer is exceptional in Jews, rare in Moslems, and common in Hindus, Chinese, and Latin Americans. Early postnatal circumcision in Jews is prophylactic in regard to balanitis and cancer. Among Indians, living in the same or adjacent areas, the low rate in Moslems as compared with the high rate in Hindus has been recorded and explained by Khanolkar‡ on the basis of the Moslem religious practice of circumcision. Among these Moslem groups, circumcision is performed at an average age of 6–8 years. Although their cancer rate is low as compared with Hindus, it is higher than that in Jews. Another pointer to the basic reason is that the penile cancer rate is quite high in Pakistani Moslems (according to Mahju§) where circumcision is practised nearer to the age of puberty. The inference from these comparative statistical premises is that circumcision confers an immunity to cancer of the penis, and the earlier it is performed, the greater the immunity.

This is not the complete answer, however. It does not explain why, among the uncircumcised, there are rates varying from 0·95 per cent (Harnett‖) in males in London to 18 per cent among Chinese (Ngai¶). Here the factor of infection is adduced to explain this discrepancy. The preceding clinical history of cancer of the penis is characteristically marked by balanitis. This infection is unknown in the circumcised; and, in the presence of a prepuce, phimosis is the common anatomical antecedent. Among the populations of the West, persistent phimosis is usually treated by circumcision; among the Chinese (as well as in others with a high cancer rate), it is not so treated, and, with concomitant defective hygiene, pyogenic infections are frequent. It is surmised that such recurrent infections eventually precipitate neoplastic change.

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* Marsden, A. T. H. (1964), 'The Pathology of Carcinoma of the Nasopharynx', *Med. Proc.*, **10**, 183.

† Yeh, S., and Cowdry, E. V. (1954), 'Incidence of Malignant Tumours in Chinese, Especially in Formosa', *Cancer*, **7**, 425.

‡ Khanolkar, V. R. (1951), 'Cancer in Relation to Race, Nutrition, and Customs', *Acta Un. int. Cancr.*, **7**, 51.

§ Mahju, M. Y. (1958), 'Incidence of Carcinoma in Pakistan in Relation to Sex and Site', *Medicos.*, **16**, 168.

‖ Harnett, W. L. (1952), *Survey of Cancer in London*. London: British Empire Cancer Campaign.

¶ Ngai, S. K. (1933), 'The Etiological and Pathological Aspects of Squamous-cell Carcinoma of the Penis among the Chinese', *Am. J. Cancer*, **19**, 259.

Cancer of the Penis, *continued.*

Two other possible causative factors call for consideration: smegma may be carcinogenic; and venereal sores, maintained in a chronic and recurrent state by secondary infection, which is more apt to occur under cover of a prepuce, may play a part.

Cancer of the Cervix.—Cancer of the cervix is a common and widespread condition. In the United States of America, O'Donnell and others* record 29,000 new cases per year, representing 11·5 per cent of all cancers in the female, and a mortality-rate of 10,000 per year. Harnett abstracts a very similar figure of almost 12 per cent from a London survey of the prevalence of cancer. These authors compute the relative incidence of uterine cervix to corpus cancer as between 2 and 3 to 1. In Vellore, South India, Gault† gives the ratio in hospital statistics as 41 cervix to 1 corpus. Here, as elsewhere among particular Indian communities, there appears to be a very high proportion of cervical cancers relative to other female cancers.

The justification for incriminating religious, social, and racial customs as part of the picture of causation of uterine cervical cancer has gathered strength from accumulating geographical and demographic statistical surveys.

The rarity of the condition in virgins has featured in many investigations, and has been brought to rather spectacular prominence by Gagnon,‡ who reports that no case of cancer of the cervix has been observed in 3280 nuns in Canada over a period of 20 years. The expected incidence in an equal population of married women, according to Stocks,§ would range from 20 to 40 cases. This points to the culpability of sexual intercourse and/or childbearing as causative factors.

In support of this suggestion there are the comparative figures for incidence in married women with children and those without. Stocks records that cancer of the cervix is 23 per cent higher in the married 'fertile' group. However, it is not only childbearing that is causatively related; 23 per cent does not account for the full measure of excess occurrence in married women. This may be inferred from the fact that the mortality-rate, as noted by Stocks,§ for cervical carcinoma is twice as high in childless married women as it is in single women; thus implicating sexual intercourse, apart from pregnancy, as a factor.

The age of marriage or the commencement of regular coitus has a bearing on susceptibility. Quite apart from the question of parity which is positively correlated with age at marriage (i.e., the earlier the marriage, the larger the family as a rule), intercourse at a young

* O'Donnell, W. E., Day, E., and Venet, L. (1962), *Early Detection and Diagnosis of Cancer.* St. Louis: Mosby.

† Gault, E. W. (1958), 'Cancer of the Uterus in India', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

‡ Gagnon, F. (1950), 'Contribution to Study of Etiology and Prevention of Cancer of Cervix, and of Uterus', *Am. J. Obstet. Gynec.*, **60**, 516.

§ Stocks, P. (1958), 'Statistical Investigations concerning the Causation of Various Forms of Human Cancer', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

age has its own contribution to the incidence of cervical malignancy. Stocks* has drawn attention to this deduction arising from the discovery that with equal parity, cancer of the cervix is more common in those married at an earlier age. Wynder and others† also deduce from their study that early sex relations seem to predispose to cervical cancer. Important additional evidence lending colour to this view derives from the works of Khanolkar‡ and Gault.§ In Deccani Hindu women, cancer of the cervix constitutes about one-half of all female cancers; in Parsee women, who marry, on an average, 10 years later than do Hindu women, it amounts to a much lesser proportion—less than one-fifth. Gault's§ review also shows an excess incidence in Vellore, Madras, Ceylon, and in fact wherever early marriage is the custom.

The next notable feature disclosed by demographic analysis is a definite racial variation. The incidence in Jewish women is low, and although not as low, it is reduced in Moslems. In Gault's hospital material, Hindu women accounted for 91 per cent and Moslem women for only 4 per cent. Wynder and others† have underlined figures brought to attention by many others in regard to the very low rate of cervical carcinoma in Jewish women, and to the lesser degree of reduced incidence in Moslem women. These racial rates are correlated with the fact that the husbands of Jewish and Moslem women are circumcised. The supposition is that the uncircumcised sexual partner conveys some carcinogenic agent to the female; and smegma, probably with the organisms it harbours, is regarded with suspicion. Gault describes the inadequate hygiene of Hindu men of the social class in which the wives suffer so often from cervical cancer. The custom is for the men to bathe in the open near a well or river whilst keeping on a garment covering the genitalia. In consequence, penile and preputial hygiene is very poor. For those with cleaner habits, the wives have a lesser cancer rate; and for the wives of the circumcised, the incidence is lower still.

In summary, the demographic evidence as to the causative factors involved in carcinoma of the cervix points to the following positive factors: Marriage; the earlier the marriage the more susceptible the woman. Childbearing; a higher rate of cancer after a greater number of pregnancies. Lack of adequate hygienic care of penis and prepuce in the male partner.

Cancer of the Breast.—Whereas cancer of the cervix tends to occur in greatest concentration among the lower social classes in poor economic circumstances, cancer of the breast has an opposite proclivity to occur with greater prevalence in the higher social

* Stocks, P. (1958), 'Statistical Investigations concerning the Causation of Various Forms of Human Cancer', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

† Wynder, E. L., Cornfield, J., Schroff, P. D., and Doraiswami, K. R. (1954), 'Environmental Study of Cancer of the Cervix', *Am. J. clin. Path.*, 24 Suppl., 8, 67.

‡ Khanolkar, V. R. (1958), 'Cancer in Relation to Habits and Customs', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

§ Gault, E. W. (1958), 'Cancer of the Uterus in India', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

Cancer of the Breast, *continued.*

grades. A further contrast between the incidence of the two cancers is that relating to marriage and parity. Cervical lesions are rare in virgins, and rise in incidence with early marriage and number of pregnancies; breast malignancy has its highest incidence in women who have not borne children or in whom childbearing has been postponed or reduced to a single event.

There is a rational correlation between these two social circumstances in which contrasting prevalences occur; viz., the lower social grades tend to have more children from an earlier age, but in the higher social strata, marriage comes at a later age, and families are smaller.

The greater liability of childless women to breast cancer is shown in the reviews of the Registrar-General* and by many surveys in different parts of the world, as, e.g., that of Clemmesen and Neilsen† in Copenhagen. It appears that it is the absence of childbearing during the first decade or decade and a half of mature sexual life that is an important factor causatively related to an excess incidence of carcinoma of the breast after the age of 45 years.

Since breast function is largely subject to the influence of hormones, particularly those elaborated by sexual and reproductive organs, it has been inferred that the breast cancer rate is correlated with unbalanced breast activity arising from the absence or inadequacy of hormones produced in quantity in pregnancy and lactation (i.e., mainly progesterone) and thus prolonged, relatively unopposed influence exercised by oestrogen.

The hypothesis may derive some support from geographical incidence figures. Japan has a very low breast cancer rate, in the neighbourhood of one-tenth or less than that of most Western countries; this is accompanied by a high birth-rate, early marriage, and prolonged lactation.

It is of interest to compare the distributional variation of breast cancer with that of fibro-adenosis, which also has a higher incidence in association with infertility. Geschickter‡ emphasizes its probable main cause as the absence or postponement of pregnancy and lactation, bringing about cessation of luteal function with persistence of oestrogenic activity. Therefore, this condition, too, has a relationship to a form of social practice. The suggestion that the incidence of breast cancer is 4–5 times higher in breasts affected by fibro-adenosis than in normal breasts, further links both pathological conditions to a set of causative conditions arising from a common social background.

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* Registrar-General of England and Wales Statistical Reviews (1949, 1951, and 1953). London: H.M.S.O.

† Clemmesen, J., and Neilsen, A. (1951), 'The Social Distribution of Cancer in Copenhagen. 1943 to 1947', *Br. J. Cancer*, 5, 159.

‡ Geschickter, C. F. (1945), *Diseases of the Breast.* 2nd ed. Philadelphia: Lippincott.

CHAPTER VIII

OCCUPATIONAL TUMOURS OF THE URINARY PASSAGES

HISTORICAL NOTE

The early history of the association of tumours of the bladder with employment in dye-making industries has been reviewed by Hueper.* The first 'aniline' tumours, so called because of exposure during the manufacture of aniline dyes, were reported in 1895 by Rehn.† The cases came from German factories which had begun dye-making from coal tar a year or two before 1860. Germany became and remained the main supplier of aniline, its derivatives, and aromatic amines to the world until 1914. Basle, where dye-making was established in 1859, became a subsidiary supplier, and also provided cases of bladder tumour.

The advent of World War I, with the cutting-off of supplies from Germany, brought about the establishment of large manufacturing plants in England, U.S.A., France, and Italy. Bladder tumours followed. In England, where small dyeworks had been in existence prior to 1914, cases of bladder growths had been reported; war-time extension of manufacture led to increasing numbers. Goldblatt‡ reported 101 instances in two factories. In the U.S.A., Gerhmann§ described 27 cases and considered that the first had appeared in 1931. The number of cases also grew in Germany and Switzerland. The International Labour Office, in a review in 1921,‖ accepted as valid over 100 cases from German chemical factories. This review stressed the importance of aromatic amines, especially benzidine and *beta*-naphthylamine, as the most active carcinogens concerned. In Switzerland, Muller¶ described the growing problem in dyestuff workers. With the cooperation of industrial management, he devised improvements in working conditions to reduce tumour incidence and mortality.

Aromatic amines are not only handled in dyeworks, they have an important place as anti-oxidants in the rubber industry. Their use in cosmetics and for medical purposes may extend their potential for harm.

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* Hueper, W. C. (1942), *Occupational Tumours and Allied Diseases*. Springfield, Ill.: Thomas.

† Rehn, L. (1895), 'Blasengeschwulste bei Fuchsinarbeitern', *Arch. klin. Chir.*, **50**, 588.

‡ Goldblatt, M. W. (1949), 'Vesical Tumours induced by Chemical Compounds', *Br. J. ind. Med.*, **6**, 65.

§ Gerhmann, G. H. (1934), 'Symposium on Aniline Tumors of the Bladder', *J. Urol.*, **31**, 126.

‖ International Labour Office (1921), Studies and Reports. Series F, No. 1.

¶ Muller, A. (1939), *Cancer of the Bladder. Occupational Tumours*. Suppl. *Occupation and Health*. Geneva, I.L.O.

EPIDEMIOLOGICAL DATA

Notably high excess rates of bladder tumours have been reported from countries in which there are large dye factories and rubber-processing plants. Case and Pearson* studied the relative correlations between the incidence of tumours and different aromatic amines and intermediate products. They found that the tumour hazard after exposure to benzidine was 19 times, commercial *alpha*-naphthylamine 16 times, and *beta*-naphthylamine was 61 times the usual rate for the whole population. Aniline alone did not appear to cause bladder cancer.

Hueper† has also emphasized highly excessive bladder-cancer rates, and has drawn attention to the wide range of employees exposed to the risk. Not only regular contact, but also intermittent, irregular exposures, e.g., among repair personnel, carry the danger.

Melick and Naryka‡ record the remarkably high bladder-cancer rate in employees in a xenylamine-producing factory in the U.S.A. The factory was in operation from 1935 to 1955, by which time the cancer rate became so obvious that the manufacture of xenylamine was stopped. The shortest duration of exposure was 131 days, when the workman concerned was found to have a bladder cancer. The latent period was, however, usually much longer: after 10–15 years, there was a 15·8 per cent incidence; after 15–20 years, the incidence rose to 35·6 per cent.

The amount of chemical absorbed is not necessarily proportional to the duration of exposure, but this provides the only convenient descriptive measure of 'dosage'. The minimum exposure time has been variously estimated as from 3 months to 1 year. Williams§ states that the latent interval may be as short as 1 year although it is rare under 7 years, or as long as 45 years. It varies with the different chemicals, with an average range of 15–22 years.

Wallace‖ records that the mortality-rate from bladder cancer is relatively low in Japan, Sweden, Italy, Norway, and New Zealand; it is higher in the U.S.A., Canada, Australia, Netherlands, and Scotland; but the highest rates are found in Denmark, England, and Wales. Here, an increasing number of younger men are affected. The reasons for the statistical differences are still matters of conjecture, but there is a tendency to try to find an explanation in exposure to certain chemical substances in use in everyday life, e.g., benzidine in printers' ink on newspapers, and dyestuff in hair-creams.

EXPERIMENTAL WORK

Progress in appreciation of the mechanism of action, development of the condition, and the chemicals responsible, stems from the discovery

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* Case, R. A. M., and Pearson, J. T. (1957), *Registrar-General Report.* London: H.M.S.O.

† Hueper, W. C. (1952), 'Environmental Cancers: A Review', *Cancer Res.*, **12**, 691; *Proceedings of 2nd National Cancer Conference. Am. Cancer Soc.*, **1**, 361; and (1962), 'Environmental and Industrial Cancers of the Urinary Bladder in the U.S.A.', *Acta Un. int. Cancr.*, **18**, 585.

‡ Melick, W. F., and Naryka, J. J. (1960), 'Xenylamine (para-aminobiphenyl) Bladder Tumours in Man', *Acta Un. int. Cancr.*, **16**, 277.

§ Williams, M. H. C. (1958), 'Occupational Tumours of the Bladder', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

‖ Wallace, D. M. (1964), 'Carcinoma of the Bladder', *Jl R. Coll. Surg. Edinb.*, **9**, 107.

by Hueper and others* that the dog's bladder was sensitive to the carcinogenic action of *beta*-naphthylamine. Thus circumstances were established in which a cancer, inducible in a laboratory animal by a known agent, resembled in site and genesis a similar tumour in man. Many workers have been excited by prospects of a revealing field of research and the hope of discovering generalizations of wide applicability to the biology of tumours.

The work of Hueper has been confirmed and extended by Bonser† and many others. In addition to *beta*-naphthylamine, benzidine and 4-aminodiphenyl are carcinogenic in the dog's bladder. Scott and Boyd‡ transplanted dog's ureters into an isolated loop of sigmoid colon; the dogs were fed with *beta*-naphthylamine; tumours did not form in either colon loop or bladder, but did at the lower ends of the ureters, just proximal to where they were constricted by the operative anastomosis. These experiments indicate that the carcinogen is carried in urine and requires sufficiently prolonged contact and sufficient concentration to induce tumours. Experiments by McDonald and Lund§ in which the bladder is divided into 2 parts, only one of which is subjected to contact with urine after the administration of *beta*-naphthylamine, have also proved that the carcinogen in transported in the urine, as only the portion giving passage to urine develops cancer, whereas the portion isolated from urine does not.

Bonser and others‖¶ studied the carcinogenic action of certain aromatic amines and their intermediate derivatives in experimental animals. Using mice, these workers elaborated a technique of intravesical surgical implantation of pellets containing the chemicals to be tested. This method overcame difficulties (mainly due to instability of amine metabolites) of ensuring sufficient concentrations of particular carcinogens by feeding mice with parent amines. Not only were different amines, their metabolites and impurities, and other compounds tested in this manner, but also the vehicles used for making the pellet, so as to take into account any separate or combined carcinogenic effect due to the pellet itself or its influence upon the release of the contained chemical. The results of these experiments indicate that the metabolites of the aromatic amines are carcinogenic agents; 2-amino-1-naphthol among other *ortho* aminophenols was particularly culpable.

The high carcinogenic properties of *N*-hydroxy derivatives of aromatic amine metabolism were shown experimentally by Miller and

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* Hueper, W. C., Wiley, F. H., and Wolfe, D. H. (1938), 'Experimental Production of Bladder Tumours in Dogs by Administration of *Beta*-naphthylamine', *J. ind. Hyg. Toxicol.*, **20**, 46.

† Bonser, G. M. (1947), 'Experimental Cancer of the Bladder', *Br. med. Bull.*, **4**, 379.

‡ Scott, W. W., and Boyd, H. L. (1955), 'A Study of the Carcinogenic Effect of *Beta*-naphthylamine on the Normal and Substituted Isolated Sigmoid Loop Bladder of Dogs', *J. Urol.*, **70**, 914.

§ McDonald, D. F., and Lund, R. R. (1954), 'The Role of the Urine in Vesical Neoplasm. I. Experimental Confirmation of the Urogenous Theory of Pathogenesis', *Ibid.*, **71**, 560.

‖ Bonser, G. M., Bradshaw, L., Clayson, D. B., and Jull, J. W. (1959), 'The Effect of Variation in Experimental Procedure in Amine Carcinogenesis', in *Carcinogenesis*. Ciba Symposium. London: Churchill.

¶ Bonser, G. M., Bradshaw, L., Clayson, D. B., Jull, J. W., and Pyrah, L. N. (1960), 'The Importance of Metabolic Mechanisms in the Aetiology of Carcinoma of the Bladder', *Acta Un. int. Cancr.*, **16**, 267.

Experimental Work, *continued.*

others.* These metabolites have been found in urine, and they are among the probable carcinogens in man.

MECHANISM OF AMINE CANCER OF THE BLADDER

Boyland† has propounded a plausible concept of the mechanism of bladder cancer arising from occupational exposure to the aromatic amines. His reasoning rests heavily on animal and laboratory experimental work, but this is correlated with the known evidence relating to human bladder cancer.

The organs exposed to carcinogenic amines in industry are the skin, lungs, and gastro-intestinal tract. Although absorption probably takes place via all three organs, none of them is affected by tumour formation. Two explanations were offered for this: insufficient concentration and duration of contact of chemical to produce tumours; or, alternatively, the initially exposed surfaces were not sensitive. In the bladder the circumstances were reversed; the chemicals were concentrated, contact was prolonged, and the epithelium was sensitive. However, it is not only a question of concentration and duration, as it is not the parent amine, but a metabolite of it, that is the carcinogenic agent. Cancerigenic derivatives are apparently not formed at the sites of introduction into the body; and, subsequently, when metabolism does produce potent agents, their potency is dependent upon the tissue and enzymatic activity of the different organs along their course.

Aromatic amine, after absorption through skin, lungs, and/or gastro-intestinal tract, reaches the liver. Here, oxidation produces *N*-hydroxy compounds and *ortho*-aminophenols. Among these, 2-amino-1-naphthol represents a highly carcinogenic compound, as has been discovered in implantation pellet procedures. The intermediate metabolites of amines are also highly toxic. They are immediately rendered inactive in the liver by conjugation with sulphate or glucuronate. The conjugates are then excreted in urine where their concentration is intensified as there is no renal reabsorption. On reaching the bladder the inactive chemical compounds are exposed to enzymes, e.g., *beta*-glucuronidase, which, in a medium of urine providing an appropriate *p*H, reaches optimum activity. Enzymatic action hydrolyses the innocuous conjugated derivatives and releases active metabolites which are carcinogenic agents.

Boyland proposes that preventive therapy be directed to inhibiting the activity of the enzyme *beta*-glucuronidase; an effect which can be achieved by oral administration of 1-4-saccharolactone.

LINK WITH SPONTANEOUS BLADDER CANCER

Human 'spontaneous' cancer of the bladder, so called because the cause is not known, is very similar in its clinical presentation and

* Miller, E. C., Miller, J. A., and Hartman, H. A. (1961), 'N-Hydroxy-2-acetylamino-fluorene: A Metabolite of 2-Acetylaminofluorene with Increased Carcinogenic Activity in the Rat', *Cancer Res.*, **21**, 815.

† Boyland, E. (1959), 'Biochemical Mechanisms of Induction of Bladder Cancer', in *Carcinogenesis.* Ciba Symposium. London: Churchill; (1960), 'Aetiology of Cancer of the Bladder', *Acta Un. int. Cancr.*, **16**, 273; and (1963), *The Biochemistry of Bladder Cancer.* Springfield, Ill.: Thomas.

pathological features to occupational amine cancer. The highly successful investigations and the resultant detailed knowledge of the genesis of amine cancers has excited hopes of discovering possible similar causative factors and mechanisms in spontaneous cancer.

Studies of metabolism of the amino-acid tryptophan, commonly ingested with usual food, reveal that certain intermediate stages do present *ortho*-aminophenols, which are detectable in human urine. Mouse bladder implantation tests by Allen and others* proved these substances to be carcinogenic. The findings are suggestive but are not proof of carcinogenesis in man.

Wallace and White† report that, in different investigations, in 20–50 per cent of patients suffering from spontaneous bladder tumours, an abnormality of tryptophan metabolism was demonstrated. Price and Brown‡ compared tryptophan metabolites in men suffering from spontaneous bladder cancer with those who had industrial bladder cancer. They found evidence of abnormal metabolism in the 'spontaneous cancer group' but not in the 'industrial cancer group'.

There is thus quite a body of evidence supporting the hypothesis that tryptophan metabolites may be a cause of cancer in man, but the evidence is by no means conclusive.

NON-INDUSTRIAL ENVIRONMENTAL FACTORS

Clemmesen and Neilsen§ found that the incidence of bladder cancer was higher in urban than in rural districts. Whilst urban conditions, with factories and chemical industries, may bring about wider and greater contact with aromatic amines, there is still an unsettled problem of the possibility of some other environmental circumstances to account for different urban and rural rates.

Clemmesen and co-workers,|| among others, report that bladder cancer is more commonly found among men who smoke than in non-smokers. A number of studies in different parts of the world indicate that the ratio of bladder cancer in smokers as compared with non-smokers is between 2 and 3 to 1. This does not remotely approximate the very much higher ratios exhibited by lung cancer in association with smoking; but the uniform results obtained do suggest some causative relationship between smoking and bladder cancer.

The relationship of bilharzial infection and bladder cancer affords another example of a probable environmental cause, and is discussed in Chapter V.

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* Allen, M. J., Boyland, E., Dukes, C., Horning, E. S., and Watson, G. J. (1957), 'Cancer of the Urinary Bladder in Mice with Metabolites of Aromatic Amines and Tryptophan', *Br. J. Cancer*, **11**, 212.

† Wallace, D. M., and White, R. W. (1963), 'Biochemical Aspects of Bladder Tumours', *Proc. R. Soc. Med.*, **56**, 931.

‡ Price, J. M., and Brown, R. R. (1962), 'Studies on the Aetiology of Carcinoma of the Urinary Bladder', *Acta Un. int. Cancr.*, **18**, 684.

§ Clemmesen, J., and Neilsen, A. (1956), 'Cancer Incidence in Denmark 1943–1953. II. Tumours of the Urinary System and Prostate', *Dan. med. Bull.*, **3**, 36.

|| Clemmesen, J., Neilsen, A., and Lockwood, K. (1960), 'On the Aetiology of Bladder Tumours', *Acta Un. int. Cancr.*, **16**, 289.

SITES OF TUMOURS

In order of frequency, the bladder, renal pelvis, ureter, and urethra are the sites of tumour growth. This is most probably dependent upon the duration of contact and action by the carcinogen on mucosal epithelium. The tumours are multicentric in origin; often beginning in the bladder, particularly near the ureteric orifices, and then presenting in renal pelvis and/or ureters near their lower ends. An alternative theory that spread occurs by implantation seeding from a single primary does not fit the known presentation and progress as well as does the concept of multifocal origin. Urethral lesions are rare but less so than has been supposed; they are found on urethroscopic examinations and they are also appearing in the form of recurrences, or delayed primary growths, following extensive operations on the bladder.

PATHOLOGY

About 90 to 95 per cent of bladder tumours arise from the mucosal lining of transitional epithelium. The patterns of reaction of bladder mucosa throw some light on the different presentations of neoplastic lesions. Mostofi* describes the following patterns of change in bladder epithelium and their relationship to the types of tumour that occur:—

1. Outward growth to form a projection. This is the commonest neoplastic form, amounting to 80 per cent.
2. Deep growth into lamina propria, sometimes forming Brunn's nests and glands; a form of reaction which occurs in some types of benign cystitis. Some epithelial tumours appear to start as infiltrating tumours; and over one-third of papillary tumours become invasive.
3. Metaplasia from transitional epithelium into squamous epithelium. About 10 per cent of cancers begin as squamous epitheliomas and 15–20 per cent of infiltrating transitional cancer show some areas of squamous metaplasia.
4. Transitional epithelium may give place to glandular epithelium with or without mucus.

Dukes,† with reference to the general pattern of the tumour, regards the distinction between 'papillary' and 'solid' tumours as fundamental to the assessment of the case, as 'solid' usually means infiltration. Sometimes papillary and solid elements are both present in one tumour. On a histological basis, Dukes differentiates six classes: (1) benign papilloma, (2) differentiated transitional-cell carcinoma, (3) anaplastic transitional-cell carcinoma, (4) squamous-cell carcinoma, (5) adenocarcinoma, and (6) other varieties.

Histological differentiation between benign and malignant tumours is often not definable. Many pathologists regard all papillary tumours as carcinoma, or as so potentially malignant as not to merit the description 'benign'. It is of value to note the criteria and grading formulated by the American Bladder Tumour Registry and to compare them with the groups classified by Dukes.

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* Mostofi, F. K. (1954), 'Potentialities of Bladder Mucosa', *J. Urol.*, **71**, 705; and (1962), 'Pathology of Cancer of the Bladder', *Acta Un. int. Cancr.*, **18**, 611.

† Dukes, C. E. (1955), The Classification of Tumours of the Bladder. (Histological Grading.) Inst. of Urol. (Univ. of London). Broadsheet No. 1.

Benign Papilloma.—The cells are indistinguishable from the normal; there are 3–5 layers of cells, which are fully differentiated and normal in arrangement; the basement membrane is distinct and intact. Three per cent of bladder tumours are classified on this basis as benign papilloma. Both systems of classification use the same nomenclature for this tumour.

Grade I.—There are more than five layers of cells; mitoses are somewhat increased, and there is lack of differentiation from base to superficial layers. This grade of low malignancy accounts for 27 per cent.

Grade II.—Intermediate in cell activity and differentiation between grades I and III. In the American Registry, 34 per cent are recorded under this heading. Grades I and II appear to match the 'differentiated transitional-cell carcinoma' of Dukes, with a marginal class appearing as more undifferentiated characters develop. In defining 'differentiated' carcinomas, Dukes adds the points that epithelial-cell pleomorphism with more deeply staining and larger nuclei appear; that the basement membrane of papillary tumours may show breaks; and, in solid tumours, there may be indications of invasion of adjoining tissues.

Grade III.—Anaplasia is marked and the mitotic index is high. About 30 per cent fall into this class. This and the next grade are comparable to 'anaplastic transitional-cell carcinoma' as described by Dukes.

Grade IV.—Used to indicate the most intensely anaplastic varieties. According to Dukes, squamous-cell carcinoma may be superimposed upon an epithelium already altered to squamous type by some preceding aetiological factor, or, as is more common, it arises without any prior non-malignant metaplasia.

Adenocarcinoma may arise from mucus cells normally present in the trigone, or from urachal remnants, ectopia vesicae and other developmental anomalies, or from metaplasia following chronic infection. Adenocarcinomas are rare, comprising about 2 per cent of all bladder tumours.

Spread.—'Aniline' tumours of the bladder often remain confined to the mucosa for periods up to several years. Spread may be direct to contiguous tissues, via lymphatic channels to lymph-nodes, and by the blood-stream.

The extent of direct spread indicates stages of progress of the tumour. Marshall* proposed a scheme of staging from which *Fig.* 15 has been modified; it is not essentially different from that more recently recommended by the World Health Organization. Stage O is carcinoma-in-situ; A is cancer restricted to submucosa; and B refers to spread to involve muscle. Stage C indicates direct spread through the bladder wall into surrounding fat; D_1 includes nodal involvement confined to the pelvis, and D_2, when para-aortic nodes are affected. D_3 has been added to indicate extension fixing

* Marshall, V. F. (1956), 'Symposium on Bladder Tumors; Current Clinical Problems regarding Bladder Tumors', *Cancer*, 9, 543.

Spread, *continued.*

the bladder to the pelvic wall; an important feature indicating a very advanced stage. Willis* records that in his series of autopsies, one-third exhibited lymph-node metastases. A similar proportion was found to have spread by the blood-stream, mainly to bones, lungs, and liver.

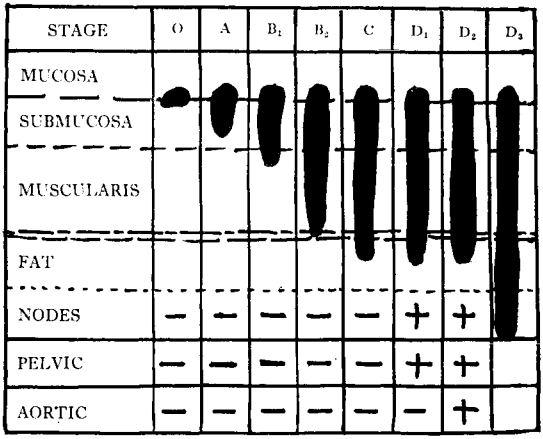

Fig. 15.—Stages of bladder cancer according to spread. (Modified from **Marshall**, 1956.)

CYSTOSCOPIC FINDINGS

Changes in the bladder indicative of its reactions to carcinogenic amines and its progress towards malignant neoplasia are visible cystoscopically. Di Maio† describes the earliest signs as patchy, intermittent, mucosal congestion associated with telangiectases. The lesions are most commonly situated in the neighbourhood of the ureteric orifices. Telangiectatic patterns are particularly noticeable as fan-shaped, large, distended, and tortuous veins, extending from an apex near the trigone to an area around the ureteric papilla. Di Maio regards these changes as precancerous.

Small haemorrhages may occur from the veins, giving rise to haematuria (and thus acquiring the description of 'haemorrhagic cystitis' used by some authors) and also to mucosal pin-point ecchymoses or larger petechiae. Muller‡ reports additional findings of ulceration and necrosis in about 14 or 15 per cent of his cases.

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* Willis, R. A. (1960), *Pathology of Tumours.* 3rd ed. London: Butterworths.

† Di Maio, G. (1949), 'Affections of the Bladder due to Amines', in *Proceedings IX International Congress on Industrial Medicine*, 476. Bristol: Wright.

‡ Muller, A. (1949), 'Occupational Diseases of the Bladder in the Industrial Area of Basle', in *Proceedings IX International Congress on Industrial Medicine*, 489. Bristol: Wright.

The next stage is the appearance of submucosal elevation with the formation of one or more sessile or pedunculated projecting tumours. They appear, mostly, in a posterolateral segment or near the base of the trigone. These may remain benign tumours and react well to fulguration therapy, but the great danger is their propensity to malignant degeneration after a variable period of some months to a decade or more. The prognosis regarding the development of cancer is rather worse in sessile than in pedunculated tumours.

CLINICAL FEATURES

The condition is often asymptomatic until well advanced. Appreciation of this fact calls for routine examination in cases of exposure to known or suspected chemical carcinogens. Routine cystoscopy has been employed in certain areas, as in North Italy, but its inconvenience has often led to workers abandoning regular attendances. Cytological examination of urinary sediment by Papanicolaou methods is less irksome to the patient and is proving increasingly valuable; it is claimed to be positive in about 80 per cent of bladder cancers.

Haematuria is the important sign of probable pathology, and it calls for thorough cystoscopic examination, retrograde pyelography, and biopsy of suspect areas.

Obstructive phenomena, either the passage of debris and clot down a ureter, or by the tumour occluding the ureter or bladder neck, may be attended by different forms of dysuria and renal colic. The pains of hydro-ureter and hydronephrosis and the symptoms and signs of secondary infection, are also reported.

CHAPTER IX

CANCER OF THE LUNG

INCIDENCE

The mortality-rate from cancer of the lung has been increasing over wide areas and in many different countries in the world. In England and Wales, a progressive, unrelenting increase in the number of certified deaths from lung cancer has marked the years since 1907, when there was a total of 297, to 1963 with a total of more than 23,000, i.e., 80 times as much. In the United States of America, the figure of 250 deaths in 1930 rose to over 30,000 in 1959. In 1957, the Medical Research Council* emphasized the growing menace of lung cancer by drawing attention to the fact that it caused approximately 1 in 18 male deaths. When deaths from 45 to 64 years are considered, the proportion is 1 in every 9. The mortality-rate has also been growing among females, although not at the same progressive speed and not at the same high level. Female deaths have risen from 123 in 1907 to 3350 in 1961; a rather less than thirty-fold increment; and accounting, in 1957, for 1 in 103 deaths.

Inferences as to the main reasons for the increase are necessarily dependent upon the premise that the increase is true and not apparent. It is therefore material closely to examine the case for this premise.

Two main arguments are advanced to support the view that the increase is apparent. First, improvement in diagnosis has uncovered most of the additional numbers of cases; and secondly, longer survival has brought more of the population into the range of cancer incidence.

It must be conceded that wider clinical knowledge and improved diagnostic techniques account for part of the phenomenal rise; but the following grounds make it unreasonable to apportion more than a small part of the increase to this factor.

The standards of professional skill and the sophistication of technical aids would apply not only to lung lesions, but equally to cancers elsewhere in the body; yet no such prodigious rise has appeared in any other cancer. In the case of lung cancer, if lack of diagnostic ability explained the smaller numbers of recorded cases during earlier years, it must be assumed that more than 95 per cent of lung cancers were missed in the first decade of this century, and, during the 1930's, approximately 5 of every 6 were misdiagnosed: assumptions that are seemingly absurd.

It is justifiable to infer that when mistaken diagnosis did occur, it involved an error in naming the pathological condition; but that the anatomical site of the lesion, namely the lungs, would be correctly described. This means that, if the claim that diagnostic error explains the increased incidence, mortality-rates during the earlier years would

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* Medical Research Council (1957), 'Tobacco Smoking and Cancer of the Lung', *Br. med. J.*, **1**, 1523.

show high figures for lung diseases even if lung cancer featured low on the list. This is not the case. On the contrary, current mortality-rates from lung cancer far outnumber the totals for all pulmonary affections recorded during earlier periods.

Oettlé,* who argues vigorously and cogently on the side of real increase, puts the further point that if improved diagnosis accounts for the increase, the number of undiagnosed cases should be falling. There are indications that this is not so. He quotes a study by Proctor† who found that the frequency of undetected primary lung carcinoma, as determined by metastatic affection of the brain, was increasing; the extent amounted to an eight-fold increase from the period prior to 1918 to that from 1934 to 1953. To this example may be added that of Kennaway and Kennaway‡ who noted a 42 per cent diagnostic error in a group of 84 autopsy-proved lung cancers. This was at a time when modern facilities were in wide and general use in England.

The second contention that the prevalence of lung cancer is only apparently increased, takes its starting point from the fact that improvement in general health and social conditions has led to an increase in the numbers of older people in the population. The rise in lung cancer incidence, it is argued, is due to the increase in numbers of cancers among these older age-groups. However, mortality-rates for lung cancer are not only increased at older ages, but in all age-groups from 45 years onwards.

A reasonable verdict in this debate would give substantial support to the thesis that much, if not most, of the increased prevalence of lung cancer is real and genuine.

TOBACCO SMOKING AS A CAUSE

Extensive investigations have revealed that there are a number of causative agents, some definite, others strongly suspect. In many geographical regions more than one agent is implicated, and in a number of cases of lung cancer more than one agent probably contributes to the pathogenesis. There are still many problems requiring solution; but what has been unravelled over the past decade or so represents a great chapter of achievement in the elucidation of cause and a major breakthrough in terms of potential preventive measures against one of the commonest of mortal cancers.

There is considerable evidence that tobacco smoking, especially cigarettes, is the principal culprit responsible for what may aptly be described as a pandemic of lung cancer.

Prior to more elaborate studies in recent years, opinions and beliefs had gained published expression from as early as just over 40 years ago. Fahr,§ in 1923, stated his opinion that the increased incidence of pulmonary carcinoma was due to an increase in cigarette smoking. In

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* Oettlé, A. G. (1963), 'Cigarette Smoking as the Major Cause of Lung Cancer', *S. Afr. med. J.*, **37**, 935 and 957.

† Proctor, N. F. S. (1962), personal communication quoted by Oettlé, A. G. (1963), 'Cigarette Smoking as the Major Cause of Lung Cancer', *S. Afr. med. J.*, **37**, 935, 957.

‡ Kennaway, E. L., and Kennaway, N. M. (1947), 'A Further Study of the Incidence of Cancer of the Lung and Larynx', *Br. J. Cancer*, **1**, 260.

§ Fahr, M. (1923), in discussion. *Verh. dt. path. Ges.*, **19**, 192. Quoted in Leading Article, *Br. med. J.* (1964), **1**, 133.

Tobacco Smoking as a Cause, *continued.*

1928, Lombard and Doering,* from a study of patients with and without lung cancer, suggested the causative relationship of smoking in lung cancer. Oschner and De Bakey,† from a review of their own clinical material, wrote of their conviction that smoking was the main cause. They found that every one of their own patients, with the exception of two women, was an excessive smoker.

Retrospective Studies.—Doll and Bradford Hill‡ reported the results of a retrospective study of just short of 5000 patients including nearly 1500 suffering from lung carcinoma. In 20 London hospitals housing these patients, questions were directed to test their background in regard to smoking habits, exposure to polluted atmosphere, and other aspects of possible relevance. One striking contrast emerged from this study: the difference in smoking habits. Those with lung cancer had a much bigger proportion of heavy smokers and a smaller proportion of light or non-smokers than did a control group without lung cancer. The relative proportions held for women on a lower scale than for men. Analysis of age incidence led to the conclusion that above the age of 45 years, the risk of developing cancer of the lung increases in proportion to the number of cigarettes smoked, and that it may be about 50§ times as much among those who smoke 25 or more cigarettes per day than among non-smokers.

Similar inquiries into the previous habits (i.e., retrospective studies) of patients with and without lung cancers were conducted in many other countries, including the U.S.A., Norway, Finland, Holland, Germany, and Switzerland. By 1957, the Medical Research Council‖ noted that 19 reports of such studies had been published, and there were an additional 10 noted in the U.S. Public Health Service Report in 1964.¶ All 29 retrospective studies disclosed consistent findings and were in general agreement with the outcome of the British investigation.

Prospective Studies.—In 1951, a different type of study was initiated. The problem was approached in a prospective manner. Doll and Bradford Hill conducted the investigation in which the smoking habits of a group of people were ascertained and their subsequent causes of deaths were analysed in relation to these habits. Questionnaires were directed to registered medical practitioners in Britain; 34,445 men and 6192 women, out of a total of 59,600, responded.

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* Lombard, H. L., and Doering, C. R. (1928), 'Cancer Studies in Massachusetts; Habits, Characteristics and Environment of Individuals with and without Cancer', *New Engl. J. med.*, **198**, 481.

† Oschner, A., and De Bakey, M. (1941), 'Carcinoma of the Lung', *Archs Surg.*, **42**, 209.

‡ Doll, R., and Bradford Hill, A. (1950), 'Smoking and Carcinoma of the Lung', *Br. med. J.*, **2**, 739; and (1952), 'Smoking and Carcinoma of the Lung', *Ibid.*, **2**, 1217.

§ This figure of 50 may be an overestimate; later studies show that it is probably somewhat less.

‖ Medical Research Council (1957), 'Tobacco Smoking and Cancer of the Lung', *Br. med. J.*, **1**, 1523.

¶ Public Health Service Publication, No. 1103 (1964), *Smoking and Health.* Washington: U.S. Govt Print. Office.

The information sought was whether: (*a*) they were, at that time, smokers, (*b*) they had previously smoked but had given up, or (*c*) they had never smoked regularly, i.e., never smoked as much as one cigarette, or its equivalent, a day, for as long as one year.

Four and a half years later, particulars of deaths supplied by the Registrars-General were analysed by Doll and Bradford Hill.* The facts emerging from the study are highly significant and the deductions are of great importance.

In males, mortality-rates increase with the smoking of more cigarettes. For ages of 35 years and over, the lung cancer death-rate per annum was:—

0·07 per 1000 in non-smokers.
0·45 per 1000 in smokers of 14 or less g.† daily.
0·86 per 1000 in smokers of 15 to 24 g. per day.
1·66 per 1000 in smokers of 25 or more g. per day.
2·76 per 1000 in persistent heavy smokers.

In the last group, consisting of men who continued to smoke more than 25 cigarettes per day, the mortality-rate fell just short of 40 times the rate among non-smokers. The following rough calculation expresses this ratio in a forcible manner; at present rates of mortality in males, the persistent heavy smoker is likely to die of lung cancer in a proportion of 1–8; whereas the non-smoker runs a risk of about 1–300. Pipe-smoking appeared to be much less harmful than cigarettes, but it, too, was associated with a higher mortality than in non-smokers.

Of equally notable import was the finding that among those who had given up smoking within the decade preceding the questionnaire, the death-rate from lung cancer was lower; and lower still, where smoking had been discontinued for more than 10 years. The Medical Research Council‡ epitomizes this aspect in the statement 'men who cease to smoke, even in their early forties, may reduce their likelihood of developing the disease by at least one-half'.

Wynder and Cornfield§ report on mortality from lung carcinoma in physicians in America. They put the ratio at a lower level than the figures in Britain, viz., the mortality from lung carcinoma in physicians who smoke at least 35 cigarettes a day is 113 per 100,000, and in non-smokers it is 10 per 100,000. Results similar to those of Doll and Bradford Hill have been reported from the U.S.A. by Hammond and Horn‖ from a prospective study involving nearly 190,000 men. Dorn¶ reports similar findings.

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* Doll, R., and Bradford Hill, A. (1956), 'Lung Cancer and Other Causes of Death in Relation to Smoking; Second Report on Mortality in British Doctors', *Br. med. J.*, **2**, 1071.

† One g. of tobacco is the approximate amount per cigarette.

‡ Medical Research Council (1957), 'Tobacco Smoking and Cancer of the Lung', *Br. med. J.*, **1**, 1523.

§ Wynder, E. L., and Cornfield, J. (1953), 'Cancer of the Lung in Physicians', *New Engl. J. Med.*, **248**, 441.

‖ Hammond, E. C., and Horn, D. (1958), 'Smoking and Death Rates—Report on Forty-four Months of Follow-up of 187,783 Men. I. Total Mortality', *J. Am. med. Ass.*, **166**, 1172; and (1958), 'Smoking and Death Rates—Report on Forty-four Months of Follow-up of 187,783 Men. II. Death Rates by Cause', *Ibid.*, **166**, 1294.

¶ Dorn, H. F. (1959), 'Tobacco Consumption and Mortality from Cancer and Other Diseases', *U.S. Publ. Hlth Service Rep.*, **74**, 581.

Prospective Studies, *continued.*

Doll and Bradford Hill* supplemented their earlier report by another, following observations of British medical practitioners for a period of 10 years, and for several years after a second questionnaire had been addressed to all the survivors. The outcome of this later analysis does not gainsay any of the deductions from the first; in fact, the evidence is more substantial in view of the longer period of observation, and in the light of several new facets which emanate from it.

In regard to the total mortality in this group of almost 41,000 doctors, the rate was 28 per cent higher among cigarette smokers, 19 per cent higher in all smokers, and 1 per cent higher among pipe or cigar smokers.

Mortality from lung cancer climbed steadily with increasing consumption of cigarettes at every age above 45 years. A linear relationship characterized this rise from 0·07 per 1000 in non-smoking men to 3·15 in men smoking 35 or more cigarettes daily. This linear rise, starting with non-smokers and advancing through the various grades of amounts smoked up to the class of heavy smokers, signifies that a smoking threshold (i.e., a minimum quantity of concentration of smoking below which lung cancer does not occur) does not exist.

Among ex-smokers, the death-rate falls progressively with increasing duration of abstinence from smoking. The death-rate from lung carcinoma per annum per 1000 varies as follows:—

Discontinued for less than 5 years	0·67
Discontinued for 5 to 9 years	0·49
Discontinued for 10 to 19 years	0·18
Discontinued for 20 years or more	0·19
Persistent smokers	1·28
Non-smokers	0·07

The relationship is a direct one, and nullifies the suggestion of the operation of a genetic factor. This suggestion supposes that smokers who give up smoking belong to a selected group who would in any event exhibit a relatively low mortality from cancer of the lung. If this were so, the lower mortality-rate would be constant in the whole group, i.e., the figure would reflect the genetic fate of the group. But the facts are at variance with this hypothesis and its implications: the facts are represented by a graduated decline in mortality-rate varying directly with duration after having given up smoking, and are most reasonably compatible with the removal of an environmental carcinogenic agent. The figures following cessation of smoking demonstrate a dramatic reduction of the risk of lung cancer; the mortality is halved by stopping for 5 years; it is cut to nearly one-third by cessation for 5 to 9 years; and after 10 years and more of discontinuance, the death-rate is reduced to between one-sixth and one-seventh of that pertaining to persistent smokers.

* Doll, R., and Bradford Hill, A. (1964), 'Mortality in Relation to Smoking: Ten Years' Observation of British Doctors', *Br. med. J.*, **1**, 1399 and 1460.

The authors accept the possibility that doctors who stop smoking may not be a completely unselected group, because those doctors with known or suspected severe and possibly fatal diseases might continue smoking, there being no point in giving up. Such a selective effect, the authors claim, will wear off after the first few years. The adjusted figures show a possible small statistical effect, but the main conclusions remain valid.

Smoking and Air Pollution.—The rise in death-rates from lung cancer in association with increasing consumption of cigarettes occurred in all areas—big towns, small towns, rural areas, and combinations of residence. Furthermore, the very low lung cancer mortality in non-smokers living in urban areas does not support the idea that air pollution *per se* is an important environmental carcinogenic agent.

Inhaling Smoke.—The effects of inhaling were elicited in the second questionnaire; the observations cover a period of about 4 years. The figures and periods are not yet extensive enough to provide information on all aspects; but in regard to cigarette smokers who inhale and who continue to smoke, after standardization for the amount smoked and for age, there was an appreciable relative excess of lung cancer mortality. Light smokers (1–14 cigarettes daily) who inhaled provided the most marked relative increase in lung cancer death-rate; among inhalers the death-rate was 1·59 per 1000, whilst there was no death recorded during the 4 years among non-inhalers at this level of smoking. Among moderate smokers, i.e., 15–24 cigarettes daily, there was an increased mortality in inhalers, though not as marked a difference as in light smokers. The data on heavy smokers did not reveal a significant difference between inhalers and non-inhalers. It is likely that at this high concentration of smoking, inhalation is virtually an invariable habit.

Mortality among Women.—The total numbers studied were much smaller than for males; and moreover, fewer women were in the age-groups of high mortality-rates as compared with the men. The figures therefore differ in important respects, and they are not as comprehensive or as informative as in males. With these reservations in mind, it is still possible to read an increased mortality-rate from lung cancer in women smokers although the rate is lower than that in men. A number of features emerging from the questionnaires addressed to women may provide some of the answers to sex differences in lung cancer rates. Women began smoking at a later average age, viz. 24 years, which was 4½ years older than the average for males. This age difference was even more marked (over 8 years) in regard to women smokers above the age of 55 years in 1951. Another notable difference appeared in regard to inhaling. At every age and at each level of smoking, women inhalers were fewer, and this was particularly so at 55 years and over, when lung cancer had its highest prevalence.

Pathology.—The increase in lung cancer in association with cigarette smoking concerns squamous-cell and anaplastic, including oat-cell, types, i.e., those classified as Kreyberg Group I. Adenocarcinoma, or Kreyberg Group II, is not affected or induced by smoking.

Pathology, *continued.*

The histological features of bronchial epithelial changes in relation to cancer, smoking, sex, residence, occupation, and age have been the subjects of a number of communications by Auerbach and others.* Their studies of material from deaths other than cancer show a high degree of correlation between cigarette smoking and certain histological characters of bronchial epithelium: hyperplasia, loss of ciliated columnar cells, metaplasia and cells with atypical nuclei. These latter cells resemble neoplastic cells, but fall short of being diagnostic of cancer in that there is no invasion of neighbouring tissue.

These cellular changes, and especially the atypical nuclei, become more widely distributed and more intense with increased smoking. Among pipe-smokers and cigar-smokers, the cellular lesions are fewer in number than in cigarette smokers, but are much more commonly seen than in non-smokers. In non-smokers, apart from finding very few such altered cells, not one lesion was found which was composed entirely of cells with atypical nuclei; the same held with regard to pipe-smokers; in cigarette smokers many such 'entire' lesions were found, especially in persistent smokers and at older ages. In non-smokers, even at old ages, and there were 5 over the age of 85 years in the series, there was little change in bronchial epithelium; in fact the findings in children were the same as in adult males who had never smoked.

Among ex-smokers, there were fewer epithelial changes, especially in regard to cells with atypical nuclei; the diminution in their numbers progressed with increase in duration of abstinence from smoking. Ex-smokers exhibited a unique type of cell, described as having a 'disintegrating nucleus'. There is no proof, but it seems likely that this cell derives from, and is a stage in the disappearance of, the 'atypical cell'. The frequency of occurrences of disintegrating cells appears to increase for perhaps 15 years after cessation of smoking, and then probably decreases gradually.

Among women, every type of epithelial abnormality, especially atypical cells, was more frequent in smokers than in non-smokers. The findings were thus parallel to those in males, but on a moderately lower plane.

Among female non-smokers, there were limited epithelial changes, mainly hyperplasia in the form of three or more cell rows with and without cilia, and but very few atypical nuclei. In women in urban areas, epithelial lesions were found in 26·3 per cent as compared with 18·8 per cent in rural areas. A difference which the authors describe as 'trivial as compared with that between smokers and non-smokers'.

* Auerbach, O., Stout, A. P., Hammond, E. C., and Garfinkel, L. (1960), 'Microscopic Examination of Bronchial Epithelium in Children', *Am. Rev. resp. Dis.*, **82**, 640; (1961), 'Changes in Bronchial Epithelium in Relation to Cigarette Smoking and in Relation to Lung Cancer', *New Engl. J. Med.*, **265**, 253; (1962), 'Changes in Bronchial Epithelium in Relation to Sex, Age, Residence, Smoking and Pneumonia', *Ibid.*, **267**, 111; and (1962), 'Bronchial Epithelium in Former Smokers', *Ibid.*, **267**, 119.

The histological changes in bronchial epithelium in relation to smoking cigarettes, cigars, and pipe; abandonment of smoking and its duration; women as compared to men smokers; non-smokers; urban and rural residence; and at different ages, all bear a remarkable and notably significant parallelism to epidemiological data on lung cancer in relation to the same circumstances.

Mechanism of Action.—The mode of action of tobacco smoking is uncertain. Carcinogenic chemicals have been abstracted from tobacco smoke. There are claims that some have been shown, e.g., by Wynder,* to produce skin cancers in laboratory animals; but this work requires further elaboration as well as demonstration that cancer production in laboratory animals necessarily incriminates the agents as carcinogenic to human bronchial epithelium. There is some evidence that they may act as promoting agents (Roe and others†). Many aspects of the mechanism of carcinogenesis are currently under investigation and numerous reports hint that the complexities are being disentangled.

The histological observations of Auerbach and others carry interesting reflections on problems of the mechanism of carcinogenesis. The authors pose, mainly on the basis of the different concentrations of atypical cells which so closely resemble neoplastic cells, the following possibilities:—

1. Cigarette smoke contains chemical components which are carcinogenic.
2. Smoking may increase susceptibility to other carcinogenic agents; it may act mechanically or as a cocarcinogen.
3. Smoking may so alter local environment as to favour survival and reproduction of such cells once they have been produced.

The first theory is feasible in view of the proven chemical carcinogenic content in tobacco smoke. The second theory may gain support, for example, by the known effect of smoke on cilia; either interfering with their action or causing their diminution or disappearance. Carcinogenic substances may thus remain in contact with bronchial epithelium for periods long enough to induce neoplastic change. The second postulate, too, is compatible with the theory of cocarcinogenic activity. The third theory is suggested particularly by the progressive diminution of atypical cells after increasingly long periods of discontinuance of smoking. As the authors remark, the theories are not mutually exclusive, all three mechanisms may operate together or at different times and intensities in the one case.

Evaluation of Evidence.—Whilst it is correct that statistical association does not establish proof of causative relation, a very reasonable and highly probable interpretation of the considerable body of evidence leads to the conclusion that cigarette smoking is the

* Wynder, E. L. (1959), 'Laboratory Contributions to the Tobacco-cancer Problem', *Br. med. J.*, 1, 317.

† Roe, F. J. C., Salaman, M. H., and Cohen, J. (1959), 'Incomplete Carcinogen in Cigarette Smoke Condensate: Tumour Promotion a Phenolic Fraction', *Br. J. Cancer*, 13, 623.

Evaluation of Evidence, *continued.*

principal cause of lung cancer. The Medical Research Council* issued a statement in 1957, which, *inter alia*, included the following conclusion:—

> Evidence from many investigations in different countries indicates that a major part of the increase is associated with tobacco smoking, particularly in the form of cigarettes. In the opinion of the Council, the most reasonable interpretation of this evidence is that the relationship is one of direct cause and effect.

The Royal College of Physicians† studied the evidence of the harmful effects of cigarette smoking, and reported that it was not only a cause of lung cancer and bronchitis but probably contributed to the development of coronary heart disease and various other less common diseases. Having taken into account the possible importance of air-pollution, the Royal College of Physicians stated, 'it is clear that at all levels of air-pollution cigarette smokers suffer a risk of lung cancer which increases with the number of cigarettes smoked'.

The British Ministry of Health is quoted in a leading article in the *British Medical Journal* in 1962,‡ to the effect that smoking, especially cigarette smoking, has been demonstrated, 'crushingly and irrefutably' as the cause of the vast majority of cases of lung cancer.

The latest authoritative review and evaluation of evidence appears in the report of the U.S. Public Health Service.§ Its report is based upon investigations of a wide and extensive scale, the very scope of which adds to the weight and soundness of the opinions and conclusions expressed. Apart from the composition of the Advisory Committee to the Surgeon-General, nearly 200 other individuals, groups, and bodies are given acknowledgement for their considerable help. Almost 1000 published papers and reports form part of the study, and they include 29 retrospective and 7 prospective analyses of the causes of lung cancer and various other diseases.

The Advisory Committee bases its judgement of the statistical evidence upon an integration of a number of criteria, viz. (1) the consistency of results in numerous studies of different types and in widespread areas in the world, (2) the quantitative strength of the statistical association; in regard to cancer of the lung there is a ten-fold increase in incidence in smokers as compared to non-smokers, (3) specificity of association between the disease and the agent, e.g., cigarette smoking and lung cancer, (4) the latent period between exposure to the agent and the onset of the disease, and (5) the coherence of the association between agent and disease against the background of knowledge of all other definite, potential, and suggested factors. Clinical and histopathological features in man

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* Medical Research Council (1957), 'Tobacco Smoking and Cancer of the Lung', *Br. med. J.*, **1**, 1523.

† Royal College of Physicians of London (1962), *Smoking and Health.* London: Pitman.

‡ British Ministry of Health (1962), quoted in Leading Article, *Br. med. J.*, **2**, 975.

§ Public Health Service Publication, No. 1103 (1964), *Smoking and Health.* Washington: U.S. Govt Print. Office.

and experimental evidence from laboratories are included in the purview. The Committee concludes that cigarette smoking is causally related to lung cancer in man and it outweighs in importance all other factors. The risks are increased for pipe and cigar smokers as compared with non-smokers, but are very much less than for cigarette smokers.

AIR-POLLUTION

There is a significant difference in lung cancer mortality-rate in urban and rural areas. Stocks* reported male mortality in towns in England and Wales to be twice as high as that in rural areas. The same author, in 1952,† found that in urban areas, the frequency of lung cancer was higher in the more densely populated suburbs. A detailed investigation by Stocks‡ in the North Wales and Liverpool Hospital region, directed to eliciting comparative lung-cancer rates in relation to both smoking and residence, shows a rise in prevalence in all three classes of population, urban, mixed, and rural, in relationship to the amount of cigarettes smoked. The rise was greatest in urban, less so in mixed, and least in rural residential areas. Notwithstanding this rate, the contrast in lung-cancer rates between smokers and non-smokers in rural areas was as striking as in urban areas.

These statistical findings have been correlated with the greater degree of air-pollution, or the lesser amount of sunshine, or both, in urban as compared with rural areas.

Stocks and Campbell§ estimate the relative culpability of tobacco smoking and air-pollution as follows: the abandonment of smoking would have reduced the lung cancer death-rate in Liverpool by 50 per cent; the removal of urban pollution of the atmosphere would reduce it by 40 per cent.

Other authorities do not accord air-pollution so high a causative influence. Clemmesen,‖ from studies of incidence in Copenhagen and other areas of habitation in Denmark, considers that air-pollution is not a factor of importance in Copenhagen, but that cigarette smoking is decisive. Doll and Bradford Hill¶ estimate that in the absence of smoking the lung-cancer rate would be only 11 per cent of that which actually prevails. These authors direct special attention to the low mortality in both sexes among non-smokers in a period of 10 years' observation (only 3 males and 1 female) in a population, almost all of whom lived in urban areas. They claim that this does not point to air-pollution as a major factor.

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* Stocks, P. (1936), British Empire Cancer Campaign Annual Report.

† Stocks, P. (1952), 'Epidemiology of Cancer of the Lung in England and Wales', *Br. J. Cancer*, **6**, 99.

‡ Stocks, P. (1958), 'Statistical Investigations concerning the Causation of Various Forms of Human Cancer', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths.

§ Stocks, P., and Campbell, J. (1955), 'Lung Cancer Death Rates among Non-smokers and Pipe and Cigarette Smokers: Evaluation in Relation to Air Pollution by Benzpyrene and other Substances', *Br. med. J.*, **2**, 923.

‖ Clemmesen, J. (1954), 'Bronchial Carcinoma: A Pandemic', *Dan. med. Bull.*, **1**, 37.

¶ Doll, R., and Bradford Hill, A. (1964), 'Mortality in Relation to Smoking: Ten Years' Observation of British Doctors', *Br. med. J.*, **1**, 1399 and 1460.

Air-pollution, *continued.*

Another line of evidence derived from geographical distribution is striking and calls for explanation. Eastcott* records that immigrants, over the age of 30 years, from Britain to New Zealand, exhibited a lung-cancer death-rate 46 per cent in excess of that of locally-born New Zealanders. The smoking habits were essentially the same in both groups. Dean† reports that British male immigrants to South Africa who died between the ages of 45 and 64 had a mortality-rate of cancer of 44 per cent higher than South African-born white men of the same age-groups and of similar smoking habits.

These findings carry a strong indication of some causative agent, apart from, or additional to, smoking in the pathogenesis of lung cancer. British immigrants appear to have brought with them the effects of some cancer-causing factor which does not exist in New Zealand or South Africa. One such agent readily suggests itself as likely, viz. air-pollution; but there are other possibilities requiring investigation—previous bronchitis and other infections, methods of smoking including inhalation, frequency and depth of draw, and length of stub.

Berkson‡ suggests another factor: the constitution or the proneness of the individual to contract the disease. However, this suggestion is incompatible with the great increase in lung cancer among people with or without the postulated constitution; nor does it explain the direct linear increase in relation to the quantity of cigarettes smoked. A similar hypothesis has been advanced by Fisher.§ Here it is supposed that those who give up smoking are part of a selected group in which mortality from cancer of the lung is low. As has already been noted, the direct relationship between reduced mortality and the duration since cessation of smoking is strong evidence against such a constitutional or genetic factor.

OCCUPATIONAL CAUSES OF LUNG CANCER

1. **Radioactive Substances.**—The examples of lung cancer in miners following exposure to radioactive gases and dust in Saxony and Bohemia have been noted in Chapter XI.

2. **Chromate Industry.**—Skin and mucous-membrane lesions have been recognized since 1827, soon after the industrial manufacture of chemicals had begun. In 1890, a case of cancer of the nostril in a chromate worker was reported. In the early 1930's, lung cancer featured as an associated condition, and reports of its excess incidence, varying from 16 to 80 times that expected in control groups, have been published by Machle and Gregorius,‖ Bidstrup,¶

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* Eastcott, D. F. (1961), in *The Air We Breathe* (Ed. Farber, S. M., and Wilson, R. H. L.). Springfield, Ill.: Thomas.

† Dean, G. (1959), 'Lung Cancer among White South Africans', *Br. med. J.*, **2**, 852; and (1961), 'Lung Cancer among White South Africans', *Ibid.*, **2**, 1599.

‡ Berkson, J. (1964), 'Smoking and Lung Cancer', *Med. Proc.*, **10**, 327.

§ Fisher, R. A. (1959), *Smoking, the Cancer Controversy.* Edinburgh: Oliver & Boyd.

‖ Machle, W., and Gregorius, F. (1948), 'Cancer of Respiratory System in United States Chromate-producing Industry', *Publ. Hlth Rep. Wash.*, **63**, 1114.

¶ Bidstrup, P. L. (1951), 'Carcinoma of the Lung in Chromate Workers', *Br. J. ind. Med.*, **8**, 302.

and Gregorius.* The cases have been reported from plants manufacturing chromate chemicals and pigments; and since workers in them are exposed at a number of stages in the process, the precise chemical responsible has not yet been determined with certainty. Some degree of differentiation of cause may be deduced in that plants producing only bichromates and chromic acid are not implicated, but monochromate production is associated with excessive cancer prevalence. Further, Walters† reports that chrome miners do not suffer ill-effects, although workers handling the products do.

The average latent period after first exposure is 21 years; and when the cancer first presents, it often does so in an area adjacent to chromium deposits in the lungs.

3. **Nickel and Cobalt Ores.**—It is uncertain whether it is exposure to the ores or to the arsenical dust with them that explains a high incidence of carcinoma of the nose, nasopharynx, and lung. The refining and extraction processes leave an arsenical residue which is often released as dust. Doll‡ has reported the occurrence of 131 cases of lung cancer and 62 cases of nasal cancer over a period of 35 years among workers in a refinery in Wales. The risk of lung cancer has been estimated as 5 times that of the general population (nasal cancer figured at 150 times the 'normal').

4. **Arsenic in Sheep-dip.**—Merewether§ referred to the evidence that workers using arsenic-containing sheep-dip seemed to be predisposed to lung cancer. Hill|| reported that in a town where the manufacture of arsenical sheep-dip was the main industry, carcinomas of the skin and lung were common and accounted for a mortality-rate twice that in the general population.

5. **Asbestos.**—Inhalation of asbestos particles often causes a diffuse reaction associated with fibrosis, the condition being referred to as asbestosis. Asbestos particles tend to become encrusted in an iron-protein complex to form 'asbestos bodies'.

Since Lynch and Smith¶ reported a case of carcinoma of the lung in association with asbestosis, there have been a number of surveys indicating a causative association.

Wyers** recorded an association between this condition and an excess incidence of lung cancer. Others have also reported a markedly significant association. The average excess rate is about 10 times that in people unaffected by asbestosis. The condition does not occur with all types of asbestos, suggesting that only certain varieties are cancerigenic. Crocidolite asbestos mined in

* Gregorius, F. (1952), 'Lung Cancer in the Chromate Industry', *Archs ind. Hyg.*, **5**, 196.

† Walters, L. G. (1963), 'Industrial Cancer in South Africa', *Med. Proc.*, **9**, 24.

‡ Doll, R. (1958), 'Cancer of the Lung and Nose in Nickel Workers', *Br. J. ind. Med.*, **15**, 217.

§ Merewether, E. R. A. (1943), *Annual Report of Chief Inspector of Factories for* 1943.

|| Hill, A. B. (1948), 'Studies in Incidence of Cancer in Factory handling Inorganic Compounds of Arsenic: Mortality Experience in Factory; Clinical and Environmental Investigations', *Br. J. ind. Med.*, **5**, 1.

¶ Lynch, K. M., and Smith, W. A. (1935), 'Pulmonary Asbestosis: Carcinoma of Lung in Asbestos-silicosis', *Am. J. Cancer*, **24**, 56.

** Wyers, H. (1949), 'Asbestosis', *Post-grad. med. J.*, **25**, 631.

Asbestos, *continued.*

the Transvaal and processed in England is one type that carries an increased risk. Lung cancer, of multicentric origin, occurs usually after an average 20-year latent period following exposure enduring from 6 months to 42 years. Crocidolite of the so-called Cape Blue variety, mined in the North-western Cape Province, has been shown by Wagner and others* to be very strongly suspect as a cause of diffuse pleural mesothelioma.

Not only miners but many other workers are increasingly exposed to occupational risks of inhaling asbestos. Asbestos is being used in an ever-widening field of industry: automobile spraying, insulating of electrical and piping equipment, tiling and roofing are examples of exposure. The employees in these kinds of work, not the users of the finished articles, are exposed to the risks. Thomson† suggests that in many industries, workers are inhaling more limited quantities of asbestos dust than is the case in those concerned in mining, packaging, and transport. The effects are not the obvious diffuse asbestosis with its characteristic radiological appearance, nor the high rate of bronchogenic carcinoma, but a basal, peripheral, inconspicuous, and readily overlooked focal fibrosis. This is the underlying lesion which is postulated as the precursor to mesothelioma of the pleura, which is so often adjacent to the lesions, and the sub-diaphragmatic peritoneum which is not much further removed. This rare tumour is being discovered more often now that its association with asbestos is under purposive investigation. Fowler and others‡ report 2 cases of mesothelioma of the pleura in association with occupational exposure to asbestos and in which focal 'asbestos bodies' were found in the lungs. Hourihane§ and Owen‖ both report reviews of material accumulated over a number of years. In both papers it is clear that the association of mesothelioma with asbestos fibres in the lungs has a frequency much too marked for it to have been fortuitous.

* Wagner, J. C., Sleggs, C. A., and Marchand, P. (1960), 'Diffuse Pleural Mesothelioma and Asbestos Exposure in the North Western Cape Province', *Br. J. ind. Med.*, **17**, 260.

† Thomson, J. G. (1962), 'Mesothelioma of Pleura or Peritoneum and Limited Basal Asbestosis', *S. Afr. med. J.*, **36**, 759.

‡ Fowler, P. B. S., Sloper, J. C., and Warner, E. C. (1964), 'Exposure to Asbestos and Mesothelioma of the Pleura', *Br. med. J.*, **2**, 211.

§ Hourihane, D. O. B. (1964), 'The Pathology of Mesotheliomata and an Analysis of their Association with Asbestos Exposure', *Thorax*, **19**, 268.

‖ Owen, W. G. (1964), 'Diffuse Mesothelioma and Exposure to Asbestos Dust in Merseyside Area', *Br. med. J.*, **2**, 214.

CHAPTER X

LATENCY AND COCARCINOGENESIS

A remarkable feature in the development of cancer is the existence of a latent interval, also called 'incubation period', between exposure to a carcinogenic agent, sometimes known but perhaps more often presumed, and the appearance of a cytologically and clinically recognizable tumour. Many provocative biological problems spring from this fact: Can it provide a lead to a knowledge of the mechanism of carcinogenesis? Is latency due to changed cells suspended in a state of dormancy until further stimuli evoke frank malignant properties? Is the process one of gradual progress over a number of years to a final, morphologically evident cellular change? Does latency represent the time taken by malignant cells to overcome host resistance? Advancing age is accompanied by an increasing incidence of neoplasms. Is this an expression of the culmination of malignant transformation after latent intervals following earlier tumorigenic influences? These are among the questions of prime importance in the understanding of the biology of cancer; they have stimulated close study in clinical practice and in the laboratory.

INACCURACIES IN ESTIMATING HUMAN LATENCY

Certain inaccuracies of timing are inherent in the circumstances of cancer in man. It is of some moment to state these qualifications so as to maintain a proper perspective.

In the earliest stages, it is not possible precisely to fix the time of initiation of cellular change. Carcinogenic action is seldom a single transitory event; it is almost invariably applied over a period of time, usually months, and often years. Even when the carcinogenic agent is known definitely, its exact or approximate date of effective influence is usually doubtful. The scar of an injury may be the seat of a carcinoma in later years; but, although the date of the traumatic episode may be established with certainty, it does not follow that the cancerigenic property began at the time of the injury; it may, in fact, have begun at some time during the course of healing, or disturbed healing, after an undeterminable interval following the trauma. This consideration encroaches on the next in the series of qualifications of timing and latency.

During intermediate stages of evolution of malignant growths, a variety of modifying influences may play a part in speeding or delaying the advent of cancer, thus vitiating calculations of the period of incubation referable to particular agents and the cancer they induce. The modifying factors are discussed later in this chapter.

At the definite cancerous stage, a third set of reservations is applicable to assessment of latent periods. The actual time of appearance of the cancer is uncertain; not only in terms of days or weeks, but quite often in

Inaccuracies in Estimating Human Latency, *continued.*

terms of months or years. This may happen even under conditions of routine examinations: a small cancer of the breast may take a long time to become clinically appreciable; carcinoma-in-situ of the cervix, or of the prostate, may elude diagnosis for a considerable interval.

Determination of duration of latency therefore must be acknowledged as necessarily inexact; but this does not completely nullify its value. Many informative lessons, important clues to biological mechanisms, and practical uses may be derived from studies of latency.

LATENCY IN HUMAN CANCER

Ionizing Radiations.—The latent period between exposure and cancer varies with a number of factors (*see* Chapter XI), of which dose, site, mode of application, and age are among the more important. Variation of this character gives rise to a wide range of intervals before the advent of cancer.

In regard to skin cancer following irradiation, a Medical Research Council Report* gives the range as from 12 to 56 years, with an average of 33 years. In fact the span of years is even greater than this: the very first case of radiation skin cancer was reported by Freiben in 1902.† It affected a man employed in the manufacture of X-ray tubes 6 years after Röntgen's discovery of X-rays. This fixes with a reasonable degree of accuracy a latent interval of at most 6 years. In this historical case, the high and intense dose was probably the reason for the relatively rapid onset of carcinoma. The knowledge that the higher the dosage, the greater the risk and the shorter the incubation period, has led to intensive and detailed prophylactic precautions, which are imposed by law in many countries of the world, to protect personnel concerned with ionizing radiations.

The age of the individual at the time of exposure has a marked influence on latency. The close association between risk and latency may be correlated with age, to the effect that the younger the age of exposure, the more sensitive are the tissues to irradiation. Consequently, doses that are small at later years represent large doses at earlier ages, and latent intervals are reduced accordingly. X-ray therapy administered to children for different skin conditions, e.g., haemangiomas, excess hair, rashes, etc., has been reported by Glucksmann and others‡ as having led to skin cancers after 12 years or so.

The marked vulnerability of young tissues is emphatically illustrated by short latent intervals in the onset of malignant lesions of organs other than skin. An example features in a series of reports on thyroid cancer following irradiation treatment of infants for 'enlarged thymus' and infected tonsils; the latent period was 7 years (*see* Chapter XI).

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* Medical Research Council (1956), *The Hazards to Man of Nuclear and Allied Radiations.* London: H.M.S.O.

† Freiben, K. (1902), 'Cancroid des Rechten Handrükens', *Dt. med. Wschr.*, 28. Verens-Beilage.

‡ Glucksmann, A., Lamerton, L. F., and Mayneord, W. V. (1952), 'Carcinogenic Effects of Radiation', in *Cancer*, Vol. 1 (Ed. Raven, R. W.). London: Butterworths.

Following exposure to a carcinogen, there appears to be a limit to the phase during which cancers develop. The limitation is not finite, as malignant lesions in reduced numbers may continue to arise at later stages, but there is a definite peak period when most tumours arise. An outstanding case in point is to be found in leukaemias consequent upon the atomic explosions in Hiroshima and Nagasaki. The date of exposure to ionizing radiation is clearly known; almost as accurate is the determination of the time of onset of leukaemia, as routine studies of exposed populations are being carefully conducted. The incidence of leukaemia has been discovered (*see* Chapter XI) to be maximal during a period of 6–8 years after the explosions. After this peak period, the numbers of cases have been diminishing. A number of other examples of peak cancer rates are quoted in a later section on latency in occupational cancer.

These observations may have a bearing on the question of the increased prevalence of cancer among aged people. The question arises whether the greater prevalence in older ages is the result of the evolution of malignancy after the termination of latent intervals following carcinogenic stimuli sustained during earlier years of life. The fact that there is not simply an increasing incidence of cancer after the latent interval, but different peak periods for different tumours, tends to run counter to the proposition posed in the question. However, since the majority of agents causing cancer in humans is not definitely known, it must be conceded that a whole series of incubation periods may culminate in the high incidence in old age.

Another instance of radiation-induced malignancy with a fairly well determined latent period is that of bone sarcoma in dial painters. As a result of ingestion of radioactive luminous paint, usually for a period of at least 6 months, and after an incubation period enduring for an average of 17 years, osteogenic sarcoma appears in a high proportion of cases. An important aspect of this example of latency is the suggestion by Aub and others* that trauma seems to play a part in bringing frank neoplasm to light. This raises issues that have been contended in law for many years: has trauma a causative role in tumours? or does injury simply draw attention to a neoplasm already in existence? That trauma may act as a precipitating agent, bringing a phase of latency to an end and provoking the final change to overt, manifest cancer, appears possible by analogy with promoting agents and cocarcinogenesis in animal experiments. The theoretical supposition is that cancer would remain latent and clinically harmless if it were not for the action of a promoting factor, in this case, trauma, in inducing progressive neoplastic behaviour. The concept of cocarcinogenesis, elaborated from observations of biological responses in laboratory animals, is discussed in a later section of this chapter.

Penile Cancer and Circumcision.—Cancer of the penis is exceedingly rare among Jews, who are circumcised in infancy; among Moslems,

* Aub, J. C., Evans, R. D., Hempelmann, L. H., and Martland, H. S. (1952), 'The Late Effects of Internally-deposited Radioactive Materials in Man', *Medicine*, **31**, 221.

Penile Cancer and Circumcision, *continued.*

who are circumcised in childhood, it is uncommon but the incidence rises with the increase in years of age at which circumcision is performed. Among uncircumcised Hindus, Chinese, and Latin Americans, penile cancer has a high prevalence rate. (*See* Chapter VII.)

As has been shown in Chapter VII, the marked difference in penile cancer rate between Western and Eastern uncircumcised men signifies that it is not the mere presence of the prepuce which is cancerigenic. There must be some additional factor. This is probably balanitis, and/or persistence of smegma; both conditions arising as a result of inadequate hygienic care. Venereal diseases, syphilis and gonorrhoea, have been blamed as pathogenic agents in cancer of the penis, but proof is lacking.

Kennaway* pointed out that, if circumcision is done after the age of 14 years, the incidence of cancer does not differ significantly between uncircumcised and circumcised men of the same racial group and social class. When carcinoma of the penis does occur after circumcision performed in the age-group 14–30 years, Lenowitz and Graham† report an average period of 22 years before the appearance of cancer. The assessment of latency is impractical when circumcision is done at ages above 30 years and cancer follows subsequently. It is clear from the records reported by Dean‡ that the calculation of averages is out of place. First, the number of such cases is small (only 7 of Dean's total of 120 cases), and secondly, because the range of time of appearance of cancer following circumcision is so great, viz., 2–24 years. It is also reasonably certain that, at the older ages, some circumcisions are done when the pre-malignant condition has become very advanced or has, in fact, evolved into frank cancer. A point of interest in Dean's series is the report that in 3 cases at least, and possibly 5 (the text and table are not clear on this), the patients stated that the tumour had originated in the circumcision scar.

It is justifiable to deduce from the evidence of the incidence and incubation period of cancer of the penis that the carcinogenic agent is removed or prevented from developing by early circumcision. When this is done in infancy, the elimination of the carcinogen is complete; when done later, up to the age of 14 years, the carcinogenic influence has already begun and its effective operation varies directly with the duration of the presence of the prepuce; after the age of 14 years, the initial cellular alteration has been established, and circumcision does not materially affect the incidence of cancer.

Carcinoma-in-situ.—Carcinoma-in-situ implies that cancer cells are already present; therefore, any latent interval which may have existed has actually terminated. However, the fact that the cancer is in a pre-invasive stage, i.e., the essential definition of carcinoma-in-situ, has relevance in the study of latency. Knowledge of the

* Kennaway, E. L. (1952), 'The Incubation Period of Cancer in Man', in *Cancer*, Vol. 1 (Ed. Raven, R. W.). London: Butterworths.
† Lenowitz, H., and Graham, A. P. (1946), 'Carcinoma of the Penis', *J. Urol.*, **56**, 458.
‡ Dean, A. L. (1935), 'Epithelioma of the Penis', *Ibid.*, **33**, 252.

time taken for the progress from an in-situ stage to an invasive one is a necessary corrective to inaccuracy in determining latency judged by the appearance of invading and symptom-producing lesions.

Carcinoma-in-situ of the cervix exemplifies the point. It is found in an age-group which is, on an average, 10 years younger than that for invasive carcinoma. While this average estimate must be treated with some reserve, because of the margin of error in diagnosis and variations from case to case, the figure may well represent a necessary addition in the calculation of the latent period following exposure to a carcinogenic influence.

Table X.—LATENCY IN INDUSTRIAL CANCER

CARCINOGEN	ORGAN AFFECTED	EXPOSURE	LATENT PERIOD	AUTHOR
Mule-spinner lubricating oil	Scrotum	Min. 16 yr.	50 yr.	Henry*
Pitch and tar	Skin	Min. 10 mth.	20–24 yr. (peak incidence)	Henry†
Shale oil	Skin	Min. 4 yr.	50–54 yr. (peak incidence)	Henry†
Benzidine	Bladder	Av. 8 yr.	16 yr.	Case and others‡
Beta-naphthylamine	Bladder	Av. 5 yr.	16 yr.	Case and others‡
Xenylamine	Bladder	Min. 131 dy.	15–20 yr. (peak incidence)	Melick and Naryka§
Arsenic	Skin	Many years	2–60 yr.	Hueper‖
Asbestos	Lung	7–21 yr.	10–12 yr.	Hueper‖
Tar	Lung	Av. 16 yr.	10 yr.	Hueper‖
Luminous dial paint	Bone	From 6 mth.	17 yr.	Martland¶

Latency in Industrial Cancer.—Many industrial tumours now regarded as attributable to work are included in tables of compensatable conditions. Latent periods, in terms of the range from the shortest to the longest intervals as well as average and peak years, are of obvious importance to industrial legislation, e.g., cancers appearing outside of the range of latency may not be accepted as

* Henry, S. A. (1946), *Cancer of the Scrotum in Relation to Occupation.* London: Oxford Univ. Press.

† Henry, S. A. (1947), 'Occupational Cutaneous Cancer Attributable to Certain Chemicals in Industry', *Br. med. Bull.*, **4**, 389.

‡ Case, R. A. M., Hosker, M. E., McDonald, D. B., and Pearson, J. T. (1954), 'Tumours in the Urinary Bladder in Workmen engaged in the Manufacture and Use of Certain Dyestuff Intermediates in the British Chemical Industry. Parts I and II', *Br. J. ind. Med.*, **11**, 75 and 213.

§ Melick, W. F., and Naryka, J. J. (1960), 'Xenylamine (para-aminobiphenyl) Bladder Tumours in Man', *Acta Un. int. Cancr.*, **16**, 277.

‖ Hueper, W. C. (1942), *Occupational Tumours and Allied Diseases.* Springfield, Ill.: Thomas.

¶ Martland, H. S. (1931), 'The Occurrence of Malignancy in Radioactive Persons', *Am. J. Cancer*, **15**, 2435.

Latency in Industrial Cancer, *continued.*

attributable to occupational causes. A knowledge of incubation periods is also of prime importance in planning régimes for routine examination and in devising methods of prevention and treatment. Another practical application of awareness of latent intervals relates to general policy of employment in certain trades. Hueper* records the recommendation of a Cancer Prevention Committee to the effect that, in regard to uncontrolled carcinogens associated with a long latent period, elderly rather than younger persons should be employed, because elderly workers would live out their lives before the advent of cancer induced by their occupational exposure.

Examples of latent intervals listed in *Table X* indicate the extent of variation with different carcinogens. It is also clear that variation in exposure time and latency results from different doses and intensities of a particular carcinogenic agent. In this regard, it is of interest to refer to the opinion of Kennaway and Kennaway† that a much more intense carcinogenic stimulus is needed to reduce the latent period than to increase the incidence.

THE CAUSES OF LATENCY

Causation and mechanism are the crucial problems of latency. Knowledge of the course of events and their origin during incubation periods could lead to a deeper understanding of cancer biology and possibly to measures to inhibit or delay the advent of overt cancer. Two lines of investigation throw some light on the problem: in man, certain modifying influences have been suggested by clinical investigations; and in animals, experiments point to mechanisms of stage carcinogenesis which may be applicable to man.

Modifying Factors

These factors are either not in themselves carcinogenic but exert an influence upon other presumed or known carcinogenic agents so as to affect the progress and development of cancer; or they are carcinogenic and modify the rate of evolution of cancer. Both types of factor may modify cancer behaviour by shortening or prolonging incubation periods.

Cellular susceptibility to carcinogenic influences is the probable major basis for determining and modifying the course of cancer. Susceptibility varies markedly with cell maturity; it is conditioned by heredity; influenced by age and sex, and the milieu provided by endogenous and exogenous biochemical and biophysical activities. When cells are exposed to carcinogenic influences, the reactions may be modified in the direction of submission or resistance, promotion or inhibition, acceleration or deceleration.

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* Hueper, W. C. (1952), 'Age Aspects of Environmental and Occupational Cancers', *Publ. Hlth Rep. Wash.*, **67**, 773.

† Kennaway, E. L., and Kennaway, N. M. (1944), 'Relation between Incidence and Incubation Period of Cancer in Man', *Yale J. Biol. Med.*, **17**, 139.

Diet.—The facts that food and drink are applied to considerable surface areas of the gastro-intestinal tract, and their metabolites are brought into contact with tissues of many kinds, lend themselves to the assumption that they provide modifying influences in the unfolding of cancerous processes. There is some suggestive evidence that this may be the case.

Rationing restrictions of food intake during World War I was accompanied by a reduction of the incidence of gastric cancer. Hindhede* and Stout† have reported and commented on the significance of these findings, which are given greater force by the advent of a rise in gastric cancer rate after 'normal' diets were restored following the war. Tannenbaum and Silverstone‡ report that insurance figures show that overeaters and the overweight are more likely to die of cancer. On the other hand, dietary deficiencies of a severity amounting to malnutrition are considered by Gillman and others§ to be causally implicated in cancer of the liver. A number of other possible dietetic factors have been discussed in Chapter IV, where the lines of investigation are indicated and the many gaps in current knowledge are emphasized.

There is some evidence that vitamin lack and nutritional anaemia are causatively associated with postcricoid carcinoma. Cancer of the thyroid is more common in areas in which goitre is endemic; as this is traceable to lack of iodine in the drinking water, the excess cancer rate may be ascribed to a dietary factor.

Apart from the last two cancers, postcricoid and thyroid, for which it is reasonable to inculpate a modifying influence in diet, dietary modifications of the development of human cancer are at present but plausible assumptions; much more study and controlled investigation will be necessary for proof.

In experimental animals, information on dietary factors is much more positive. Cowdry's‖ review of the subject indicates that dietary restriction decreased the incidence of some tumours, of spontaneous, induced, and transplanted types; in others, tumour growth was retarded. High fat diets seemed to increase the tumour rate. Both over- and under-feeding of amino-acids and vitamin B elements brought about marked changes in cancer progress.

Environmental Temperature and Cryptorchidism.—A review of the literature (Kark¶) on maldescent of the testis confirmed that the incidence of tumours in cryptorchids of all types is 35–50 times greater than in normally placed testes; and the intra-abdominally retained testis is 4 times more susceptible to malignant change than the inguinal testis, i.e., the comparative rates of occurrence of

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* Hindhede, M. (1925), 'Cancer Statistics. Cancer and Diet', *Acta med. scand.*, **62**, 379.

† Stout, A. P. (1932), *Human Cancer*. Philadelphia: Lea & Febiger.

‡ Tannenbaum, A., and Silverstone, H. (1952), 'Nutrition and the Genesis of Tumours', in *Cancer*, Vol. 1 (Ed. Raven, R. W.). London: Butterworths.

§ Gillman, J., Gillman, T., and Gilbert, C. (1950), 'Observations on the Aetiology of Cancer of the Liver', *J. natn. Cancer Inst.*, **11**, 653.

‖ Cowdry, E. V. (1955), *Cancer Cells*. Philadelphia: Saunders.

¶ Kark, W. (1962), 'The Problem of Cryptorchidism and Malignancy', *Med. Proc.*, **8**, 83.

Environmental Temperature and Cryptorchidism, *continued.*

tumours in abdominal and scrotal testes is about 170 : 1. This excess incidence has been correlated with the environmental temperature of the testis: in the scrotum, environmental temperature is lower than in the groin, which in turn is lower than that within the abdomen. Harrison and Weiner* record that in man, the abdominal temperature is 2·2° C. higher than that in the scrotum. The differences lead to the inference that the higher the environmental temperature, the greater the risks of malignancy.

Experimental observations on animals by Moore and colleagues†‡ disclosed that higher surrounding temperatures caused testicular tubular epithelial degeneration. In man, there are a number of observations showing, morphologically and functionally, similar disturbances of the testis. Wangensteen§ reported mild degenerative changes in the testes of men dying of febrile diseases. Rea‖ records the reduced functional capacity of undescended testes. MacLeod and Hotchkiss¶ reported reduced sperm counts following high febrile illness and also following purposive insulation and warming of the scrotum.

A theory to explain the influence of raised environmental temperature upon cancer incidence is linked to the functional disturbances of the testis. Burrows and Horning** postulate that reduction in testicular androgen production stimulates persistent oversecretion of pituitary gonadotrophin; the effect of this is sustained hyperplasia of testicular cells and ultimate malignant change.

An intriguing facet of the biology of testicular tumours derives from the possibility of discovering an association between their latent periods and causative mechanisms. The peak age of onset of seminomas in normally situated testes is from 40 to 45 years; in cryptorchids, it is a decade less. This suggests that in cryptorchids the carcinogenic agencies are either more powerful or operate from an earlier age. The first alternative is possible but it should be recalled that Kennaway and Kennaway†† consider that a much more intense stimulus is needed to reduce incubation periods than to increase incidence; the second seems a more acceptable choice, as cryptorchidism affects the testis from about the age of 5 years. Normally at this age, tubules increase in size and become tortuous, and cells assume an orderly arrangement, becoming identifiable as

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* Harrison, R. G., and Weiner, J. S. (1948), 'Abdomino-testicular Temperature Gradients', *J. Physiol., Lond.*, **107**, 48.

† Moore, C. R., and Oslund, R. (1924a), 'Experiments on the Sheep Testis; Cryptorchidism, Vasectomy and Scrotal Insulation', *Am. J. Physiol.*, **67**, 595.

‡ Moore, C. R., and Quick, W. J. (1924b), 'The Scrotum as a Temperature Regulator for the Testis', *Ibid.*, **68**, 70.

§ Wangensteen, O. H. (1927), 'The Undescended Testis; an Experimental and Clinical Study', *Archs Surg., Chicago*, **14**, 663.

‖ Rea, C. E. (1939), 'Functional Capacity of the Undescended Testis', *Ibid.*, **38**, 1054.

¶ Macleod, J., and Hotchkiss, R. S. (1941), 'The Effect of Hyperpyrexia upon Spermatozoa Counts in Men', *Endocrinology*, **28**, 780.

** Burrows, H., and Horning, E. S. (1952), *Oestrogens and Neoplasia.* Springfield, Ill.: Thomas.

†† Kennaway, E. L., and Kennaway, N. M. (1944), 'Relation between Incidence and Incubation Period of Cancer in Man', *Yale J. Biol. Med.*, **17**, 139.

spermatogenic and Sertoli entities. Cooper,* Pace,† Nelson,‡ and Robinson and Engle§ have contributed to knowledge of the histological deviations of undescended testes from the normal pattern. From the age of 5 or 6 years to 9 or 10, there is a lag in normal developmental differentiation; and from about 10 years to puberty, maturation is defective.

The theory invoking a feed-back mechanism between androgen and gonadotrophin to account for the relative frequency of cancer in undescended testes has a difficult hurdle to overcome. This arises from reports that orchidopexy does not reduce the risks of malignancy, and the latent period is, if anything, reduced. Gilbert's‖ review refers to 65 cases, in which an average period of 12 years elapsed between orchidopexy and the diagnosis of seminoma. The theory is therefore acceptable only on the basis of one or more of several assumptions on the following lines: by the time orchidopexy is performed, the hormonal reciprocal mechanism has already been established, viz., deficient androgen and consequent excess gonadotrophin has already initiated cellular changes which will culminate in cancer. Or that potential malignancy is initiated by some other biological mechanism, and hormonal imbalance acts as a later trigger.

It is also interesting to speculate on similarities between circumcision *vis-à-vis* penile cancer and orchidopexy *vis-à-vis* testicular cancer. The later the circumcision, the higher the incidence of penile cancer, until after the age of 14 years when the incidence is equal to that in the uncircumcised; perhaps by the time orchidopexy is done, it is already too late to prevent the effects of cancerigenic stimuli, and that earlier operative replacement of an undescended testis is needed to eliminate cancer inducing agents. More extensive and more detailed clinical records may convert speculative theorizing into something more substantial. Recorded cases are, as yet, few in number, and in many, the ages at which orchidopexy was performed is not given.

Multiple Cancers.—The incidence of a second cancer (exclusive of multifocal lesions of one cancer) is greater than the incidence of a first cancer. On the basis of statistical studies of autopsy material, Warren and Ehrenreich¶ deduced that multiple cancers occurred 11 times as often as might be expected by chance alone. Other investigators, too, have reported that the person with one cancer has, by comparison with an unaffected person, a greater proclivity

* Cooper, E. R. A. (1929), 'Histology of Retained Testis in Human Subjects at Different Ages: Comparison with Scrotal Testis', *J. Anat.*, **64**, 5.

† Pace, J. M. (1935), 'Histologic and Pathologic Anatomy of the Retained Testis' *Proc. Staff Meet. Mayo Clin.*, **10**, 726.

‡ Nelson, W. O. (1951), 'Mammalian Spermatogenesis. Effect of Experimental Cryptorchidism in the Rat and Non-descent in the Testis in Man', *Rec. Proc. Hormone Res.*, **6**, 29.

§ Robinson, J. N., and Engle, E. T. (1954), 'Some Observations on the Cryptorchid Testis', *J. Urol.*, **71**, 726.

‖ Gilbert, J. B. (1941), 'Studies in Malignant Testis Tumors: V. Tumors developing after Orchidopexy. Report of 2 Cases and Reviews of 63', *Ibid.*, **46**, 740.

¶ Warren, S., and Ehrenreich, T. (1944), 'Multiple Primary Malignant Tumours and Susceptibility to Cancer', *Cancer Res.*, **4**, 554.

Multiple Cancers, *continued.*

to the development of another cancer; but most reports give an excessive rate of less than 11; 6 seems a more general estimate.

The deduction that this comparative incidence betokens a greater susceptibility by the different cells of one individual has been challenged by some authorities. They point to possible fallacies in the collection and classification of data, and particularly to the following order of frequency in the occurrence of a second cancer. It occurs most frequently in the same organ as the first cancer; next, in order of frequency, a second cancer arises in a paired member of the first organ affected, e.g., a cancer of a kidney following upon an earlier neoplasm of the opposite kidney; lower still in the scale of occurrence of second cancers are those arising in the same organ system, e.g., a cancer of part of the colon arising as a separate entity after a first cancer in a different portion of the large bowel. Lastly, in the order, comes second cancers affecting unrelated tissues. This consecutive distribution of frequency of second cancers, it is claimed, signifies the evolution of a series of changes with different latent periods in groups of cells which have been exposed to a carcinogen at an earlier stage.

Carcinogenesis and Two-stage Mechanism

The concept of a two-stage mechanism of carcinogenesis, arising from complementary actions of two agents, derives mainly from experimental work by Berenblum,* using croton resin and oil on pre-treated mouse skin, and Rous and Kidd,† studying the association of trauma and tar tumours of rabbit skin.

The term 'cocarcinogen' is used in a restricted sense. Although the various definitions applied to it are not altogether finite, and certain weaknesses of clarity will probably require modification and correction, it is necessary to appreciate the limitations of the term. A cocarcinogen is not a 'modifying influence' as used in the immediately preceding section of this chapter; remote factors like diet and temperature are not included in the definition; nor are the different effects of solvents, which carry carcinogens, part of the mechanism. When a carcinogen is applied in a form or dosage that does not itself produce tumours, or the latent period is prolonged, a cocarcinogen is a second agent which potentiates the appearance of tumours, or augments their numbers, or shortens the latent interval. If the second agent inhibits tumorigenesis of the carcinogen, the action is one of anticarcinogenesis.

Berenblum's experiments revealed that initial application of a known carcinogenic tar to mouse skin in doses short of tumour production, followed by subsequent local application of croton oil or resin, produced a marked growth of tumours. A remarkable feature was that croton oil was not itself a carcinogen (later work showed that it was in fact weakly carcinogenic, but not in the dose and application period used in the early experiments). The evidence led to the inference that carcinogenesis

* Berenblum, I. (1941), 'The Cocarcinogenic Action of Croton Resin', *Cancer Res.*, **1**, 44.
† Rous, P., and Kidd, J. G. (1941), 'Conditional Neoplasms and Subthreshold Neoplastic States: Study of Tar Tumors of Rabbits', *J. exp. Med.*, **73**, 365.

was a two-stage process: the first stage of *initiation*, producing non-apparent or 'latent' tumour cells, and a second stage of *promotion*, potentiating tumour development. The use of the words 'initiation' and 'promotion' was introduced by Rous and his associates.

Rous and Kidd also reported their experiments in 1941. Tar application to rabbit ears produces tumours after a certain dosage. Under circumstances of tar initiation, prior to the advent of tumours, small disks were punched out of the ears for histological examination; at an early stage, there was no sign of tumour formation; later, especially at and near the healing edge of a disk, tumours appeared in increasing numbers. Here again the evidence pointed to an initial stage of latent neoplastic potentiality, which another agent at a later stage, viz., trauma or the healing of a wound, brought to frank tumour formation.

The work of Deelman in 1923* is regarded as being in line with cocarcinogenesis, and as its first experimental demonstration: deep scarification of skin augments tar carcinogenesis. These experiments disclosing trauma as a cocarcinogen are similar to those employing 'disking' of rabbit ears. Many other combinations of agents and dosage variations have been tested, and the volume of information is reaching large dimensions. A recent review by Salaman and Roe† indicates the growing field of discovery in cocarcinogenesis. It applies not only to skin, where a number of new agents of initiation and promotion are reported, but also to subcutaneous tissues and skeletal muscle, liver, lung, urinary bladder, gastro-intestinal tract, and the reticulo-endothelial system. Some substances, e.g., urethane, may act as initiators in some organs and as promoters in others. In addition to chemical substances and trauma, teflon sheet implants, X-rays, and viruses are listed as agents: urethane and X-radiation, virus plus chemical agent, virus plus hormone, virus plus virus, and virus plus X-rays, all act as cocarcinogenic 'pairs' and fit into the concept of a multistage mechanism.

Anticarcinogenesis is an important corollary of this concept. It may prove to be more important from a practical point of view in the control and management of human cancer, as preventive and curative therapy come within its ambit. Berenblum‡ detailed a number of irritants which did not have cocarcinogenic effects, but exercised a contrary influence; some factors promoted tumours at times (e.g., X-ray in small doses) and were inhibitory at others (e.g., X-rays in large doses). Crabtree's review of the subject§ provides pointers to the lines of research undertaken in more recent years. Mustard gas as an inhibitor is included in Crabtree's review; certain organic peroxides checked the carcinogenic action of 3 : 4-benzpyrene on mouse skin; and many other agents and combinations of carcinogens are also listed as affecting the evolution of tumours of the skin. Experimental liver cancer by azo-compounds comes under the influence of diet: a normal diet with adequate vitamin and protein content has an anticarcinogenic effect.

The possible applications of cocarcinogenesis and the two-stage (or multistage) mechanism to the development of human cancer are obvious,

* Deelman, H. T. (1923), 'Quelques Remarques sur le Cancer Experimental du Goudron', *Bull. Ass. fr. Étude Cancer*, **12**, 24.
† Salaman, M. H., and Roe, F. J. C. (1964), 'Cocarcinogenesis', *Br. med. Bull.*, **20**, 139.
‡ Berenblum, I. (1944), 'Irritation and Carcinogenesis', *Archs Path.*, **38**, 233.
§ Crabtree, H. G. (1947), 'Anti-carcinogenesis', *Br. med. Bull.*, **4**, 345.

Two-stage Mechanism, *continued.*

but they are as yet largely hypothetical. Friedewald and Rous* reported on extensions of earlier work on tar tumours and trauma. Methylcholanthrene (MC) paint on rabbit ears produces tumours after a very much longer latent interval than does tar, and the tumours are much more often benign. On cessation of exhibition of MC, the tumours often regress, and then reappear with resumption of the tumorigen. Tumours are readily promoted by healing 'disk' lesions. The authors analyse the differences in tar and MC tumours: tar both initiates and promotes neoplastic change, whereas MC has much weaker promotive activity and requires augmentation by trauma, which may be applied a considerable time after the last exposure to MC. This initiation by MC of a subthreshold cytologically unrecognizable tumour is compared to human tumours with a long incubation period, during which the tumour is latent but potential, and is triggered by a second-stage promoter.

It is by analogy with trauma as a promoting agent in experimental tumours, that the possibility arises of human bone sarcomas, and perhaps other tumours too, being influenced to overt, spreading lesions by a traumatic event. If such a possibility is accepted, trauma becomes a contributory cause of cancer; a matter involving weighty legal implications. It is necessary, however, to emphasize that up to the present, no positive or acceptable proof of trauma as a causative agent has been demonstrated in human cancer.

Berenblum and Shubik† report on certain of the properties of initiators and promoters. Initiation can be induced by a very small dose, even a single application, of carcinogen; it occurs fairly rapidly and is lasting and irreversible; and it determines the number of tumours that ultimately appear. The latent period between initiation and overt tumour is determined by the strength of the promoter, the action of which is gradual and may take a long time. Friedewald and Rous have shown that tumour promotion, in some cases, is reversible; and Tannenbaum‡ demonstrated that a low-calorie diet affected the promoting, but not the initiating, stage. The two stages are distinct and specific, as shown by failure to produce tumours when the sequence of initiation and promotion are reversed; application of a promoter prior to an initiator does not give rise to tumours.

The effects of the initiating stimulus, especially the rapid and irreversible changes in cells which are not apparent morphologically, are suggestive of a mutagenic mechanism, whereby heritable changes are wrought in the genes of a group of cells. This possibility leads Walpole§

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* Friedewald, W. F., and Rous, P. (1950), 'The Pathogenesis of Deferred Cancer', *J. exp. Med.*, **91**, 459.

† Berenblum, I., and Shubik, P. (1947), 'Role of Croton Oil Applications, associated with Single Painting of Carcinogen, in Tumour Induction of Mouse's Skin', *Br. J. Cancer*, **1**, 379; (1947), 'New, Quantitative Approach to Study of Stages of Chemical Carcinogenesis in Mouse's Skin', *Ibid.*, **1**, 383; and (1949), 'Experimental Study of Initiating Stage of Carcinogenesis and Re-examination of Somatic Cell Mutation Theory of Cancer', *Ibid.*, **3**, 109.

‡ Tannenbaum, A. (1944), 'Dependence of Genesis of Induced Skin Tumors on Caloric Intake during Different Stages of Carcinogenesis', *Cancer Res.*, **4**, 673; and (1944), 'Importance of Differential Consideration of Stages of Carcinogenesis in Evaluation of Cocarcinogenic and Anticarcinogenic Effects', *Ibid.*, **4**, 678.

§ Walpole, A. L. (1959), 'Initiation and Promotion in Carcinogenesis', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

to speculate that just as complete carcinogenesis may be 'spontaneous', so, too, may initiation be 'spontaneous'; so that mutation occurring by pure chance, or by stimuli, unrecognized at present, of endogenous or exogenous origin, gives rise to a clone of cells susceptible to the action of a promoter.

Berenblum*† thinks that although initiation may represent mutagenesis, there is no evidence to support the thesis. As for promotion, this does not appear to act by stimulating 'dormant' cells to undergo division, but by causing delay in their maturation from the 'stem' cell phase, leading to imbalance between rates of division and death, and so to progressive growth which eventually becomes self-perpetuating.

The hypothesis that promotion is essentially a stage of hyperplasia has excited many theories relating neoplasia to a form of decontrolled, ungoverned, or unrestricted hyperplasia. Studies of cocarcinogenic effects tend to negate these theories. While hydrocarbon initiators may give rise to hyperplasia of skin epithelium, urethane, another proven initiator, has no such action. Not only is this so on local application to the skin, but also when it operates systemically (i.e., urethane given by mouth followed by croton oil paint to skin produces rich skin tumour crops). This means that hyperplasia is not necessary to the stage of initiation. Promoting agents may cause epithelial hyperplasia, but all agents causing epithelial hyperplasia do not promote tumours. One agent may promote tumours in a mouse, but not in a rabbit, although it stimulates hyperplasia in both. Therefore, in the stage of promotion, hyperplasia does not seem to play an essential part. Berenblum* considers that hyperplasia *per se* has a minor role in promotion. Notwithstanding these considerations, the place of hyperplasia in tumour formation is not yet settled, and further research may throw new light on the problem.

Tumour Progression.—The idea that tumour development is a result of a progressive acquisition of properties was propounded by Rous and Beard in 1935,‡ and in more recent years a theory of progression has been elaborated by Foulds.§ In 1949, this author expounded on his observations of spontaneous breast cancers in mice: they appeared and grew during pregnancy; usually regressed after parturition; and then reappeared with the next pregnancy. The phase of progression was described by the author as 'responsive', because the growth of tumours occurred only under circumstances of hormonal stimulation, i.e., the tumours were hormone dependent. With subsequent pregnancies, a stage was reached where there was

* Berenblum, I. (1954), 'A Speculative Review: the Probable Nature of Promoting Action and its Significance in the Understanding of the Mechanism of Carcinogenesis', *Cancer Res.*, **14**, 471.

† Berenblum, I. (1959), 'Some New Implications of the Two-stage Mechanism in the Study of Skin Carcinogenesis', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

‡ Rous, P., and Beard, J. W. (1935), 'The Progression to Carcinoma of Virus-induced Papillomas (Shope)', *J. exp. Med.*, **62**, 523.

§ Foulds, L. (1949), 'Mammary Tumours in Hybrid Mice: Growth and Progression of Spontaneous Tumours', *Br. J. Cancer*, **3**, 345; (1961), 'Progression in Carcinogenesis', *Acta Un. int. Cancr.*, **17**, 148; and (1964), 'Tumour Progression and Neoplastic Development', in *Cellular Control Mechanisms and Cancer* (Ed. Emmelot, P., Mühlbock, O.). Amsterdam: Elsevier.

Tumour Progression, *continued.*

no longer regression after parturition and the tumours were no longer dependent upon oestrogen influence; they grew independently and autonomously. The change from dependence to autonomy often appeared to be abrupt. Some tumours developed to an independent status without passing through the phase of responsiveness, i.e., these tumours advanced directly to unresponsiveness.

In terms of the mechanism of progression in neoplastic development, latency would represent a period during which there was progress from an inapparent potentially malignant phase up to the time of recognizable tumour formation. There appears to be a close link between the concepts of progression and two-stage mechanisms. Foulds suggests that 'initiation establishes a region of diffuse incipient neoplasia with a new capacity for neoplastic development'. The subsequent course varies from an absence of further change to responsive or dependent tumours, to unresponsive and autonomous lesions. Salaman,* working with the skin carcinogen, 9 : 10-dimethyl-1 : 2-benzanthracene and the cocarcinogen croton oil, found the action of the latter agent to be a gradual one, requiring repeated applications. During the course of its promotion, the effects were reversible (comparable to 'responsiveness' as described by Foulds) for a considerable period. Irreversibility (cf. unresponsiveness and autonomy) became established shortly before tumours appeared.

Immune Mechanisms and Latency.—The latent period is explicable on the basis of immune mechanisms. Immunological tolerance (*see* p. 251 *et seq.*), induced or facilitated by a promoter, may change a previously potential, but inapparent tumour, to overt cancer.

The deletion of tissue specific antigen, as postulated in Green's immunological theory of carcinogenesis (*see* p. 255), by continued exhibition of a carcinogen, which is 'complete' in the sense that it is itself both initiator and promoter, or by the application of a promoter, will induce tumour formation and so bring the incubation period to an end.

Other deletion concepts (regarding enzymes and cellular protein constituents) may be offered in explanation of latency. These, together with the others already noted in this chapter, make up a sizeable list of theories, all of which are plausible, but none of which is proven. Latency remains a reality of cancer behaviour that is still an enigma.

* Salaman, M. H. (1952), 'The Latent Period of Co-carcinogenesis', *Br. J. Cancer*, 6, 155.

CHAPTER XI

IONIZING RADIATION AND CARCINOGENESIS

Nature of Ionizing Radiation.—The rays are capable of penetrating into and through tissues; during their passage, electrically charged ions are left in the tissues where they induce physicochemical changes in cellular protoplasm and intercellular material.

There are two classes of such radiation: particulate and high-energy electromagnetic. The common types in the particulate class are:—

ALPHA RAYS.—The particles are rapidly moving nuclei of helium atoms carrying a positive electric charge. They are emitted by such radioactive elements as radium and polonium. When applied to the body from without they have little effect because of very limited penetrating power. However, when the particles are discharged from radioactive materials within the body, the destructive effects may be considerable.

BETA RAYS are streams of rapidly moving electrons carrying a negative charge. The particles are emitted by many radioactive elements or are created by electrical currents passed through cathode-ray tubes. Their penetrating power varies but is greater than that of alpha particles.

NEUTRONS are the constituents of atomic nuclei, the mass number of which is 2 or more. As their name implies, they are neutral electrically. Neutron particles are produced in uranium and plutonium 'piles' and 'bombs' where they may be liberated with tremendous force and energy.

PROTONS are nuclei of hydrogen atoms carrying a positive charge. Their penetration of tissues resembles that of alpha rays in being minimal.

Electromagnetic radiant energy is non-particulate and forms waves varying in length from 10,000 metres or more (e.g., electrical) at the long extreme, through the spectrum of light waves, to the short extreme of one ten-millionth of a millimetre or less in gamma and X-rays. Ionization effects of practical magnitude occur with waves at the short end of the scale.

GAMMA RAYS have a high energy with marked penetrating powers; the stronger of them may pass through the complete width of the body without much absorption along its track. The radiations emanate from both natural and artificial atomic breakdown.

X-RAYS are created by electrical apparatus. The power of the electrical energy used in this apparatus determines the penetrating

Nature of Ionizing Radiation, *continued.*

capacity of the rays; with high voltages, the resultant X-rays approach the powers of gamma rays. In the tissues X-rays liberate electrons, so that their action is the same as that of beta particles.

Mode of Action.—There are two theories explaining the mode of action of ionizing radiations; they are not necessarily mutually exclusive; on the contrary, they are probably complementary.

TARGET THEORY.—In its passage through tissues, an ionizing ray strikes a target, i.e., an atom, which consists of a positively charged nucleus with negatively charged electrons in orbit around it. The ray dislodges an electron, which attaches itself as a satellite to another atom. The result is two electrically unbalanced atoms known as 'ion pairs': one a positively charged atom from which an electron has been dislocated, and the other possessing an additional negative electron. This ionization of atoms affects the molecules to which they belong, initiating chemical and protoplasmic changes.

Ion pairs are measurable by an apparatus (Geiger-Müller counter) containing positive and negative plates to attract negative and positive atoms.

TOXIC THEORY.—This supposes that water in the tissues is ionized by radiation with the production of oxidizing substances toxic to cell metabolism. Molecules other than water may be affected producing different toxins which diffuse widely in the tissues.

The target theory is applicable to ionization of cellular components such as chromosomes so as to induce mutations, or to mitochondria affecting specific enzymes. The toxic theory provides an acceptable explanation for more general effects, e.g., radiation sickness and the fatal effects of massive generalized exposure.

Apart from these direct actions, ionizing radiations also have indirect influences upon tissues. Damage to vessels, glandular cells, and connective tissues, may disturb nutrition and function of neighbouring or distant cells. In addition, the products of autolysis of a large number of cells destroyed by radiation act as general toxins.

Risks of Exposure.—

1. Medical personnel. Medical and technical staff operating X-ray machines, radium and other radioactive substances, for purposes of therapy, diagnosis, and research. In this form of occupational exposure, X-rays and gamma rays are mainly concerned, and the main organs affected are skin and bone-marrow.
2. Patients receiving such emanations for therapy, and to a minor degree for diagnosis. Infants and children are much more vulnerable.
3. Customers and shop-attendants using X-ray fluoroscopy for shoe-fitting.

4. Personnel concerned in the manufacture and testing of X-ray tubes and radioactive substances.
5. Employees painting luminous dials. The paint contains radium, radiothorium, and mesothorium, and is ingested when the painter points the brush by licking it. The radioactive substances are deposited in the skeleton and give rise to bone sarcomas.
6. Miners breathing radon gas and radioactively charged dust particles in the air of mines where the ores contain radioactive elements. The Schneeberg and Joachimsthal (Jachymov) mines in South-Eastern Europe are well-known examples with a high incidence of lung cancer.
7. Personnel at atomic energy establishments. Radioactive 'waste' from such plants may be particularly dangerous if the precautions adopted within the laboratories and workshops are relaxed when contaminated 'waste' is dumped elsewhere.
8. Industrial users of X-rays, e.g., for the detection of metal flaws.
9. Nuclear fission explosives. The release of high-energy neutrons which combine with elements to produce radioactive compounds constitutes the danger. The fall-out of radioactive material may contaminate food and water-supplies as well as surface ground. The quantities of fall-out can be of serious proportions near the explosion. A further potential danger in the vicinity of an explosion arises from a concentrated large radioactive particle.

Nuclear war explosives, as used in Hiroshima and Nagasaki, were effective mainly because of the blast created. Subsidiary damage was due to heat and ionizing radiation released by the explosion. Blast caused severe effects up to 5 miles; heat flash burns occurred up to 3 miles; and severe ionizing radiation up to 1 mile. These measurements refer to the particular strength of nuclear bombs exploded high in the air over Hiroshima and Nagasaki. Explosions near the ground surface cause much more intense and widespread radiation dosages: a test explosion of this nature is reported to have produced a lethal or near-lethal human dose over an area of about 7000 square miles (M.R.C.*).

10. Articles in everyday life:—
    a. Watches with luminous dials which contain radioactive substances. The emanations are too weak to cause ill-effects, but certain of the more elaborate instruments, e.g., those for skin-divers, have a much increased quantity of luminous paint and may reach a harmful level.
    b. Television receivers emit soft X-rays, but they are nearly always adequately screened.
11. Natural sources:—
    a. Cosmic radiation from 'outer' space. Most of the emanations are filtered by the earth's atmosphere; thus the higher above

* Medical Research Council (1956), *The Hazards to Man of Nuclear and Allied Radiations*. London: H.M.S.O.

Risks of Exposure, *continued.*

sea-level, the greater the amount of radiation. However, the additional dose even at high-altitude flying is regarded as insignificant.

b. Radioactive elements in the earth occur naturally. The chief sources are thorium and uranium. Their emanations and those of their daughter atoms are small. Occasional concentrations where the elements are mined may produce higher quantities.

c. Internal radiation. Some normal constituents of the body emanate small quantities of radiation. Some foodstuffs, e.g., breakfast cereals and nut kernels, also have a very mild activity. Drinking water from regions of rock and granite is a source of radioactive substances. It is unlikely that these internal sources amount to a sufficient quantity to cause damage.

Biological Effects.—The effects depend upon a number of factors:—

1. DOSE.—Various units are in use: 'Rads' express the amount of radiation absorbed in the tissues; 1 rad equals 100 ergs energy per gramme tissue. 'Roentgen', or 'r', is a unit of X-ray dosage producing a standard quantity of ions of positive or negative charge. 'Curie' and a millionth of a curie, or microcurie, indicate the energy released by disintegration per second of radioactive substances.
2. DURATION OF EXPOSURE.
3. TISSUE VARIATIONS.—E.g., human lymph-nodes, gonads, haematopoietic cells, skin, and small intestinal mucosa are highly sensitive; but muscle and nerve-cells are relatively resistant.
4. CELL ACTIVITY.—Cells undergoing active growth are more readily affected.
5. AGE.—Children are more sensitive.
6. EXTENT OF EXPOSURE.—The greater the area of exposure, the more severe the early reactions; this probably also applies to the delayed and later effects.
7. SITE OF EXPOSURE.—With equal dosage and duration of application, upper abdominal exposure produces more severe and earlier effects than those following exposure of a limb.

According to information derived from studies of bomb victims at Hiroshima and Nagasaki, the M.R.C.* reports that general whole-body irradiation of from 400 to 500 r causes acute illness in all people so exposed. Sudden nausea, vomiting, and diarrhoea occur within a half to several hours. In about half the population, the effects are lethal, in some, within a week. Liebow and others† have described the pathological effects of atom bombs. In those

* Medical Research Council (1956), *The Hazards to Man of Nuclear and Allied Radiations.* London: H.M.S.O.

† Liebow, A. A., Warren, S., and De Coursey, E. (1949), 'Pathology of Atomic Bomb Casualties', *Am. J. Path.*, **25**, 853.

who recover from the initial acute effects, there is a fall in white blood-cells; and in 2–4 weeks, loss of hair is an early herald of a further series of signs which include cutaneous and mucous haemorrhages, oropharyngeal and intestinal ulceration, wasting, fever, and progressive anaemia. A lesser dose, as from the fall-out following a test-bomb in the Marshall Islands zone amounting to an average whole-body dose of 175 r, produced milder effects without any deaths.

Delayed and late effects in survivors are atrophy and scarring of the skin and subcutaneous tissues, bone and cartilage necrosis, cataract if the lens has been exposed, and certain general disturbances, of which anaemia and leukaemia are the most striking. The genetic effects, noted in Chapters XXII and XXIII, are of great importance in terms of biological and carcinogenic consequences.

All these later sequelae are not only related to the products of nuclear fission bombs, but may also follow occupational, industrial, and therapeutic radiation exposure. The malignant consequences are noted in the following sections.

Carcinogenic Effects.—

1. SKIN CANCER.—The main victims are radiologists, radiological technicians, and patients treated by X-rays or radium. Stricter and wider application of precautions and preventive measures is reducing the incidence of skin cancer caused by ionizing radiation. The pathology of these cancers is noted in Chapter VI.
2. CANCER OF THE THYROID GLAND has been reported by Winship,* Duffy and Fitzgerald,† Simpson and others,‡ Wilson and co-workers,§ and others. There is suggestive evidence of the occurrence of cancer of the thyroid gland in children as a result of X-ray therapy for a variety of conditions: 'enlarged' thymus, infected tonsils and adenoids, and glands of the neck. The dosage was usually low; but the age was also very low, in some cases less than one year. Cancer followed after a short latent period; in one series, the average latent period is reported as 7 years.
3. CANCER OF THE PHARYNX AND LARYNX.—This has also been reported as having followed therapy for tuberculous adenitis of the neck. The latent interval was about 20 to 30 years, and it is therefore probable that the initial dosage was much higher than that in current use.
4. CARCINOMA OF THE LUNG.—Apart from isolated cases, as reported by Abrahamson and others,‖ of lung cancer following

* Winship, T. (1952), Symposium on *Thyroid Tumours; Carcinoma of Thyroid in Children*, 1951. *Trans. Am. Goiter Ass.*, 365.

† Duffy, B. J., jun., and Fitzgerald, P. J. (1950), 'Thyroid Cancer in Childhood and Adolescence; Report on 28 Cases', *Cancer*, **3**, 1018.

‡ Simpson, C. L., Hempelmann, L. H., and Fuller, L. M. (1955), 'Neoplasia in Children treated with X-rays in Infancy for Thymic Enlargement', *Radiology*, **64**, 840.

§ Wilson, G. M., Kilpatrick, R., Eckert, H., Curran, R. C., Jepson, R. P., Blomfield, G. W., and Miller, H. (1958), 'Thyroid Neoplasm following Irradiation', *Br. med. J.*, **2**, 929.

‖ Abrahamson, L., O'Connor, M. H., and Abrahamson, M. L. (1950), 'Bilateral Alveolar Lung Carcinoma associated with Injection of Thorotrast', *Ir. J. med. Sci.*, **293**, 229.

Carcinogenic Effects, *continued.*

radiations emanating from thorotrast given by injection for arteriography, it appears that radiation cancer of the lungs requires inhalation of radioactive substance. It is a remarkable fact that notwithstanding the great numbers of breast cancers treated by irradiation with its known effect upon the lungs, as evidenced by fibrosis and vascular changes, the occurrence of primary lung cancer following such treatment is quantitatively negligible. Inhalation of radon gas and radioactive particulate dust by miners in Schneeberg and Joachimsthal (Jachymov), on the other hand, causes a high incidence of lung cancer. About half the deaths among those miners are from such tumours. Other points of interest in these cases are that the average latent period between exposure and diagnosis is 17 years; and the pathological types of cancer are epidermoid or oat-cell in about equal proportions, with an occasional sarcomatous variety.

5. BONE TUMOURS, following ionizing irradiations, occur in three main groups of cases: the occupational group of luminous dial painters; patients receiving irradiation therapy; and those treated by radium salts.

a. BONE SARCOMA IN DIAL PAINTERS.—Martland* records the occurrence of osteogenic sarcoma in girls employed as dial painters in a New Jersey factory. The paint, a phosphorescent zinc sulphide, contains traces of one or more of the following radioactive substances: radium, mesothorium, and radiothorium. It was estimated that by licking the brush to give it a sharp point, the working girl swallowed about 5 mg. radioactive material in the course of 6 months. This often caused anaemia and necrosis of the jaw within 2–5 years; and the cases of osteogenic sarcoma occurred after an average of 17 years. Jaffe† estimates the incidence of sarcoma in this occupational group as 20 per cent, but with the passage of time, the numbers of reported cases are increasing.

The sarcomas occur chiefly in the hip and knee regions. Initially, the changes consist of the formation of irregular osseous tissue between trabeculae, with radiologically increased density. The later effects have been described by Aub and others.‡ There is an increase in fibrous tissue which replaces areas of bone destruction; a phase accompanied by irregular porotic areas on radiological examination. The bones become more vulnerable to trauma, which frequently appears to precipitate, or brings to light, frank neoplastic change.

b. BONE TUMOURS FOLLOWING THERAPEUTIC EXHIBITION OF SALTS OF RADIUM OR MESOTHORIUM.—The effects of such therapy have been included in the paper by Aub and others, who

* Martland, H. S. (1931), 'The Occurrence of Malignancy in Radioactive Persons', *Am. J. Cancer*, **15**, 2435.

† Jaffe, H. L. (1958), *Tumors and Tumorous Conditions of the Bones and Joints*. Philadelphia: Lea & Febiger.

‡ Aub, J. C., Evans, R. D., Hempelmann, L. H., and Martland, H. S. (1952), 'The Late Effects of Internally-deposited Radioactive Materials in Man', *Medicine*, **31**, 221.

record 5 instances of bone tumours. The cases had received oral or intravenous radioactive substance for hypertension, arthritis, or anaemia. Looney and others* also report on similar cases. Such misguided therapy has passed its fashionable phase and is seldom, if ever, used in present practice.

c. BONE TUMOURS FOLLOWING EXTERNALLY APPLIED RADIATION.—These are rare, but there are records by Cahan and others† and Sabanas and others‡ of sarcoma following radiation therapy, e.g., for joint tuberculosis, benign bone tumours, and healthy bone exposed in the field of radiation. Both fibrosarcoma and osteogenic sarcoma occur after latent intervals of 5–10 years.

d. ATOMIC EXPLOSION FALL-OUT.—Radioactive fission products are currently under research and a number are suspect in terms of potential causes of bone sarcomas. According to the M.R.C. report (1960),§ Strontium 90 is produced in relatively great quantity, it is readily absorbed in the body and stored in bone, and is thus one of the suspected isotopes.

Caesium 137, Iodine 131, and Carbon 14 are other fission products with characters that suggest potential danger.

6. LEUKAEMIA.—The grounds for regarding radiation as a cause of leukaemia are substantial, especially when the evidence derived from a number of sources is considered collectively.

a. INCIDENCE IN MEDICAL PERSONNEL.—March‖ has shown that the mortality-rate from leukaemia among radiologists is about 9–10 times greater than it is among medical practitioners not specially connected with medical radiology. The high incidence in medical personnel is also reported by Henshaw and Hawkins¶ and by Peller and Pick.**

b. LEUKAEMOGENESIS OF X-RAY THERAPY.—Court-Brown and Doll†† investigated the incidence of leukaemia in over 13,000 patients treated by X-rays for ankylosing spondylitis. Their survey showed that the death-rate from leukaemia in this group was about 10 times greater than in a normal population

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* Looney, W. B., Hasterlik, R. J., Brues, A. M., and Skirmont, E. (1955), 'A Clinical Investigation of the Chronic Effects of Radium Salts administered Therapeutically', *Am. J. Roentg.*, **73**, 1006.

† Cahan, W. G., Woodard, H. Q., Higinbotham, N. ‖L., Stewart, F. W., and Coley, B. L. (1948), 'Sarcoma arising in Irradiated Bone. Report of Eleven Cases', *Cancer*, **1**, 3.

‡ Sabanas, A. O., Dahlin, D. C., Childs, D. S., jun., and Ivins, J. C. (1956), 'Post-radiation Sarcoma of Bone', *Ibid.*, **9**, 528.

§Medical Research Council (1960), *The Hazards to Man of Nuclear and Allied Radiations. A Second Report.* London: H.M.S.O.

‖ March, H. C. (1944), 'Leukaemia in Radiologists', *Radiology*, **43**, 275; (1947), 'Leukaemia in Radiologists', *J. Am. med. Ass.*, **135**, 179; and (1950), 'Leukaemia in Radiologists in a 20-year Period', *Am. J. med. Sci.*, **220**, 282.

¶ Henshaw, P. S., and Hawkins, J. W. (1944), 'Incidence of Leukaemia in Physicians', *J. natn. Cancer Inst.*, **4**, 339.

** Peller, S., and Pick, P. (1952), 'Leukaemia and other Malignancies in Physicians', *Am. J. med. Sci.*, **224**, 154.

†† Court-Brown, W. M., and Doll, R. (1957), 'Leukaemia and Aplastic Anaemia in Patients irradiated for Ankylosing Spondylitis', *Med. Res. Council Spec. Report Series* No. 295. London: H.M.S.O.

Carcinogenic Effects, *continued.*

of the same sex and age composition. Abbatt and Lea* studied ex-service men suffering from ankylosing spondylitis. Those who had received X-ray therapy (1627) exhibited much the same leukaemia rate as reported by Court-Brown and Doll; those who had not had X-ray treatment (399) did not provide one case of leukaemia. Although the evidence is weakened by the small numbers in the untreated group, these figures point to the absence of an association between ankylosing spondylitis and leukaemia, and strengthen the positive association between ionizing radiation and leukaemia.

Further evidence supporting this latter connexion derives from the incidence of leukaemia in relation to dosage. Court-Brown and Doll found that at moderate and low doses the incidence of leukaemia increases in proportion to the dose of X-rays.

Whilst these surveys on leukaemia following irradiation for ankylosing spondylitis are the most extensive that have been conducted, there are significant reports on other conditions submitted to ionizing radiation therapy. Lynch† reports the occurrence of acute myeloid leukaemia after treatment of benign skin lesions. Simpson and others‡ refer to leukaemia as a probable sequel to X-ray treatment of enlarged thymus in infancy. Radioactive iodine has been incriminated as a cause in isolated cases; among others by Abbatt and co-workers,§ Pochin and co-workers,‖ and Seidlin and co-workers.¶

c. DIAGNOSTIC X-RAYS.—Stewart and others** investigated deaths in children from malignant conditions over a 3-year period 1953–55, in relation to previous diagnostic and therapeutic X-rays. A control group of living children, born in the same localities and of similar age range, served as a contrast. In deaths ascribed to leukaemia and other malignant disease, twice as many mothers of the children concerned had been submitted to X-ray abdominal examinations during pregnancy as was the case in the control group, thus suggesting the risk of exposure of the foetus to diagnostic X-ray applied to the mother.

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* Abbatt, J. D., and Lea, A. J. (1956), 'Leukaemia in Ankylosing Spondylitis treated with X-rays', *Lancet*, **2**, 1317.

† Lynch, F. W. (1954), 'Leukaemia as a Possible Complication of Radio-dermatitis', *Archs Derm. Syph.*, **63**, 503.

‡ Simpson, C. L., Hempelmann, L. H., and Fuller, L. M. (1955), 'Neoplasia in Children treated with X-rays in Infancy for Thymic Enlargement', *Radiology*, **64**, 840.

§ Abbatt, J. D., Farran, H. E. A., and Green, R. (1956), 'Acute Myeloid Leukaemia after Radioactive Iodine Therapy', *Lancet*, **1**, 782.

‖ Pochin, E. E., Myant, N. B., and Corbett, B. D. (1956), 'Leukaemia following Radio-active Treatment of Hyperthyroidism', *Br. J. Radiol.*, N.S., **29**, 31.

¶ Seidlin, S. M., Siegel, E., Malamed, S., and Yallow, A. A. (1955), 'Occurrence of Myeloid Leukaemia in Patients with Metastatic Thyroid Carcinoma following Prolonged Massive Radio-iodine Therapy', *Bull N.Y. Acad. Med.*, **31**, 410.

** Stewart, A., Webb, J., Giles, D., and Hewitt, D. (1956), 'Malignant Disease in Childhood and Diagnostic Irradiation in Utero', *Lancet*, **2**, 447.

Another type of study sponsored by the Medical Research Council and referred to in a Report in 1960* consisted of a survey of children of 40,000 mothers who had been exposed to X-rays during pregnancy. The leukaemia rate was no different from that in the general population of children. The evidence is, therefore, conflicting and uncertain, and further observations are being undertaken in an effort to obtain greater clarity.

d. ATOMIC EXPLOSIONS.—The incidence of leukaemia among survivors of the atomic explosions at Hiroshima and Nagasaki in 1945 provides strong testimony on the side of radiation as a leukaemogenic agent. Folley and others† submitted the results of the first survey of the incidence of leukaemia in the 3-year period 1948, 1949, and 1950. The study is continuing and further reports published by Lange and others‡ in 1954, Moloney and Kastenbaum in 1955,§ together with those of the United States Atomic Bomb Casualty Commission (quoted by the M.R.C. 1960), have brought the following facts to light.

The incidence in bomb survivors is 4–5 times in excess of that in an unexposed Japanese population of comparable size, age, and sex distribution.

The excess incidence is progressively greater, the nearer the survivors were to the centre of the explosion; i.e., the higher the dose, the greater the incidence.

A peak period of leukaemia rate occurred in 1951, 1952, and 1953, i.e., from 6–8 years after the explosions; this has been followed by a diminishing frequency. These findings may be coordinated with that of the study of irradiated patients with ankylosing spondylitis, to provide a reasonable conclusion that the risk of leukaemia lessens after the eighth year from the time of exposure and becomes much reduced after a decade.

e. EXPERIMENTAL EVIDENCE.—In regard to experimental leukaemogenesis by ionizing radiation, it is of some moment to recognize the difference between leukaemia in laboratory animals and man, and to temper any deductions suggested by experiments in the light of such recognition.

Mice and birds have been the animals most extensively studied, and in them leukaemia arises spontaneously as well as by induction. The leukaemias are usually of the lymphatic type, originating from lymphoid tissue. The myeloid type, arising in bone-marrow, is rare in mice. In man both types occur spontaneously, and may be subacute or chronic in

* Medical Research Council (1960), *The Hazards to Man of Nuclear and Allied Radiations. A Second Report.* London: H.M.S.O.

† Folley, J. H., Borges, W., and Yamawaki, T. (1952), 'Incidence of Leukaemia in Survivors of the Atomic Bomb in Hiroshima and Nagasaki, Japan', *Am. J. Med.*, **13**, 311.

‡ Lange, R. D., Moloney, W. C., and Yamawaki, T. (1954), 'Leukaemia in Atomic Bomb Survivors', *Blood*, **9**, 574.

§ Moloney, W. C., and Kastenbaum, M. A. (1955), 'Leukaemogenic Effects of Ionizing Radiation in Atomic Bomb Survivors in Hiroshima City', *Science*, **121**, 308.

Carcinogenic Effects, *continued.*

clinical progress; a less common fulminating course occurs and is accompanied by cells which are not distinctly of either lymphoid or myeloid variety. Radiation-induced leukaemia in man falls into this acute category or into the chronic myeloid form; whereas in mice, radiation nearly always induces a fairly acute lymphatic type. The lymphatic and myeloid leukaemias may be fundamentally different diseases, and the different presentations in men and mice may invalidate comparison and correlation.

Induction of leukaemia in mice of known non-spontaneous-leukaemic strains is readily achieved by radiation. It appears from the work of Mole* that both intensity and spacing of dose are important factors in causation. Upton† suggests that such a complex time-intensity relationship underlies an 'optimum dose rate' for lymphoma induction. He notes, too, that the incidence of leukaemia with greatly reduced dose rates in mice, though small, is higher than control levels, and compares this with the incidence in radiologists. It may be significant that partial shielding of the mouse from whole-body irradiation, or injection of marrow, inhibits the induction of lymphoma (i.e., lymphosarcoma), but the production of myeloid leukaemia is not entirely eliminated.

A feature of considerable interest, and one carrying intriguing suggestions on biological mechanisms, is that studied and elaborated by Kaplan.‡ Lymphosarcoma (of which lymphatic leukaemia is part) in the mouse has a predilection for affection of, and in fact often originates in, the thymus. All agents, including radiation, known to induce this tumour injure the thymus; such injury is probably followed by repair, which appears to be dependent upon available bone-marrow and appropriate hormonal activity. Repair fails and lymphomas develop in the absence of either, or both of these two factors, i.e., when the hormone status is one leading to thymic hypertrophy, and/or bone-marrow has been damaged by whole-body irradiation. That the neoplastic change is not simply a disturbance in the mechanism of repair but seems to be strongly influenced by the stimulus to repair or by some other indirect medium, is deducible from the fact that lymphoid tumours develop in non-irradiated thymic tissue transplanted into irradiated hosts. This raises the possibility that irradiation activates, or releases from control, some leukaemogenic agent, e.g., a virus, which will flourish and produce a malignant condition in suitable host tissue. These concepts are the subjects of intensive study; but decisive proof is not yet in sight.

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* Mole, R. H. (1956), in *Progress in Radiobiology. Proc. IV Int. Conf. Radiobiol.* (Ed. Mitchell, J. S., Holmes, B. E., and Smith, C. L.). Edinburgh: Oliver & Boyd.

† Upton, A. C. (1959), 'Studies on the Mechanism of Leukaemogenesis by Ionizing Radiation', in Ciba Symposium on *Carcinogenesis.* London: Churchill.

‡ Kaplan, H. S. (1959), 'The Nature of the Neoplastic Transformation in Lymphoid Tumour Induction', in Ciba Symposium on *Carcinogenesis.* London: Churchill.

The following note on the relative incidence of leukaemia, spontaneous and radiation-induced, is added to maintain a proper perspective of its place in the whole field of human cancer. Although the death-rate from leukaemia in England and Wales has risen from 17 per million living persons in 1931 to 56 per million in 1959 (M.R.C. Report 1960*), and its rate of increase comes third to that of cancer of the lung and coronary thrombosis (Hewitt†), it is still a relatively uncommon disease. This is emphasized by comparing its mortality-rate of 56 per million with that from lung cancer which amounted to 464 per million of the population in 1959 (M.R.C. Report 1960*).

* Medical Research Council (1960), *The Hazards to Man of Nuclear and Allied Radiations. A Second Report.* London: H.M.S.O.

† Hewitt, D. (1955), 'Some Features of Leukaemia Mortality', *Br. J. prev. soc. Med.*, **9**, 81.

CHAPTER XII

CHEMICAL CARCINOGENESIS. CYCLIC HYDROCARBON GROUP

HISTORICAL NOTE

The first suggestion, based on clinical observation, that a form of human cancer was due to a chemical agent, was propounded by Percivall Pott in 1775.* The common cancer of the scrotum in chimney sweeps, presenting after 20 years or so of their occupational engagement, was ascribed by Pott to the effects of soot. During the following century, the booming growth of industrial mechanization brought many thousands of employees into the factories and machine-shops; and increasing numbers of occupational cancers induced by chemical substances were identified and recorded. Scrotal cancer affected the mule spinner, as a result of the lodgement of mule-spinning lubricating oil on the scrotum. Skin cancers of the hands, arms, and face affected workers employed in various stages of tar distillation and in the shale-oil refineries of Scotland.

A number of outstanding discoveries of chemical cancers induced by occupational exposure to polycyclic hydrocarbons are listed and discussed in Chapter VI.

The horizons for understanding the scope and mechanism of chemical carcinogenesis were immeasurably widened by the first experimental production of cancer by a chemical substance by Yamagiwa and Ichikawa in 1915 (published in 1918†). These investigators induced tumours on rabbit ears by the application of tar. In 1918, too, Tsutsui‡ produced skin cancers in mice, so facilitating research and leading to a prodigious extension of laboratory work.

The first chemically pure carcinogen was discovered by Kennaway.§ Its identification, by studies of the fluorescence of a number of polycyclic aromatic hydrocarbons, was reported by Hieger,‖ and Kennaway and Hieger,¶ in the same year. The compound, 1,2–5,6-dibenzanthracene, when painted in a benzene solution on mouse skin, induces

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* Pott, P. (1775), *Chirurgical Observations relative to the Cataract, the Polypus of the Nose, the Cancer of the Scrotum, the Different Kinds of Ruptures, and the Mortification of the Toes and Feet.* London: Hower Clarke & Pollins.

† Yamagiwa, K., and Ichikawa, K. (1918), 'Experimental Study of the Pathogenesis of Carcinoma', *J. Cancer Res.*, **3**, 1.

‡ Tsutsui, H. (1918), 'Ueber das Kunstlich Erzeugte Cancroid bei des Maus', *Verh. jap. path. Ges.*, **8**, 253.

§ Kennaway, E. L. (1930), 'Further Experiments on Cancer-producing Substances', *Biochem. J.*, **24**, 497; and (1930), 'The Identification of a Carcinogenic Compound in Coal Tar', *Br. med. J.*, **2**, 749.

‖ Hieger, I. (1930), 'The Spectra of Cancer-producing Tars and Oils and of Related Substances', *Biochem. J.*, **24**, 505.

¶ Kennaway, E. L., and Hieger, I. (1930), 'Carcinogenic Substances and their Fluorescence Spectra', *Br. med. J.*, **1**, 1044.

papillomata and epitheliomata; and, when injected in an oily medium under the skin or into peritoneum, induces sarcoma.

Cook and others,* a few years later (1933), using similar methods of study, and taking advantage of the intense fluorescence of 3,4-benzpyrene, isolated and identified this hydrocarbon as an active constituent in pitch and tar.

The next beacon lighting the path of research was that of Yoshida† who produced the first experimental hepatoma in laboratory animals (rats) by administering *o*-aminoazotoluene. This was followed by Kinosita‡ demonstrating butter yellow as a cause of liver cancer.

Rehn in 1958§ opened a chapter of brilliant research and rewarding discovery with his report on bladder tumours among aniline dye workers. Clinical observations and laboratory studies have led to an appreciation of the pathology; to the identification of the chemical compounds responsible; to an understanding of their modes of action; to lines of investigation which hold fair promise of an explanation of the origin of 'spontaneous' tumours of the bladder; and, most notably, to prophylactic control and therapeutic management of these tumours. The subject is discussed in Chapter VIII.

Many other chemical substances have been examined for their carcinogenic and cocarcinogenic properties, and the list of proven active agents now amounts to several hundreds. Some are classifiable into broad groups, making it practicable to discuss them under appropriate headings. Other chemicals are not amenable to such division and have to be grouped together arbitrarily.

POLYCYCLIC HYDROCARBON CARCINOGENS

The first pure hydrocarbon, 1,2–5,6-dibenzanthracene, found to be carcinogenic, induced sarcomas after injection into subcutaneous tissues or into the peritoneum, and epitheliomas when applied to the skin surface. This compound was synthesized by its discoverers (see introductory Historical Note) and was later found to be present in a very restricted field of materials to which man is exposed. The next hydrocarbon with similar effects was 3,4-benzpyrene. It was first isolated from coal tar, and it has a widespread distribution in materials in common use, e.g., mineral oils, processed rubber, soot, motor engine exhausts (and therefore a possible component of polluted atmosphere), cigarette smoke, and in freshly mined asbestos cobs (Harrington‖). In laboratory animals, its skin-cancer effects are more potent than its sarcoma production.

In view of the similarity of chemical structure between the 'anthracene' hydrocarbons and natural steroids and bile acids in man, the theoretical expectation that aromatic polycyclic hydrocarbons might

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* Cook, J. W., Hewett, C. L., and Hieger, I. (1933), 'The Isolation of a Cancer-producing Hydrocarbon from Coal-tar', *J. chem. Soc.*, **30**, 395.

† Yoshida, T. (1932), 'Ueber die Experimentelle Erzeugung von Hepatoma durch die Fütterung mit o-amino-azotoluol', *Proc. imp. Acad. Japan*, **8**, 464.

‡ Kinosita, R. (1937), 'Studies on Cancerogenic Chemical Substances', *Acta Soc. path. jap.*, **27**, 665.

§ Rehn, L. (1895), 'Blasengeschwulste bei Fuchsinarbeitern', *Arch. klin. Chir.*, **50**, 588.

‖ Harrington, J. S. (1962), 'Occurrence of Oils containing 3 : 4 benzpyrene and Related Substances in Asbestos', *Nature, Lond.*, **193**, 48.

Polycyclic Hydrocarbon Carcinogens, *continued.*

be derived from their interaction and metabolism, received part confirmation from *in vitro* experiments. Cook and Haslewood* produced methylcholanthrene from deoxycholic acid; this compound, even in very small quantities, has proved to be a highly potent carcinogen, and the question of the formation of a cholanthrene compound by some metabolic change in the human body remains open; this chemical may provide the link between human spontaneous cancer and experimental chemical cancers.

The group of polycylic hydrocarbon carcinogenic compounds, benzanthracene and derivatives, benzpyrene, and cholanthrene, have been used for intensive and wide investigations, not only to establish the cause of certain cancers affecting man, but to unravel biological problems of tumours and to discover mechanisms of cancer production. A voluminous literature contains much that is indicative of the success that has attended this work, but it also points to many obvious gaps in our knowledge. Some of the work is selected here to indicate its scope and potential.

Alteration of Structure.—Seemingly minor alterations and modifications of chemical structure have a profound effect upon carcinogenic potency. Fieser† reported that the substitution of methyl radicles conferred upon a very weak carcinogen, 1,2-benzanthracene, a high grade of activity in the form 5 methyl 1,2-benzanthracene. Other minor substitutions were found to nullify activity. The inferences are that chemical structure is the important factor in the induction of tumours, and that irritation as a main causative mechanism has but little support.

The theory of mechanical irritation as a cause had lost ground by earlier findings, recorded by Ross in 1918,‡ of an absence of correlation between irritative properties and carcinogenic capacities of coal, tar, soot, and their products. In fact, an inverse relationship was suggested by the following observations:—

Substance	*Irritative Powers*	*Cancer Incidence*
Coal dust	Most marked	Nil
Blast furnace pitch	Marked	Nil
Gas tar pitch	Slight	High
Tar	Very slight	Marked
Soot	Absent	Most marked

Sites of Tumour Formation.—Painting of the skin, injection under the skin, or into the peritoneum, and implantation of pellets containing the chemical, all gave rise to tumours locally at the site of application of the carcinogen. Such local reactions were carcinomatous or sarcomatous, depending upon the tissue exposed. The breadth of the spectrum of tumours may be seen from the following

* Cook, J. W., and Haslewood, G. A. D. (1933), quoted by Haddow, A. (1958), 'Chemical Carcinogens and their Modes of Action', *Br. med. Bull.*, 14, 79.

† Fieser, L. F. (1938), 'Carcinogenic Activity, Structure and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons', *Am. J. Cancer*, 34, 37.

‡ Ross, H. C. (1918), 'Occupational Cancer', *J. Cancer Res.*, 3, 321.

examples: gliomas followed implantation of methylcholanthrene in the brain; leukaemia was caused by intrasplenic injection; implanted pellets in liver, uterus, or kidney produced cancers of these organs.

The carcinogens were also found to act at distant sites, remote from the vicinity of their application. This was first observed in an increased lung-cancer rate in mice under treatment by skin painting. Magnus* demonstrated that intragastric dibenzanthracene in mice did not cause tumours in the gastro-intestinal tract, but did, in marked degree, induce lung tumours. Shimkin† reviewed the subject and reported his own experiments of intratracheal and intravenous administration of carcinogens which produced a high incidence of lung cancer.

In a description of a series of tumours induced by subcutaneous injection of hydrocarbons, Bonser and Orr‡ record a majority of sarcomatous lesions, but also a significant number of adeno-carcinomas in female mice. These tumours were most probably of mammary epithelial origin, and arose by action upon epithelial cells in contact with, or close to, the injected chemical carcinogen. A more remarkable form of provocation of mammary carcinoma was reported by Orr;§ in his experiments, methylcholanthrene was instilled intranasally, precautions being taken to prevent spillage or direct application to the breast.

The remote production of breast cancer has been elaborated by Huggins and others.|| Methylcholanthrene and dibenzanthracene fed orally to rats of a special strain were known to induce, within 2 months, 100 per cent mammary cancer rate in females. These cancers had been shown, in previous experiments, to be hormone influenced because ovariectomy had been followed by tumour regression. The experiments in the 1961 paper disclosed cancer development after a single feed of the chemical compound. The tumours affected two sites: adenocarcinoma of the breast and of the external acoustic duct. The breast lesion was suppressed by giving steroid hormones (progesterone or oestradiol), and by pregnancy.

These experimental findings contain features of great interest and substantial biological importance. At least three causative agents are concerned in the breast tumours; they are associated in a complex of causation, wherein each contributes a fundamental aetiological factor. The hormone dependence of breast tumours (*see also* Chapter XIV, p. 145 *et seq.*) emerges very clearly; hormones are sufficiently powerful to affect the formation of the tumour or bring about its regression. Viral agents are intimately concerned. It has been shown (*see* Chapters XVIII and XIX) that viruses, in

* Magnus, H. A. (1939), 'Experimental Production of Malignant Papillomata of Lung in Mice with 1 : 2 : 5 : 6 dibenzanthracene', *J. Path. Bact.*, **49**, 21.

† Shimkin, M. B. (1939), 'Production of Lung Tumours by Intratracheal Administration of Carcinogenic Hydrocarbons', *Am. J. Cancer*, **35**, 538.

‡ Bonser, G. M., and Orr, J. W. (1939), 'The Morphology of 160 Tumours induced by Carcinogenic Hydrocarbons in the Subcutaneous Tissues of Mice', *J. Path. Bact.*, **49**, 171.

§ Orr, J. W. (1943), 'Mammary Carcinoma in Mice following Intranasal Administration of Methylcholanthrene', *Ibid.*, **55**, 483.

|| Huggins, C., Grand, L. C., and Brillantes, F. P. (1961), 'Mammary Cancer induced by a Single Feeding of Polynuclear Hydrocarbons, and its Suppression', *Nature, Lond.*, **189**, 204.

Sites of Tumour Formation, *continued.*

most if not all experimental breast cancers, probably mediate the tumorigenic influence of chemical agents. Therefore in the experiments of the remote production of breast cancer by polycyclic hydrocarbons, it would seem reasonable to infer that the chemical compound, after absorption through respiratory or gastro-intestinal mucosa, reached the breast via circulatory vessels and released existing, but latent, viruses from some form of control, so as to render them carcinogenically active and potent in the presence of a suitable hormonal milieu.

This explanation is, in appreciable degree, theoretical, but it has been the inspiration of extensive research to test its validity. Whilst the complete theory itself has not yet been proved or disproved, a number of aspects of the concept have been verified and many other facets of the nature of cancer have been brought to light.

Host Susceptibilities.—Experiments have been devised to demonstrate the importance of age as an element in the sensitivity of cells to chemical carcinogens. Roe and others* have shown the marked sensitivity of neonatal mice to carcinogenic hydrocarbons. Similar circumstances exist in regard to ionizing radiation and virus infection as agents provoking neoplasia. It is possible that the sensitivities in all three groups of carcinogenesis are explicable on the basis of immunological reactions, which are discussed in Chapters XVIII and XXIV.

Hereditary susceptibility of laboratory animals to chemical carcinogens has often been demonstrated. Strains, both resistant and sensitive to different chemical compounds, have been produced by inbreeding. The place of genetic constitution in the genesis and behaviour of tumours has not been determined, but it has an important bearing which is discussed in Chapter XXIII.

Somatic Mutation.—A regular finding in chemically induced malignancy is the acquisition of autonomy by the tumour; once it has developed malignant properties it becomes independent of the presence of the causative chemical agent. This has been one of the main props of the theory of somatic mutation, in which it is supposed that the altered genetic pattern produces a clone of cells, reproducing itself without further exogenous influence.

Combined Action and Cocarcinogenesis.—Experimental trials of combining two or more chemical carcinogens of the aromatic polycyclic hydrocarbon group have revealed, fairly uniformly, mutual support in producing tumours. Such tumours appear sooner and in greater numbers when the agents are combined at one time or are given in sequence. In another group of carcinogenic agents, viz., the azo compounds, both synergistic and antagonistic actions have been disclosed (*see* p. 134).

* Roe, F. J. C., Rowson, K. E. K., and Salaman, M. H. (1961), 'Tumours of Many Sites induced by Injection of Chemical Carcinogens into Newborn Mice, a Sensitive Test for Carcinogenesis: The Implications for Certain Immunological Theories', *Br. J. Cancer*, **15**, 515.

The phenomenon of synergism may be related to cocarcinogenesis and the two-stage mechanism of cancer formation. This important concept was framed on the basis of experimentation with chemical carcinogens and is discussed in Chapter X.

Mechanism of Action.—One of the prime objectives of the very extensive research into the action of hydrocarbon carcinogens is the elucidation of their mode of action. The objective has not yet been achieved, with the result that a number of conjectures and hypotheses are offered in explanation of the limited data available.

It is not known whether the chemicals are altered in living tissues and if they produce cellular aberrations by their metabolites, or whether they act in their administered form. Knowledge of the active carcinogenic elements in the aromatic amines is much more complete. It has been established (*see* Chapter VIII, p. 82) that with these chemical substances, the metabolites are the tumorigenic agents. In the aromatic polycyclic hydrocarbons, metabolites have not been shown to be the active agents (Boyland*). This author suggests that they act in their original structure.

Enzymes in Malignant Tissues.—In a number of generalizations, Greenstein† summarizes the results of his work on the alterations of enzyme activity in cancerous tissues. Each normal tissue is characterized by a specific pattern of enzymatic activity; this is reduced or eliminated in the cancers arising from the tissues. This represents the basic alteration; and it is confirmed by analytical studies showing that the difference between the enzymes of cancer and normal cells is quantitative and not qualitative. The range of enzymatic activity of cancer cells is narrowed, and cancer tumours of different kinds come to resemble one another in terms of their enzyme activity, i.e., they converge towards a common biochemical behaviour, whilst diverging from that of their parent tissues.

Weinhouse‡ expounds the proposition that tumour cells are not biochemically fixed or static, but that they undergo continual enzyme alterations with progressive cell proliferation and with serial transplantations. This may explain tumour progression (*see* Chapter X, p. 101). Certain enzymes are reduced during pre-malignant and early phases, when tumours are dependent or responsive; later, apparently sudden transformation to malignant autonomy may be the result of further enzymatic disturbance which becomes associated with somatic mutation.

The various theories of deletion, whether this involves key proteins of the genetic code (Potter§), or proteins essential for control of normal cell growth (Miller and Miller‖), or a certain class

* Boyland, E. (1964), 'Polycyclic Hydrocarbons', *Br. med. Bull.*, **20**, 121.

† Greenstein, J. P. (1954), *Biochemistry of Cancer*. 2nd ed. New York: Academic Press.

‡ Weinhouse, S. (1960), 'Enzyme Activities and Tumor Progression', in *Amino Acids, Proteins and Cancer Biochemistry*. New York: Academic Press.

§ Potter, V. R. (1957), 'The Present Status of the Deletion Hypothesis', *Univ. Mich. med. Bull.*, **23**, 400.

‖ Miller, J. A., and Miller, E. C. (1953), 'The Carcinogenic Amino-azo-dyes', *Adv. Cancer Res.*, **1**, 339.

Enzymes in Malignant Tissues, *continued.*

of soluble liver proteins (Soroff and Cohen*), are readily understandable in terms of Greenstein's generalizations; but the question arises whether the enzymatic changes are links in the causative chain of malignancy, or incidental effects of some fundamental, although as yet unknown, agency.

Miller† recorded the occurrence of chemical binding between hydrocarbons and epidermal tissue proteins; and Heidelberger and Moldenhauer‡ reported work suggesting that carcinogenic potency was related to the degree of such binding. Heidelberger§ states further that binding, which was found in every case studied, appears to be a necessary reaction for the initiation of malignancy. This author supports the deletion theory of carcinogenesis on the basis of the experimental findings on protein binding with carcinogen. It is assumed that certain enzymes exercise a control over growth; these enzymes are deleted by the binding; cells lacking the particular enzymes are genetically produced; these cells, having lost their growth control factor, become autonomous, unrestricted, and cancerous in their behaviour.

A case against this theory finds substance in the fact that certain chemicals bind with, and upset, proteins, yet do not lead to cancer. Further argument countering the theory of deletion by binding is noted later in this chapter in relation to chemically induced liver tumours.

The precise target substance within the cell is the subject of much research, and, as yet, much speculation. Boyland and Green|| report work on the interaction of polycyclic hydrocarbons and nucleic acids. The finding that non-carcinogenic hydrocarbons react in a similar manner tends to weaken theories explaining carcinogenesis on the basis of chemical deletion of DNA or RNA (*see* Chapter XXII, p. 230). In a review, Haddow¶ gives more weight to the possibility of the combination of carcinogen with cellular substrates and binding with nucleoprotein rather than with nucleic acid. The theory has it that consequent elimination of 'growth' proteins or enzymes may lead to primitive reactions with disturbed cell division.

Electron donation and acceptance in chemical carcinogens were studied by Allison and Nash.** Powerful activity, both donor and

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* Soroff, S., and Cohen, P. P. (1951), 'Electrophoretic and Ultra-centrifugal Studies on the Soluble Protein of Various Tumours and of Livers from Rats fed 4-dimethylaminoazobenzene', *Cancer Res.*, **11**, 376.

† Miller, E. C. (1951), 'Studies on the Formation of Protein-bound Derivatives of 3, 4-benzpyrene in the Epidermal Fraction of Mouse Skin', *Ibid.*, **11**, 100.

‡ Heidelberger, C., and Moldenhauer, M. G. (1956), 'The Interaction of Carcinogenic Hydrocarbons with Tissue Constituents. IV. A Quantitative Study of the Binding to Skin Proteins of Several C14-labelled Hydrocarbons', *Ibid.*, **16**, 442.

§ Heidelberger, C. (1959), 'The Relation of Protein Binding to Hydrocarbon Carcinogenesis', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

|| Boyland, E., and Green, B. (1962), 'Interaction of Polycyclic Hydrocarbons and Nucleic Acids', *Br. J. Cancer*, **16**, 507.

¶ Haddow, A. (1958), 'Chemical Carcinogens and their Modes of Action', *Br. med. Bull.*, **14**, 79.

** Allison, A. C., and Nash, T. (1963), 'Electron Donation and Acceptance by Carcinogenic Compounds', *Nature, Lond.*, **197**, 758.

acceptor, was exhibited by polycylic aromatic hydrocarbons, steroids, stilbenes, aromatic amines, and heterocyclic nitrogen compounds. It is suggested that the interaction between carcinogens and enzyme systems is explicable on an electronic transfer basis.

Polycylic hydrocarbon carcinogenesis is explained by an immunological theory by Green; this is discussed in Chapter XXIV, p. 247.

Another theory, stimulated by chemical induction of breast tumours which later become transmissible by cell-free extracts, surmises that chemical carcinogens act by deletion of repressors of viral activity. This has been referred to earlier in this chapter and is also discussed in Chapters XVIII and XIX.

THE AROMATIC AMINES

These cyclic hydrocarbons arise in the manufacture of dyes. Their current use extends beyond the dyestuff industry, e.g., to rubber works, chemical laboratories, and cosmetic preparations. The induction of cancers in man and experimental animals by aromatic amine metabolites is discussed in Chapter VIII. The exciting prospect of an explanation for the occurrence of 'spontaneous' bladder cancer in man as a result of irregular trytophan metabolism is also noted in the same chapter. The elucidation of the cause and its probable mode of action in human bladder cancer provide grounds for justifiable optimism for an extension of the knowledge and for similar unravelling of the puzzles of other tumours.

Boyland* considers that *o*-aminophenols or arylhydroxyamines, the active carcinogenic metabolites of aromatic amines, probably act by destruction of the factor controlling cell growth. This factor may be compared to the 'suppressor' present in bacteria, or to the 'repressor', the place of which is described by Lwoff† on the following lines: molecular balance in a micro-organism is controlled by repressors, produced by regulator genes; absence, or deletion, of repressors leads to anarchic synthesis of enzymes and circumstances in which one system outgrows the others. The same type of regulation of balance occurs in the cells of more complex organisms. There is some evidence pointing to bacterial suppressors being part of RNA. If this proves correct, it furnishes a probable example of binding between a chemical carcinogen and RNA.

FLUORENE COMPOUNDS

This is another group of polycyclic hydrocarbons, not derived from anthracene, but related to this group. The fluorene compounds were developed as insecticides during World War II, and their 'domestic' use for this purpose carries a danger in view of their cancerigenic properties. One of the most potent is 2-acetylaminofluorene, which induces a wide spectrum of tumours. It has also been used experimentally to test synergistic action with other substances, e.g., with thiourea, it produces carcinoma of the thyroid; with stilboestrol, pituitary tumours are induced.

* Boyland, E. (1963), *The Biochemistry of Bladder Cancer*. Springfield, Ill.: Thomas.

† Lwoff, A. (1960), 'Tumor Viruses and the Cancer Problem: A Summation of the Conference, in a Symposium on the Possible Role of Viruses in Cancer', *Cancer Res.*, **20**, 820.

AZO DYES AND LIVER TUMOURS

The azo dyes are also cyclic hydrocarbon derivatives, and are used in industry and in the preparation of foods. While it is not known whether they cause cancer in man, they are regarded with suspicion in view of their potent carcinogenic activity in experimental animals.

As already indicated in the Historical Note, Yoshida in 1932 produced the first experimental hepatoma by using *o*-aminoazotoluene in rats; and this was followed in 1937 by the work of Kinosita showing 4-dimethyl aminoazobenzene ('butter yellow') as a cause of similar tumours.

Following oral feeding, there is an initial phase of degeneration and necrosis of liver cells, which is succeeded by regeneration, advancing to hyperplasia with the formation of nodules, and ultimate malignant tumour development.

Weiler* summarizes the results of immunological investigation of azo dye-induced hepatoma in rats. Tissue-specific antigen, normally present in healthy liver, was found to be absent in hepatomas. The presumption is that the antigen has been deleted by the carcinogen. The loss of antigen was found to have begun before the advent of malignancy; carrying the possible implication of a causative relationship between loss of antigenic control and uninhibited cell proliferation.

Miller and Miller,† on the basis of their experiments with aminoazo-induced hepatoma, advance the theory that the cancer is attributable to deletion of key proteins which are essential for normal growth. These workers and Heidelberger (*see above*) ascribe the deletion to chemical binding between carcinogen and protein. However, experimental work on azo-dye tumorigenesis has produced a forceful argument against the thesis of binding as a causative mechanism of cancer. Hypophysectomy prevents liver cancer formation notwithstanding the occurrence of chemical binding. There are also other hormonal influences which have been proved experimentally. Therefore chemical binding, by itself, cannot explain the carcinogenic mechanism.

MacDonald and others‡ and Arcos and Griffith§ demonstrated that certain combinations of the azo dyes act synergistically in hepatic tumour induction. Contrariwise, Crabtree|| reported that some chemicals retarded the carcinogenic potency of azo cancer induction. The concepts of cocarcinogenesis and anticarcinogenesis (*see* Chapter X, p. 110 *et seq.*) are involved in these experimental results, but the sites of action of synergists and antagonists are not yet known.

NITROSO COMPOUNDS

Dimethylnitrosamine, one of the group of nitroso compounds derived from amines, was found to be carcinogenic by Magee and Barnes.¶

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* Weiler, E. (1959), 'Loss of Specific Cell Antigen in Relation to Carcinogenesis', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

† Miller, J. A., and Miller, E. C. (1953), 'The Carcinogenic Amino-azo Dyes', *Adv. Cancer Res.*, **1**, 339.

‡ MacDonald, J. C., Miller, E. C., Miller, J. A., and Rusch, H. P. (1952), 'The Synergistic Action of Mixtures of Certain Hepatic Carcinogens', *Cancer Res.*, **12**, 50.

§ Arcos, J. C., and Griffith, G. W. (1961), 'Ratio Depeudent Synergism in Azo Dye Carcinogenesis', *Br. J. Cancer*, **15**, 291.

|| Crabtree, H. G. (1955), 'Retardation of Azo-carcinogenesis by Non-carcinogenic Azo-compounds', *Br. J. Cancer*, **9**, 310.

¶ Magee, P. N., and Barnes, J. M. (1956), 'Production of Malignant Primary Hepatic Tumours in Rat by Feeding Dimethylnitrosamine', *Ibid.*, **10**, 114.

Other members of the group proved to have similar properties, inducing neoplastic conditions in different organs and in different species, e.g., liver adenomas and cancers in rats and guinea-pigs; kidney, oesophageal, and bladder tumours in rats; lung tumours in rats and hamsters; and sarcomas in mice. In rats, liver tumours present clinical and pathological features resembling those in man. A further provoking suggestion is that cigarette smoke contains nitrosamines.

In a review by Magee and Schoental,* the suggestions emerge that the compounds act via metabolites by alkylation, i.e., the introduction of an alkyl group (a radical of the methane series). The action appears to be at microsomal level, affecting DNA and RNA as well as other cellular constituents. Mutagenic activity by the compounds has been demonstrated in simple organisms, and it is supposed that this may be the mechanism of carcinogenesis.

ALKYLATING AGENTS

The chemical introduction of an alkyl group into nucleic acid and other cell components characterizes chemical compounds of the nitrogen mustard series. Other chemical agents also possess alkylating powers, e.g., diepoxides, certain lactones, polyethyleneimines, and probably the nitrosamines. These agents are carcinogenic, and this property, it is postulated, derives from their characteristic chemical reaction.

The agents are radiomimetic (*see* Chapter XI, p. 115), and act, according to Haddow,† directly upon dividing cells during their interphase, becoming manifest by irregularities of mitosis. The common occurrence of marked nuclear abnormalities suggests that carcinogenesis is effected via mutagenesis; the mutation, in turn, being due to alkylation of DNA; a reaction causing the deletion of certain base pairs from the DNA sequence, and so removing growth control factors. Lawley‡ quotes another line of action and an alternative in which it is visualized that the alkylated DNA molecule releases a nucleoprotein virus-like particle, which effects mutation and carcinogenesis.

A number of alkylating agents are being used for their cytotoxic effects as therapeutic measures in neoplasia. In this sense, the description 'radiomimetic' becomes more appropriate, as ionizing radiation is a known cause as well as a recognized treatment for cancer. In radiation, the effect is largely determined by dose and site of application; in alkylating agents, the effect varies mainly with chemical structure. Loveless and Ross§ reported that bifunctional agents exhibited greater tumour-growth inhibition than did monofunctional types.

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* Magee, P. N., and Schoental, R. (1964), 'Carcinogenesis by Nitroso Compounds', *Br. med. Bull.*, **20**, 102.

† Haddow, A. (1958), 'Chemical Carcinogens and their Modes of Action', *Ibid.*, **14**, 79.

‡ Lawley, P. D. (1962), 'Effects of Alkylating Agents on Nucleic Acids and their Relation to Other Mutagens', in Symposium on *The Molecular Basis of Neoplasia*. Austin: Univ. Texas Press.

§ Loveless, A., and Ross, W. J. C. (1950), 'Chromosome Alteration and Tumour Inhibition by Nitrogen Mustards: The Hypothesis of Cross-linking Alkylation', *Nature, Lond.*, **166**, 1113.

Summary of Theories of Mechanisms of Chemical Carcinogenesis

1. Somatic mutation resulting from interaction of chemical compound and DNA.
2. Deletion of enzymatic or growth control factor, due to chemical binding, or some form of combination, with extrachromosomal microsomes, e.g., repressor substances. The deleted factor may reside in RNA.
3. Immunological factors arising from antigenic properties of combined chemical compound and cell protein.
4. Viral activation, as a result of deletion of repressor or controlling factor by the chemical substance.

The theories are not necessarily mutually exclusive; and as none of them can be considered as proven, they may all turn out to be wrong.

CHAPTER XIII

CHEMICAL CARCINOGENESIS. INORGANIC AND SIMPLE COMPOUNDS

METALS

The carcinogenic properties of many metals have been investigated in relation to occupational tumours. Other sources of exposure arise from the use of metals for domestic purposes and from their prescription in medicinal and cosmetic preparations. In most instances of possible metal-induced cancer in man, the evidence is not conclusive, but only suggestive of culpability of the metal. The following notes include examples of metals and metalloids with carcinogenic properties in experimental animals or man, or both.

Iron.—In recent years, since the observations of Richmond,* reports have been accumulating that iron-dextran produces sarcomas in experimental animals. In addition to inducing sarcomas following repeated subcutaneous or intramuscular injection in rats, mice, and hamsters (Roe and Lancaster†), Haddow and others‡ have produced such tumours in rabbits. These authors report that 2 of 6 treated rabbits developed sarcomas after latent intervals of 3½ and 4 years, respectively. Roe and Lancaster record in a review that dextran alone was non-carcinogenic; that the iron-dextran tumour cells included many exhibiting a content of iron; that the tumours became transplantable; and that remote tumours were also induced.

These experimental findings are of importance, not only in their own right but also because iron-dextran and other iron preparations are widely and commonly used in medical and veterinary practice. Robinson and others§ report a case of a 74-year-old female patient who developed a sarcoma at the site of an injection of iron-dextran 3 years previously. These authors carefully qualify their observation: the histological appearances differed from those in rat sarcomas induced by iron-dextran; proof of the cause of the sarcoma in their patient was lacking; and the latent interval was short. Nevertheless, the case carries a message of warning.

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* Richmond, H. G. (1959), 'Induction of Sarcoma in the Rat by Iron-dextran Complex', *Br. med. J.*, **1**, 947.

† Roe, F. J. C., and Lancaster, M. C. (1964), 'Natural, Metallic and Other Substances, as Carcinogens', *Br. med. Bull.*, **20**, 127.

‡ Haddow, A., Roe, F. J. C., and Mitchley, B. C. V. (1964), 'Induction of Sarcomata in Rabbits by Intramuscular Injection of Iron-dextran ("Imferon")', *Br. med. J.*, **1**, 1593.

§ Robinson, C. E. G., Bell, D. N., and Sturdy, J. H. (1960), 'Possible Association of Malignant Neoplasm with Iron-dextran Injection', *Ibid.*, **2**, 648.

Iron, *continued.*

The recommendation by some that iron-dextran complex should not be given to man has been opposed by others. Lane* and Cox† have criticized the recommendation mainly on the grounds that the amounts used in experiments far exceed the dosage in treating humans. However, a note of caution is not unreasonable, especially as the drug has been in common use for about 10 years, whereas the expected latent interval is 15–20 years.

Another aspect of the subject may prove to be of importance. In man, certain conditions in which disturbed iron metabolism has given rise to iron deposition in tissue, or in which an excess of iron has been inhaled, are associated with a high incidence of cancer. Willis‡ drew attention to a particular type of liver cirrhosis accompanying haemochromatosis which was especially prone to hepatoma formation; and Faulds and Stewart§ reported an increased frequency of lung cancer in haematite miners. Iron may prove a fundamental factor in cancer production, and much current research is directed to its investigation.

MODE OF ACTION OF IRON.—Richmond‖ quotes work showing that dextran is split off from the iron after injection; the free iron becomes chelated with cell protein, forming haemosiderin. In the histiocytes containing iron pigment, there are also globules of ceroid material, which is known to accumulate in cells as a result of deficiency of vitamin E. The suggestion thus arises that iron may act by blocking the antioxidant activity of vitamin E, leading either to products which are carcinogenic, or to deletion of certain cell growth-controls necessary for normal activity. The fact that iron is included in the chemical composition of asbestos and chromate may prove to be relevant in the hypothesis that iron is carcinogenic.

Haddow¶ discusses whether the chelation process of iron and protein may offer a model for the cancerigenic activity of other metals, and possibly reach beyond metal-plus-protein chemical interactions to a relationship with the cellular protein effects of the amine metabolites in urinary tract carcinogenesis and also with the disturbances of cell constituents and functions by ionizing radiations.

Aluminium.—Haddow and colleagues**†† obtained sarcomas by injecting aluminium-dextran in mice. This carcinogenic activity

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* Lane, R. S. (1964), Correspondence, *Brit. med. J.*, **2**, 119.

† Cox, J. S. G. (1964), Correspondence, *Ibid.*, **2**, 120.

‡ Willis, R. A. (1941), 'Haemochromatosis, with Special Reference to Supervening Carcinoma of the Liver', *Med. J. Aust.*, **2**, 666.

§ Faulds, J. S., and Stewart, M. J. (1956), 'Carcinoma of the Lung in Hæmatite Miners', *J. Path. Bact.*, **72**, 353.

‖ Richmond, H. G. (1959), 'Induction of Sarcoma in the Rat by Iron-dextran Complex', *Br. med. J.*, **1**, 947.

¶ Haddow, A. (1959), 'The Possible Role of Metals and of Metal Chelation in the Carcinogenic Process', in Ciba Symposium on *Carcinogenesis* London: Churchill.

** Haddow, A., and Horning, E. S. (1960), 'On the Carcinogenicity of an Iron-dextran Complex', *J. natn. Cancer Inst.*, **24**, 109.

†† Haddow, A., Dukes, C. E., and Mitchley, B. C. V. (1961), in *39th Rep. Brit. Emp. Cancer Campgn*, 74.

appears to be restricted to experimental animals, and, in the Western World up to the present time, there have not been reports of an excess incidence of lung or other cancers among workers employed in aluminium industries.

It is of interest to note that investigations into two large aluminium plants in Russia by Litvinov and others* revealed that considerable quantities of 3,4-benzpyrene were discharged into the atmosphere, and that respiratory diseases, including cancer, occurred more frequently in the areas affected than in other neighbourhoods. The benzpyrene, and not aluminium, is suggested as the pathogenic agent.

Arsenic.—The place of arsenic as an occupational hazard, causing carcinomas of the skin and lungs, is noted in Chapters VI and IX.

The evidence for arsenic as a cause of skin cancer is strongly based. Neubauer† reviewed 137 cases of skin carcinoma complicating arsenical dermatitis which had resulted from oral arsenic therapy. About two-thirds of the cases had been treated for skin diseases, and the remainder for various conditions such as anaemia, debility, etc. The age incidence of the arsenical skin cancer was significantly lower than that of skin cancer from other causes; and 60 per cent of Neubauer's patients were under 50 years of age. Many other reports contribute supporting testimony to arsenic as a causative agent in skin cancer, but the case for its carcinogenicity in lungs is subject to some doubt.

On the one side of the debate, Roth‡ reports an excess rate of skin cancer, bearing the marks and characters of cancer superimposed on arsenical dermatitis, together with a coincident excess rate of lung cancer among vineyard workers. The exposure in these cases arises from the arsenic content of pesticide sprays used in the vineyards. Robson and Jelliffe§ describe 6 patients, all with typical arsenical skin changes and in all of whom lung cancer developed. The patients had been treated by arsenical preparations: 4 had Fowler's solution for psoriasis, and 2 had arsenic-containing tonics. The drugs had been administered periodically for from 3 to 15 years; and the latent interval prior to the appearance of the lung cancer varied from 17 to 54 years. The cancers in 5 cases submitted to histological examination were anaplastic. While these reports do not definitely incriminate arsenic, the evidence cannot be ignored, and may be added in support of the strongly argued case put by Buechley|| that arsenic is the basic agent effecting lung cancer among Schneeberg miners and cigarette smokers.

On the other hand, most authorities ascribe the excess pulmonary carcinoma incidence in Schneeberg miners to inhalation of

* Litvinov, N. N., Goldberg, M. S., and Kimina, S. N. (1963), 'Morbidity and Mortality in Man caused by Pulmonary Cancer and its Relation to the Pollution of the Atmosphere in the Areas of Aluminium Plants', *Acta Un. int. Cancr.*, **19**, 742.

† Neubauer, O. (1947), 'Arsenical Cancer; Review', *Br. J. Cancer*, **1**, 192.

‡ Roth, F. (1956), 'Über die Chronische Arsenvergiftung der Moselwinzer unter Besonderer Berucksichtgung des Arsenkrebs', *Z. Krebsforsch.*, **61**, 287.

§ Robson, A. O., and Jelliffe, A. M. (1963), 'Medicinal Arsenic Poisoning and Lung Cancer', *Br. med. J.*, **2**, 207.

|| Buechley, R. W. (1963), 'Cigarettes, Arsenic and Lung Cancer', *Acta Un. int. Cancr.*, **19**, 718.

Arsenic, *continued.*

radioactive material; and, in general, many investigators are cautious in expressing views about arsenical pathogenesis in lung cancer (Leading Article*).

Chromates and Asbestos are noted in Chapter IX, p. 88. The premises upon which they are considered to have definitive roles in the induction of lung tumours in man are founded on good evidence; but with neither chromates nor asbestos has experimental neoplasia been produced and there are thus important gaps in knowledge of the two carcinogens and their modes of action.

Cobalt.—Heath,† consequent on observations on the effects of cobalt upon cells in tissue-culture, injected powdered cobalt intramuscularly in rats. A high proportion developed sarcomas, mostly rhabdomyosarcomata, but also other types. In 1960, the same investigator reported on detailed, step-by-step histological changes in the muscle cells affected by cobalt. The initial reaction consisted of an extensive and continuing dedifferentiation of muscle-fibres into more primitive, free myoblast cells. This was followed by further changes from myoblasts to malignant sarcomatous cells.

Lead.—Zollinger's‡ experiments, in which renal tumours in rats resulted from repeated subcutaneous injections of lead phosphate, were followed by others, e.g., van Esch and others,§ demonstrating the same type of tumour after prolonged oral feeding with lead acetate. These latter investigators report that they found no indication of radioactivity in the lead used, nor any evidence that viruses had been activated by the metal or its conjugates within the tissues.

Boyland and others|| confirmed the induction of renal cortical adenomas and adenocarcinomas by lead acetate. Arising from the known effect of lead, viz., the disturbance of haemoglobin metabolism and the increase in excretion of porphyrin, these authors discuss the possibility that porphyrin is the immediate carcinogen. The whole question of the mechanism of action is still open and is the subject of planned research.

Dukes¶ remarks upon the improving state of knowledge in regard to renal tumours. Up to recent years, very little was known about possible causative agents; now, a beginning has been made along a number of different lines. In man, one set of agents derived from dyestuff manufacture is known to cause mainly urinary bladder tumours, but also occasional papillomatous lesions of the renal

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* Leading Article (1964), 'Arsenic and Lung Cancer', *Br. med. J.*, **1**, 1656.

† Heath, J. C. (1956), 'The Production of Malignant Tumours by Cobalt in the Rat', *Br. J. Cancer*, **10**, 668; and (1960), 'The Histogenesis of Malignant Tumours induced by Cobalt in the Rat', *Ibid.*, **14**, 478.

‡ Zollinger, H. U. (1953), 'Durch Chronische Bleivergiftung Erzeughte Nierenadenome und-carcinoma bei Ratten und ihre Bezeihungen der Entsprechenden Neubildungen des Menschen', *Arch. path. Anat.*, **323**, 694.

§ Van Esch, G. J., van Genderen, H., and Vink, H. H. (1962), 'The Induction of Renal Tumours by Feeding of Basic Lead Acetate to Rats', *Br. J. Cancer*, **16**, 289.

|| Boyland, E., Dukes, C. E., Grover, P. L., and Mitchley, B. C. V. (1962), 'The Induction of Renal Tumours by Feeding Lead Acetate to Rats', *Ibid.*, **16**, 285.

¶ Dukes, C. E. (1961), 'Clues to the Causes of Cancer of the Kidney', *Lancet*, **2**, 1157.

pelvis (*see* Chapter VIII, p. 79). In experimental animals, different agents have been used to induce kidney tumours—a virus is the probable cause of cancer in the leopard frog (Lucké*). Kirkman and Bacon† discovered that oestrogens produced renal carcinoma in male hamsters. This experimental result could not be achieved in other animals, the rat, mouse, or guinea-pig, leading to the surmise that these latter animals possess livers capable of inactivating oestrogens, whereas inactivation by the liver did not take place in the hamster. A further notable experimental finding was the induction of renal tumours in female hamsters by the exhibition of oestrogens after ovariectomy, or prior to the stage of reproductive maturity, i.e., in circumstances of low progesterone production. Therefore, this hormone may be antagonistic to oestrogen, thereby preventing renal cancer in female mice under ordinary conditions. Experimental and therapeutic trials are being conducted to test the validity of this property.

Finally, it has also been shown that chemical substances induce renal cancers in the laboratory. Subcutaneous injections of 3,4-benzpyrene provoke renal carcinomas in hamsters, and lead affects the kidneys of rats.

Boyland and others‡ draw attention to the increasing mortality-rate from renal neoplasms among males in England and Wales, and note, too, the increase in the amount of lead pollution of the atmosphere along roads; a feature which has been aggravated by the addition of lead tetraethyl to motor-car fuels.

Dukes points to the known renal lesions accompanying lead poisoning. The arteriosclerotic nephritis indicates the renal damage, and, may, in some circumstances and combinations, be a precursor to malignant change.

Nickel.—The increased incidence in exposed industrial workers of cancer of the nose, its accessory sinuses, and of the lung has been documented by Doll.§ Among others, Gilman and Herschen‖ have produced tumours experimentally.

Goldblatt¶ discusses two theories of nickel carcinogenesis. The 'arsenic theory', which implies that the arsenic content of the dust arising from processing nickel is the carcinogen, has not much to sustain it, because there is no associated arsenical dermatitis or skin cancer, and because other known industrial arsenical dusts do not produce ethmoid tumours. The second theory, the 'metal theory', attributing pathogenesis to nickel itself, appears more substantial. The evidence in human cases as well as that from experimental work are in favour of nickel being the causative agent.

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* Lucké, B. (1952), 'Kidney Carcinoma in the Leopard Frog: A Virus Tumor', *Ann. N.Y. Acad. Sci.*, **54**, 1093.

† Kirkman, H., and Bacon, R. L. (1950), 'Malignant Renal Tumours in Male Hamsters (*Cricetus auratus*) treated with Estrogen', *Cancer Res.*, **10**, 122.

‡ Boyland, E., Dukes, C. E., Grover, P. L., and Mitchley, B. C. V. (1962), 'The Induction of Renal Tumours by Feeding Lead Acetate to Rats', *Br. J. Cancer*, **16**, 285.

§ Doll, R. (1958), 'Cancer of the Lung and Nose in Nickel Workers', *Br. J. ind. Med.*, **15**, 217.

‖ Gilman, J. P. W., and Herschen, H. (1963), 'The Effect of Physical Form of Implant on Nickel Sulphide Tumourigenesis in the Rat', *Acta Un. int. Cancr.*, **19**, 615.

¶ Goldblatt, M. W. (1958), 'Occupational Carcinogenesis', *Br. med. Bull.*, **14**, 136.

Selenium.—Nelson and others* induced hepatic cirrhosis and tumours in rats by feeding them with selenium. Tscherkes and others† compare the metabolic disturbances brought about by selenium with those due to ethionine which also induce liver cancer (Farber‡), and reason that there may be certain cytochemical links in their neoplastic activity that is worthy of research.

Beryllium causes different types of tumour, e.g., pulmonary carcinoma, reticulin-cell sarcoma, and osteogenic sarcoma, in a number of experimental animals, such as rabbits and monkeys, when given by various routes and combined in different salts. It has not been proved to have caused cancer in man, although suspicion has been raised in some cases.

PHARMACEUTICAL AND CHEMICAL AGENTS

Urethane.—The induction of lung tumours in mice by urethane was reported by Nettleship and Henshaw in 1943.§ At first, the chemical was given by injection or with oral feeds; later, skin painting was discovered to be effective. Larsen‖ demonstrated that urethane could be transferred from mother to offspring via the placenta to produce pulmonary tumours in the new generation. De Benedictis and others¶ added evidence of yet another route. They reported that urethane, administered via an intragastric tube to a lactating female, was passed with breast milk to suckling mice, which later developed a high pulmonary tumour incidence.

Urethane was at one time regarded as a specific carcinogen in the lung, but it is now known to be tumorigenic in other tissues as well. Tannenbaum and Silverstone** used it as a skin paint and found that it caused epitheliomas. It also causes breast cancer, mesenchymal tumours, and adenomas of the lacrimal duct. Toth and others,†† using urethane in drinking water, induced a high incidence of malignant lymphomas in Swiss mice.

Urethane has been used widely in experimental work, e.g., its use in elaborating the concept of a two-stage mechanism is noted in Chapter X and the effects of starvation and dietary modifications on tumour formation have been studied in terms of urethane carcinogenesis. Its action on the lungs of mice was used as part of the supporting evidence for the theory devised by Warburg.

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* Nelson, A., Fitzburgh, O. G., and Calvey, H. (1943), 'Liver Tumors following Cirrhosis caused by Selenium in Rats', *Cancer Res.*, **3**, 230.

† Tscherkes, L. A., Volgarev, M. N., and Aptekar, S. G. (1963), 'Selenium-caused Tumours', *Acta Un. int. Cancr.*, **19**, 632.

‡ Farber, E. (1956), 'Carcinoma of the Liver in Rats fed Ethionine', *Arch. Path.*, **62**, 445.

§ Nettleship, A., and Henshaw, P. S. (1943), 'Induction of Pulmonary Tumors in Mice with Ethyl Carbamate (Urethane)', *J. natn. Cancer Inst.*, **4**, 309.

‖ Larsen, C. D. (1947), 'Pulmonary-tumor Induction by Transplacental Exposure to Urethane', *Ibid.*, **8**, 63.

¶ De Benedictis, G., Chieco-Bianchi, L., Maiorano, G., and Fiore-Donati, L. (1963), 'Influence of Age and Route of Administration on Lung Carcinogenesis by Urethan in Swiss Mice', *Acta Un. int. Cancr.*, **19**, 695.

** Tannenbaum, A., and Silverstone, H. (1958), 'Urethan (Ethyl Carbamate) as a Multipotential Carcinogen', *Cancer Res.*, **18**, 1225.

†† Toth, B., Della Porta, G., and Shubik, P. (1961), 'The Occurrence of Malignant Lymphomas in Urethan-treated Swiss Mice', *Br. J. Cancer*, **15**, 322.

Table XI.—Examples of Carcinogenic Drugs and Agents used for Medicinal, Agricultural, and Domestic Purposes

Drug	Tumour	Author
Thiourea and thiouracil	Carcinoma of face in rats	Rosin, A., and Rachmilewitz, M. (1954), *Cancer Res.*, **14**, 494
	Eyelids and auricular region in rats	Rosin, A., and Ungar, H. (1957), *Ibid.*, **17**, 302
	Thyroid cancer in rats	Doniach, I. (1950), *Brit. J. Cancer*, **4**, 223. and (1953), *Ibid.*, **7**, 181
Natural goitrogens (iodine peroxidase)	Thyroid cancer in rats	Alexander, N. M. (1959), *J. biol. Chem.*, **234**, 1530.
Sulphonamides	Multiple	Hansen, P. B., and Bichel, J. (1952), *Acta radiol. Stockh.*, **37**, 258
Carboxymethylcellulose, polyvinyl-pyrolidone, and polyoxyethylene	Subcutaneous fibrosarcomas: especially as promotor in mice	Lusky, L. M., and Nelson, A. A. (1957), *Fed. Proc.*, **16**, 318; Hueper, W. C. (1957), *Cancer, N.Y.*, **10**, 8
Tannin	Hepatic tumours in rats, by subcutaneous injection	Korpassy, B., and Mosonyi, M. (1950), *Brit. J. Cancer*, **4**, 411
Carbon tetrachloride	Carcinoma of liver in mice	Edwards, J. E. (1941), *J. nat. Cancer Inst.*, **2**, 197
'Dulcin'. Synthetic sweetening agent (*p*-phenetylurea)	Tumours of liver in rats by feeding	Fitzhugh, O. G., and Nelson, A. A. (1950), *Fed. Proc.*, **9**, 272
Senecio alkaloids	Tumours of liver in rats by feeding	Cook, J. W., Duffy, E., and Schoental, R. (1950), *Brit. J. Cancer*, **4**, 405
Isonicotinic acid hydrazine (isoniazid)	Pulmonary tumours in mice by injection	Juhász, J., Balo, J., and Kendrey, G. (1957), *Krebsforsch.*, **62**, 188
	,, ,, ,, by feeding	Biancifori, C., and Ribacchi, R. (1962), *Nature, Lond.*, **194**, 488
D.D.T.	Hepatoma in rats by feeding	Fitzhugh, O. G., and Nelson, A. A. (1947), *J. Pharmacol.*, **89**, 18
Aldrin and dieldrin	Hepatoma in mice by feeding	Davis, K. J., and Fitzhugh, O. G. (1962), *Toxicol. Appl. Pharmacol.*, **4**, 187
Aramite	Biliary tract tumours in dogs and rats	Oser, B. L., and Oser, M. (1962), *Ibid.*, **4**, 70
Concentrated sodium chloride solution	Sarcoma by subcutaneous injection in mice and rats	Tokoro, Y. (1940), *Gann*, **34**, 149
Concentrated glucose solution	Sarcoma by subcutaneous injection in mice and rats	Nishiyama, Y. (1935), *Ibid.*, **29**, 1
Concentrated fructose solution	Sarcoma by subcutaneous injection in mice and rats	Takizawa, N. (1940), *Ibid.*, **34**, 1
Arachis oil	Sarcoma by subcutaneous injection in mice	Walpole, A. L., and others (1954), *Brit. J. Pharmacol.*, **9**, 306

Warburg's Theory.—Warburg, first in 1930* and again in 1956,† advanced the theory that neoplasia arose from a transformation of carbohydrate metabolism. This author had reported (1923‡) his findings of a higher metabolic rate of anaerobic glycolysis in cancer cells than in normal tissues. He theorized that urethane, acting as a narcotic as well as a carcinogen, initially inhibits respiration, resulting in selective survival and proliferation of cells which maintain their structure and derive their energy by anaerobic fermentation. This leads to morphological change with conversion of differentiated cells into undifferentiated cancer cells.

The theory has been radically criticized and its very basis of altered aerobic and anaerobic glycolysis has been brought into question (Berenblum§ and Hieger‖). As it is quite often resuscitated, particularly as an addendum to other biochemical theories, it is noted here.

A number of drugs, prescribed medicinally or used for agricultural and domestic purposes, have been found to be carcinogenic in laboratory animals. The range of these substances is growing and an indication of this may be seen from the selected list in *Table XI.*

* Warburg, O. (1930), *Metabolism of Tumours.* (Trans. by Dickens, F.) London: Constable.

† Warburg, O. (1956), *The Origin of Cancer Cells.* (Trans. by Burk, D.) *Science*, **123**, 309.

‡ Warburg, O. (1923), 'Versuche an Uberlebendem Carcinomgewebe', *Biochem. Z.*, **142**, 317.

§ Berenblum, I. (1940), 'Metabolism of Normal and Neoplastic Skin Epithelium', *Am. J. Cancer*, **38**, 367.

‖ Hieger, I. (1961), *Carcinogenesis.* London: Academic Press.

CHAPTER XIV

HORMONES AND CANCER. ENDOCRINE GLAND TARGETS

THE HORMONAL SYSTEM

The hormonal system of the body possesses receptor and effector mechanisms. The major primary receptor centre for stimuli from endogenous and exogenous sources, and transmitted by humeral and nervous pathways, probably resides in the hypothalamus. Its responses are transmitted via a portal system mainly to the pituitary gland, which reacts by secreting one or more of a series of hormones that act either directly upon an effector mechanism, or do so indirectly via another endocrine organ and its secretion (*Fig.* 16).

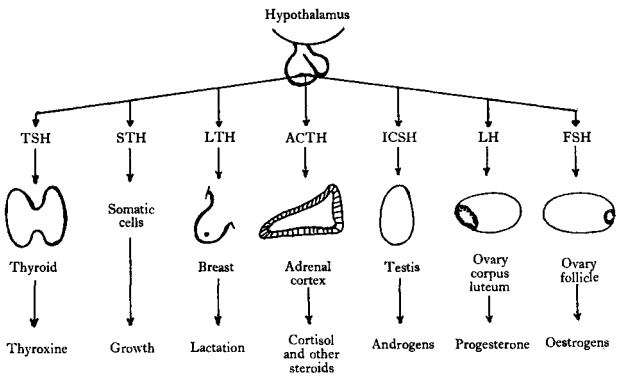

Fig. 16.—Anterior pituitary hormones.

The pituitary secretes hormones which regulate the hormonal activity of the ovaries (by FSH, follicle-stimulating hormone, and LH, luteinizing hormone), testes (by LH, or ICSH which is the same hormone and represents interstitial cell-stimulating hormone), adrenal cortices (by ACTH, adrenocorticotrophic hormone), and the thyroid (by TSH, thyroid-stimulating hormone). The main influence regulating the activity of the pituitary in regard to these hormones is the secretion of the target organs themselves, with the result that a reciprocal harmonizing mechanism, or feed-back mechanism as it is often called, maintains homeostasis or meets the demands of various stimuli (*Fig.* 17). An illustrative example is the regulation of the secretion of thyroxine.

The Hormonal System, *continued.*

Circulating thyroxine suppresses pituitary secretion of thyrotrophic (thyroid-stimulating) hormone, TSH. Lack of circulating thyroxine stimulates the production of TSH which has the function of activating the thyroid gland and thus bringing about an increased secretion of thyroxine. This feed-back mechanism of the TSH-thyroxine axis is only partly autonomous; in addition to the influence of the hypothalamus, other hormones may have an effect.

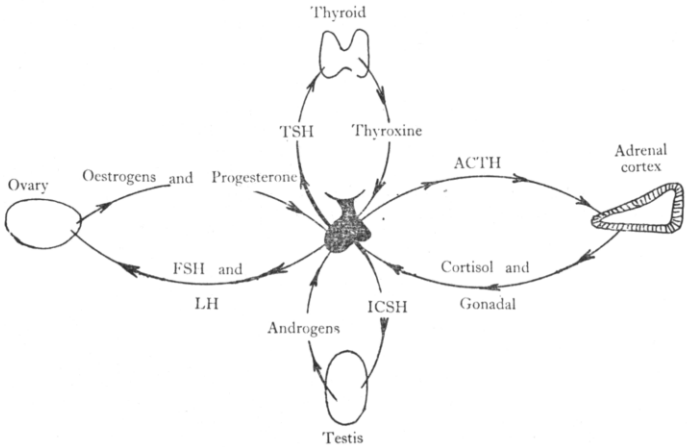

Fig. 17.—The pituitary axis and the feed-back mechanisms.

The hormonal system includes a variety of pathways of action. Some anterior pituitary (adenohypophysis) secretions act directly upon target organs: prolactin or luteotrophic (LTH) hormone acts directly on breast tissues, stimulating breast development and milk secretion; and growth or somatotrophic hormone (STH) affects all somatic cells. There are other autonomous endocrine glands, some with and some without a feed-back system. Those that are related to tumour genesis and behaviour are discussed in this chapter.

Many basic concepts of the mode and route of hormonal influence upon, or the induction of, tumours have been learnt from animal experiments. The following is an outline of some of the main associations between hormones and tumours.

Direct Tumour Formation.—Pituitary-thyroid interaction serves to exemplify a fundamentally important mode of tumour formation in endocrine glands. If thyroxine secretion were suppressed, as by the continued presence of a goitrogenic substance, the deficiency of circulating thyroxine would lead to increased TSH production; TSH would activate thyroid epithelium, and would continue to do so because the production of thyroxine was blocked. Continued

stimulation of the thyroid gland leads to hyperplasia and hypertrophy, then, under suitable circumstances, to benign tumours, which may remain hormone-dependent for a variable period, and then finally to carcinoma. Reciprocally, chronic thyroid hormone deficiency, by stimulating persistent secretory activity of the pituitary, may induce tumour formation of its thyrotrophic elements.

Indirect Tumour Formation.—Hormonal tumour induction may progress along channels more complex than the direct action of one hormone upon its reciprocal partner. The target organ may be affected indirectly via the intervention of an intermediate endocrine secretion. An instance occurs in a pituitary-ovarian-uterine chain: continued secretion of ovarian oestrogens induces endometrial hyperplastic changes, followed in some by polypoid adenomatous growths and carcinoma. The ovarian secretory imbalance may itself be due to persistent pituitary gonadotrophic hormone; so that the cause of the uterine cancer is initially due to gonadotrophins acting upon the ovaries.

Similar mediating hormonal activity may be surmised from experiments in which a hormone induces a tumour in a target organ only when the pituitary is intact; hypophysectomy prevents the formation of the particular lesion notwithstanding the exact duplication of all other factors in the experiment.

Conditional or Dependent Tumours.—Many tumours induced by hormones, and affecting endocrine organs or other tissues, are temporarily or permanently conditional upon the continued imbalance of the hormonal milieu. Breast tumours in mice may regress on withdrawal of oestrogens, and reappear when the stimulus is reapplied.

Similarly, when antagonistic hormones are administered, the tumour may respond by receding or disappearing. In this manner, a tumour usually occasioned by oestrogen may be prevented from occurring, or induced to retreat once it has formed, by the exhibition of progesterone.

The concept of progression, framed by Foulds* (*see* Chapter X, p. 113), involves the dependence of the tumour, during the stage of responsiveness, upon oestrogens.

Permissive Tumours.—Another group of tumours associated with hormones is known as 'permissive'. In this group the hormone is not an inducing agent, directly or indirectly, but exercises an influence upon a tumour formed from non-hormonal causes. Azo-dye carcinogenesis in the liver is prevented by hypophysectomy, and then restored by administration of ACTH. This manner of action is akin to cocarcinogenesis.

Synergism and Activation.—In some strains of mice, tumours induced by oestrogens become transmissible by cell-free extracts (*see* Chapters XVIII and XIX). The inference is that the viruses have

* Foulds, L. (1949), 'Mammary Tumours in Hybrid Mice: Growth and Progression of Spontaneous Tumours', *Br. J. Cancer*, **3**, 345.

Synergism and Activation, *continued.*

been latent, and that the hormone has either provoked their activity or deleted a factor controlling viral action. In like manner, this mechanism of hormonal oncogenesis may be associated with chemical or ionizing radiation agents.

ANTERIOR PITUITARY TUMOURS

Thyrotrophic Tumours occur in mice and rats following persistent decrease of thyroid hormone secretion. After the discovery by Chesney and others* of an endemic 'cabbage goitre' in rabbits (*see below* under THYROID TUMOURS), experiments showed that continued insufficiency of thyroid hormone due to goitre gave rise to a hypertrophic 'tumour' of the anterior pituitary. This was similar to the lesions found in thyroidectomized animals (Furth and others†), and also in human cases of cretinism and some goitres. Gorbman‡ reported pituitary tumours following depression of thyroid secretion by irradiation with I^{131}. In addition to cabbage, which proved to be goitrogenic in rabbits, other goitrogens, discovered by Hercus and Purves§ among others, and diets deficient in iodine (Bielschowsky‖), all of which depressed thyroxine, gave rise to pituitary thyrotrophic hyperplasia.

Further aspects of the intimate reciprocal relationship between pituitary TSH-secreting tumours and thyroid hormone were described by Furth and others.¶ Growth of transplants of such tumours was initially prevented in animals with functioning thyroid tissue, but took and grew on thyroidectomized animals. At this stage, the pituitary tumours were responsive, regressing in the presence of thyroxine. At a later stage, after passing through some generations of athyroid mice, the tumours could be grown in mice with functioning thyroids, i.e., the tumour had become independent.

It is of considerable moment to note that pituitary tumours arising as a result of diminution of circulating thyroxine do not secrete TSH only but a variety of trophins (Gorbman‡).

The view that TSH-secreting tumours arise from a breakdown of the feed-back mechanism is strongly supported by the evidence; as

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* Chesney, A. M., Clawson, T. A., and Webster, B. (1928), 'Epidemic Goitre in Rabbits. I. Incidence and Characteristics', *Bull. Johns Hopkins Hosp.*, **43**, 261.

† Furth, J., Dent, J. N., Burnett, W. T., jun., and Gadsden, E. L. (1955), 'The Mechanism of Induction and Characteristics of Pituitary Tumors induced by Thyroidectomy', *J. clin. Endocrin.*, **15**, 81.

‡ Gorbman, A. (1949), 'Tumorous Growths in the Pituitary and Trachea following Radiotoxic Doses of I^{131}', *Proc. Soc. exp. Biol. Med.*, **71**, 237; and (1956), 'Pituitary Tumors in Rodents following Changes in Thyroid Function: a Review', *Cancer Res.*, **16**, 99.

§ Hercus, G. E., and Purves, H. D. (1936), 'Studies on Endemic and Experimental Goitre', *J. Hyg., Camb.*, **36**, 182.

‖ Bielschowsky, F. (1953), 'Chronic Iodine Deficiency as a Cause of Neoplasia in Thyroid and Pituitary of Aged Rats', *Br. J. Cancer*, **7**, 203.

¶ Furth, J., Burnett, W. T., Gadsden, E. L., and Dent, J. N. (1952), 'Conditions of Transplantation and Hormonal Secretions of Pituitary Tumors induced by I^{131}', *Cancer Res.*, **12**, 263.

a complement to this thesis, Bielschowsky* has added the idea that the tumour is a compensatory reaction to the deficiency of thyroxine.

Adrenocorticotrophic Tumours.—The status of the 'basophil adenoma', at one time considered to be the essential cause of 'Cushing's syndrome', is now uncertain, because the syndrome is associated with lesions of the adrenal cortex, and it often occurs in the absence of a pituitary tumour. However, the coexistent pituitary adenoma in many cases of adrenocortical dysfunction indicates the existence of a reciprocally acting axis between the anterior pituitary and the adrenocortical glands.

Furth† recorded that total irradiation of mice induced ACTH-secreting pituitary tumours. Normal mice accepted transplants of these tumours and exhibited effects, such as polyuria and atrophy of lymphoid tissues. These effects were abolished by adrenalectomy, which at the same time promoted more rapid progress of the transplant. The inferences are, firstly, that the pituitary tumour stimulated excess adrenocortical secretions, so causing the clinical effects; and, secondly, that adrenalectomy removed a restraining influence upon tumour cell growth.

Gonadotrophic Tumours.—In 1936, Cramer and Horning,‡ and Zondek,§ among others, reported enlargement and tumorous lesions of the anterior lobe of the pituitary gland following prolonged oestrogen administration. The concept of an hypophysial-ovarian relationship, in which the normal rhythmic alternation of oestrogen and progesterone stimulates a corresponding rhythmic secretion of FSH and LH, provides a possible explanation of the phenomenon.

Bielschowsky and Horning‖ record pituitary gonadotrophic adenoma, with ICSH-secretory properties, following experimental cryptorchidism; the changes in the adenohypophysis resembling those following gonadectomy.

A further example of oestrogenic stimulation of pituitary tumour formation is that following adrenocortical tumours arising from gonadectomy in certain strains of mice at an early age. It is discussed below under the heading of mammotrophic tumours.

Somatotrophic Tumours.—Pituitary STH has a broad target; apart from special effects upon epiphysial cartilage, it affects virtually all somatic cells. Eosinophil adenoma with excess STH causes acromegaly in adult man and gigantism if it begins earlier in life. The extreme rarity of growth hormone-secreting tumours in experimental animals explains the relative lack of knowledge about its control and mechanism of action.

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* Bielschowsky, F. (1955), 'Neoplasia and Internal Environment', *Br. J. Cancer*, **9**, 80.

† Furth, J. (1955), 'Experimental Pituitary Tumors', in *Recent Prog. Horm. Res.*, **2**, 221.

‡ Cramer, W., and Horning, E. S. (1936), 'The Effect of Oestrin on the Pituitary Gland', *Lancet*, **1**, 1056.

§ Zondek, B. (1936), 'Tumour of the Pituitary induced with Follicular Hormone', *Ibid.*, **1**, 776.

‖ Bielschowsky, F., and Horning, E. S. (1958), 'Aspects of Endocrine Carcinogenesis', *Br. med. Bull.*, **14**, 106.

Mammotrophic Tumours.—A complex set of factors is probably involved in tumour induction by LTH or prolactin-secreting tumours. Woolley and others* established that in mice of certain strains, ovariectomy shortly after birth gave rise within a few months to adrenocortical adenomatous tumours. Hypophysectomy prevented their development, proving their dependence upon pituitary secretion. However, dependence gave way to autonomy, as was shown by Woolley and Little,† who reported the advent of carcinoma after about 1 year of the existence of adenoma. The production of excess oestrogen by these adrenocortical lesions is followed by hypophysial tumours and adenocarcinomas of the developing breast. These hypophysial tumours are the same as those obtained by the exhibition of oestrogen (*see above* under GONADOTROPHIC TUMOURS).

The suggestion that the pituitary tumours so formed secrete increased prolactin finds support in the work of Bielschowsky.‡ He showed that in the presence of acidophilic pituitary adenoma, hyperplastic secreting mammary tissue coexisted with an atrophic uterus and vagina, thus demonstrating the absence of oestrogenic stimulation and the positive effect of LTH. Further proof that LTH-secreting tumours are induced by oestrogens came from the transplantation experiments of Furth and his associates in 1956.§ Pituitary tumours arising in mice submitted to whole-body irradiation, and those arising in rats submitted to stilboestrol stimulation and becoming autonomous after successive transplants, both exhibited prolactin secretion, as was attested by their mammary effects.

THYROID TUMOURS

The observation by Chesney and others‖ that rabbits, on a diet consisting mainly of cabbage, developed goitres, led to the discovery of a goitrogenic substance in seeds of the Crucifer family, of which cabbage is a member. Another member, rape seed, was extensively used in the laboratory for the production of goitres. The active principle of rape seed is probably a derivative of urea, the goitrogenic properties of which were reported by Kennedy.¶ An extensive experimental field was opened which showed that in rats and mice, almost every cause of chronic hypothyroidism or blockage of thyroxine manufacture or

* Woolley, G. W., Fekete, E., and Little, C. C. (1939), 'Mammary Tumor Development in Mice ovariectomized at Birth', *Proc. natn. Acad. Sci., U.S.A.*, **25**, 277.

† Woolley, G. W., and Little, C. C. (1945), 'The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain. III. Observations on Adrenal Glands and Accessory Sex Organs in Mice 13 to 24 Months of Age', *Cancer Res.*, **5**, 321.

‡ Bielschowsky, F. (1954), 'Functional Acidophilic Tumours of Pituitary of Rat', *Br. J. Cancer*, **8**, 154.

§ Furth, J., Clifton, K. H., Gadsden, E. L., and Buffett, R. F. (1956), 'Dependent and Autonomous Mammotropic Pituitary Tumors in Rats; their Somatotrophic Features', *Cancer Res.*, **16**, 608; and Furth, J., Gadsden, E. L., Clifton, K. H., and Anderson, E. (1956), 'Autonomous Mammotropic Pituitary Tumors in Mice. Their Somatotropic Features and Responsiveness to Estrogens', *Ibid.*, **16**, 600.

‖ Chesney, A. M., Clawson, T. A., and Webster, B. (1928), 'Epidemic Goitre in Rabbits. I. Incidence and Characteristics', *Bull. Johns Hopkins Hosp.*, **43**, 261.

¶ Kennedy, T. H. (1942), 'Thioureas as Goitrogenic Substances', *Nature, Lond.*, **150**, 233.

secretion, produced thyroid lesions which culminated in neoplastic tumours. Drugs like thiourea, ionizing radiation, surgical extirpation, and iodine-deficient diets, all brought about a reduction of circulating thyroxine. The effect of this was to stimulate TSH secretion, and, by continuing to do so, caused lesions of the adenohypophysis. These are discussed above, under ANTERIOR PITUITARY TUMOURS, where relevant detailed references are noted. The persistent TSH induces thyroid hyperplasia, followed by benign adenoma and ultimate carcinoma, thus completing the cycle of reciprocal thyroid-pituitary-thyroid effects.

There is suggestive evidence that a similar mechanism operates in the formation of a proportion of thyroid tumours in man. The fact that cancer of the thyroid has a substantially higher prevalence rate in regions where goitres are more common than in other areas has been amply demonstrated in the statistical analyses of Segi and Kurihara.* In Switzerland, Austria, Denmark, and Sweden, the rates are high, whereas in Japan, the rates are much lower. These findings are reinforced by many others published during a period of nearly fifty years, e.g., Bérard and Dunet† found that 2·5–4 per cent of all malignant tumours comprised those affecting the thyroid in goitre areas, compared to a relative incidence of less than 0·5 per cent in other areas. Another aetiological factor adding weight to the evidence of an association between human thyroid cancer and goitre is the higher incidence of cancer in females, by about 3 : 1 as compared to males, and in correlation with the relative sex incidence of goitre. Most of these cancers in goitres occur in association with non-toxic 'epidemic' hypothyroid goitres. The inference is that deficiency of iodine in the diet, which is the common cause of endemic goitre, leads to insufficient thyroxine production. This activates TSH, which ultimately induces tumour formation in the thyroid.

An interesting comparison of mortality-rates from thyroid carcinoma in England and the U.S.A. has been noted by Campbell and others.‡ Below the age of 25 years, the rate in the U.S.A. is markedly higher than it is in England. Above this age, up to 35 years, the difference becomes progressively smaller, until after 35 years, there is virtual equality. The authors point out that thyroid cancers at young ages are often attributable to irradiation therapy to the neck in infancy (a subject discussed in Chapter XI, p. 115). They suggest that, as there is the same mortality after the fourth decade of life and therefore probably no major difference in aetiological factors during adult life, the discrepancy in mortality at young ages may be traceable to the more extensive use in the U.S.A. of radiotherapy in infancy and childhood. Irradiation of the thyroid destroys secreting epithelium, and the younger the cells, the greater the destruction. It would appear, therefore, that the consequent thyroxine deficiency with its sequel of excessive, persistent TSH, gives rise to the thyroid cancer in these young people.

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* Segi, M., and Kurihara, M. (1962), *Cancer Mortality for Selected Sites in 24 Countries (1958–1959)*. No. 2. Department of Public Health, Tohoku University School of Medicine. Japan: Sendai.

† Bérard, L., and Dunet, C. (1921), 'Le Goitre Metastatique Existe-t-il?', *Rev. Chir.*, **59**, 521.

‡ Campbell, H., Doll, W. R. S., and Letchner, J. (1963), 'The Incidence of Thyroid Cancer in England and Wales', *Br. med. J.*, **2**, 1370.

Thyroid Tumours, *continued.*

Corroboration of the place of ionizing radiation as a causative agent in thyroid cancer appears in a study of the prevalence of the condition among those exposed in 1945 to the atomic explosions at Nagasaki and Hiroshima. Socolow and others* record that carcinoma of the thyroid occurred in a younger age-group, at an average of 20 years earlier than that seen in the unaffected population of the same area, and also that its prevalence was significantly higher than in the non-irradiated population. The study further revealed that those more heavily irradiated showed a higher prevalence rate.

Prophylactic and therapeutic measures provide further testimony supporting the thesis that thyroid cancer is hormone induced. Where the incidence of goitre has been lowered by adding iodine to the diet, the cancer rate has also been lessened. Dunhill† recorded partial regression of thyroid tumours on the exhibition of thyroid extract. Crile‡ also treated thyroid cancer by desiccated thyroid; and in 1957, he described some cases of thyroid carcinoma that were partially hormone-dependent. The lack or cessation of administration of thyroid, permitting a residual hypothyroid condition, may result in an increase in the rapidity of progress of the cancer.

OVARIAN TUMOURS

A series of experiments elegantly demonstrates the feed-back relationship between the ovary and the anterior pituitary (*Fig.* 18). Furth and Butterworth§ showed that irradiation destruction of ovarian oestrogen-production in prepubertal mice led to persistent overproduction of pituitary gonadotrophin in adult life with consequent ovarian tumour formation. This was followed by the work of Golden and Sveringhaus,‖ who proved that oestrone was inactivated by the liver. They transplanted a residual ovary into the spleen, so that hormones secreted by it were drained via portal veins to the liver; inactivation by the liver was manifest in the development of classical castration effects upon the uterus and vagina as well as upon the anterior pituitary, notwithstanding the continued viability and functional activity of the transplanted ovary. The next stage in the experimental series came in 1944 with the work of Biskind and Biskind,¶ who showed that when the transplanted ovary was maintained in the spleen for more than 10 months, it became the

* Socolow, E. L., Hashizume, S., Neriishi, S., and Niitani, R. (1963), 'Thyroid Carcinoma in Man after Exposure to Ionizing Radiation: Summary of Findings in Hiroshima and Nagasaki', *New Engl. J. Med.*, **268**, 406.

† Dunhill, T. (1937), 'The Surgery of the Thyroid Gland', *Trans. med. Soc. Lond.*, **30**, 234.

‡ Crile, G. (1955), 'Treatment of Cancer of the Thyroid with Desiccated Thyroid' *Cleveland Clin. Q.*, **22**, 161; and (1957), 'The Endocrine Dependency of Certain Thyroid Cancers and the Danger that Hypothyroidism may stimulate their Growth', *Cancer*, **10**, 1119.

§ Furth, J., and Butterworth, J. S. (1936), 'Neoplastic Diseases occurring among Mice subjected to General Irradiation with X-rays. II. Ovarian Tumors and Associated Lesions', *Am. J. Cancer*, **28**, 66.

‖ Golden, J. B., and Sveringhaus, E. L. (1938), 'Inactivation of Estrogenic Hormone of the Ovary by the Liver', *Proc. Soc. exp. Biol. Med.*, **39**, 261.

¶ Biskind, M. S., and Biskind, G. R. (1944), 'Development of Tumors in the Rat Ovary after Transplantation in the Spleen', *Ibid.*, **55**, 176.

seat of hypertrophy and tumour formation. Li* reported this occurrence in an ovary transplanted into the spleen of a male mouse.

The rationale is that the anterior pituitary reacts to the absence of circulating oestrogens by persistent hypersecretion of gonadotrophic hormone; this affects the viable but transplanted ovary, first stimulating normal follicular and corpus luteal formation, then hypertrophy and adenomatous nodules, and finally, in some cases, cancerous tumours.

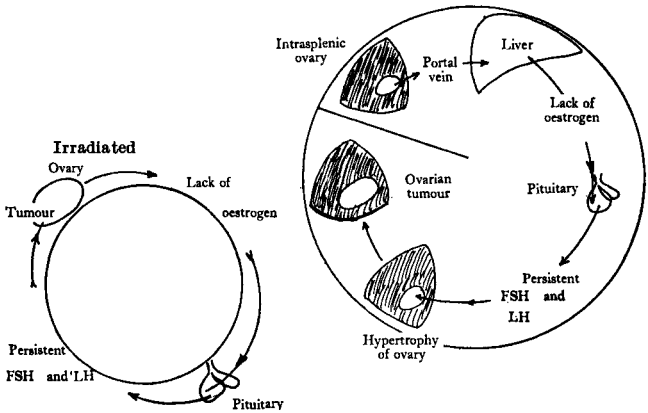

Fig. 18.—Hormonal induction of ovarian tumours.

The feed-back mechanism as an explanation of the phenomenon is supported by the absence of hyperplasia in an ovary transplanted into the spleen of an hypophysectomized castrate (Biskind and others†); i.e., proving the necessity of pituitary hormones for the morphological changes in the ovary. The evidence was further augmented some years later by Biskind and others‡ when they showed that the administration of exogenous gonadotrophins accelerated the appearance of neoplastic lesions in the intrasplenic ovary.

TESTICULAR TUMOURS

The suggestion by Burrows and Horning§ that the raised incidence of cancer of the undescended testis in man is due to a breakdown in the reciprocal testicular androgen-pituitary ICSH mechanism, is noted in

* Li, M. H. (1948), 'Malignant Granulosa-cell Tumor in an Intrasplenic Ovarian Graft in a Castrated Male Mouse', *Am. J. Obstet. Gynec.*, **55**, 316.

† Biskind, G. R., Pencharz, R., and Biskind, M. S. (1948), 'The Pathogenesis of Intrasplenic Ovarian Tumors in Rats with a Note on the Effect of Hypophysectomy and Unilateral Castration', *Acta Un. int. Cancr.*, **6**, 97.

‡ Biskind, G. R., Bernstein, D. E., and Gospe, S. M. (1953), 'The Effect of Exogenous Gonadotrophins on the Development of Experimental Ovarian Tumors in the Rat', *Cancer Res.*, **13**, 216.

§ Burrows, H., and Horning, E. S. (1952), *Oestrogens and Neoplasia.* Springfield, Ill.: Thomas.

Testicular Tumours, *continued.*

Chapter X, p. 107 *et seq.* The experimental background and clinical investigations, indicating environmental temperature as the crucial factor reducing testicular function, are also discussed in the same chapter. In the context of the present chapter, it is necessary to add other experimental and clinical evidence bearing on endocrine influences in testicular tumours.

Natural or synthetic oestrogen in mice gives rise to testicular interstitial-cell tumours (Burrows,* and Bonser and Robson†). The appearance of interstitial- or Leydig-cell tumours is associated with suppression of seminiferous cells and atrophy of the tubules, i.e., a castration-like effect due to the oestrogen. The oestrogen probably also stimulates production of anterior pituitary ICSH, which acts upon interstitial cells. The tumour induction is highly selective, probably on the basis of genetic susceptibility, as it does not take place in other species under the same circumstances. However, the administration of gonadotrophin can produce Leydig-cell tumours in the rat, and tumours in the transplanted intrasplenic rat testis may also be produced when unopposed secretion of gonadotrophin is experimentally arranged (Biskind and Biskind‡).

In man, interstitial-cell tumours are among the least common of the testicular tumours, accounting for 1·4 per cent (Collins and Pugh§). Leydig cells secrete steroids including androgenic hormones, which are regulated by a feed-back mechanism with pituitary ICSH. Hyperplasia and neoplasia of these interstitial cells, when they do occur, usually follow absence of maturation of seminiferous epithelium, atrophy due to injury, irradiation, or senile changes. It is to be noted that although hyperplasia of Leydig cells is a common feature of undescended atrophied testes, there is no special proclivity for these hyperplastic cells to become malignant (Collins and Cameron‖). In fact, seminomas and teratomas are the common types of tumours affecting cryptorchids.

Assays of urinary hormonal content in testicular tumours in man, as reported by Symington and Wallace,¶ show significant results only in regard to gonadotrophins, which were raised in seminomas and teratomas, and particularly so in the latter. The authors discuss the possible reasons for this raised level; it might be due to increased pituitary gonadotrophic secretion resulting from some feed-back mechanism, or it may be secreted by the tumour itself. More extensive investigations will be required to answer the problems.

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* Burrows, H. (1935), 'Changes induced in the Interstitial Tissue of the Testis of the Mouse by Certain Oestrogens', *J. Path. Bact.* **41**, 218.

† Bonser, G. M., and Robson, J. M. (1940), 'The Effects of Prolonged Oestrogen Administration upon Male Mice of Various Strains: Development of Testicular Tumours in the Strong A Strain', *Ibid.*, **51**, 9.

‡ Biskind, M. S., and Biskind, G. R. (1945), 'Tumor of the Rat Testis produced by Heterotransplantation of Infantile Testis to Spleen of Adult Castrate', *Proc. Soc. exp. Biol. Med.*, **59**, 4.

§ Collins, D. H., and Pugh, R. C. B. (1964), 'Classification and Frequency of Testicular Tumours', in *The Pathology of Testicular Tumours.* Edinburgh: Livingstone.

‖ Collins, D. H., and Cameron, K. M. (1964), 'Interstitial-cell Tumour', in *The Pathology of Testicular Tumours.* Edinburgh: Livingstone.

¶ Symington, T., and Wallace, N. (1964), 'Hormone Investigations in Cases of Testicular Tumours', in *The Pathology of Testicular Tumours.* Edinburgh: Livingstone.

ADRENAL CORTICAL TUMOURS

Adrenocortical tumours following gonadectomy in male and female mice have been reported by Woolley and Little.* They arise initially as a compensatory hyperplasia, replacing the loss of ovarian oestrogenic or testicular androgenic secretion.

Hypophysectomy, according to Ferguson and Visscher,† prevents the formation of the tumour. This evidence together with that concerning the advent of adenohypophysial tumours some months after the adrenocortical adenomas have appeared, suggests the operation of a feed-back mechanism: probably consisting of gonadectomy—stimulation of pituitary gonadotrophins—stimulation of adrenal cortex to hyperplasia and then neoplasia. It does not appear that ACTH plays a part in this cycle (Bielschowsky and Horning‡).

* Woolley, G. W., and Little, C. C. (1945), 'The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain. III. Observations on Adrenal Glands and Accessory Sex Organs in Mice 13 to 24 Months of Age', *Cancer Res.*, **5**, 321; and (1945), 'The Incidence of Adrenal Cortical Carcinoma in Male Mice of Extreme Dilution Strain over 1 Year of Age', *Ibid.*, **5**, 506.

† Ferguson, D. J., and Visscher, M. B. (1953), 'The Effect of Hypophysectomy on the Development of Adrenal Tumours in C3H Mice', *Ibid.*, **13**, 405.

‡ Bielschowsky, F., and Horning, E. S. (1958), 'Aspects of Endocrine Carcinogenesis', *Br. med. Bull.*, **14**, 106.

CHAPTER XV

HORMONES AND MAMMARY CANCER

ENDOCRINE PHYSIOLOGY OF THE BREAST

Ovarian Hormonal Influence on Mammary Development.—In most animals, oestrogen and progesterone are necessary for normal development of the mamma. Initial development and differentiation in the female are, in the main, effects of oestrogen. The changes are characterized by growth and branching of ducts, the epithelial-cell lining of which hypertrophies; the epithelial increase extends to lobular terminations, which become enlarged; and there is marked proliferation of fat and fibrous tissue in the stroma. In the human female adolescent breast, the changes are essentially the same except for the absence of lobule formation, which requires corpus luteum hormone, i.e., progesterone.

To a lesser degree, oestrogen stimulates breast development in males and it seems that androgens do the same in smaller measure and in varying degree in different species including man.

Corpus luteum in human females is a feature of sexual maturity; it follows ovulation and persists during the second half of the menstrual cycle. Its production of progesterone thus alternates with high-level oestrogen, produced mainly by the Graafian follicle during the first half of the cycle, and also to a lesser extent by the corpus luteum. Progesterone stimulates the development of lobules in the breast during the premenstruum. Such lobular growth is marked in pregnancy, when persistence of corpus luteum with continuous secretion of progesterone, plus the major contribution of this hormone by the placenta, are the responsible factors.

Anterior Pituitary Influence.—The production of oestrogen and progesterone by the ovaries is under the control of pituitary FSH and LH respectively. These gonadotrophins do not act directly upon the breast, but, in stimulating the production of ovarian hormones, they exercise an indirect effect. In addition, the anterior pituitary has another role in relation to the effects of ovarian hormones: in the female castrate, administration of oestrogen alone or in combination with progesterone affects the mammary gland only in the presence of an intact hypophysis; leading to the inference that some hypophysial secretion is a necessary part of ovarian hormonal influence.

The anterior pituitary secretes prolactin or LTH, which has a mammogenic action, stimulating growth of alveolar epithelium, and a lactogenic action. In an ovariectomized and hypophysectomized rat, the combination of hormones, oestrogen, progesterone, and prolactin, does induce lobule-alveolar development, but not to the same extent as oestrogen plus progesterone to the ovariectomized

but hypophysis-present animal. Another pituitary hormone, namely growth hormone or STH, is required for full development.

Adrenocortical Influences.—It appears likely that oestrogens and androgens produced by the adrenal cortex are the only hormones from this endocrine organ affecting mammary growth and function. The evidence concerning the influence of other adrenocortical hormones is conflicting and uncertain.

Fig. 19 indicates the mammary target tissues influenced by 'direct' and 'indirect' hormones.

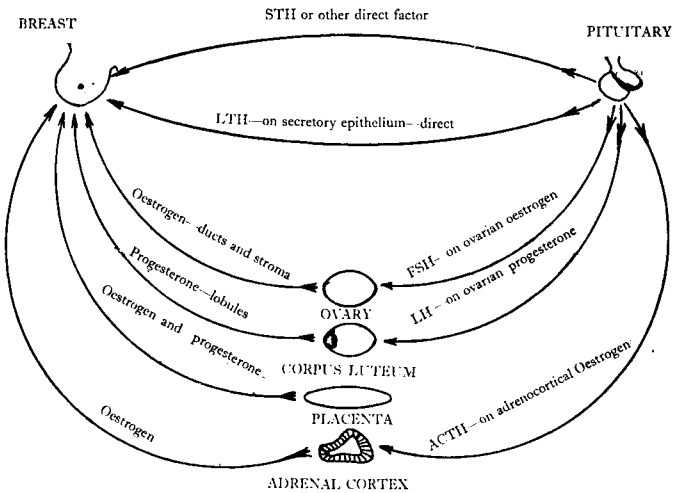

Fig. 19.—Hormonal influences on breast development.

EXPERIMENTAL FINDINGS IN BREAST TUMOURS

Ovarian hormones, oestrogen and progesterone, similar adrenocortical hormones, and adenohypophysial prolactin (LTH) and growth (STH) hormones, play roles in the induction of cancer of the breast. Other factors enter the picture, and in some species, viral, chemical, or physical factors are required as coexistent or synergistic agents for the carcinogenic action of hormones. Apart from the resulting complexity and multiplicity of pathogenic factors, it is clear that findings and deductions in one species cannot be extrapolated to another. Genetic specificities and variations even in different strains of one species exercise marked influences. Nevertheless, the biological lessons from experimental investigations are of fundamental character, and they are profoundly affecting the understanding and management of human breast cancer.

The references to be used here to mark the discoveries of experimental investigations and their important place in clarifying biological problems are severely limited. The work quoted represents but a fraction of the prodigious literature that is accumulating on the subject.

Oestrogens and Breast Cancer.—Loeb,* in 1919, reported experiments pointing to the influence of oestrogen upon tumour formation. Ovariectomy, before the age of 4 months in mice of high-breast cancer strain, prevented the development of the tumour; when the operation was done at a later age, from 4 to 7 months, the cancer rate was lowered; after 7 months, ovariectomy had no influence. The next significant advance was delayed until 1932, when Lacassagne† reported his work on oestrogens and breast cancer. In female mice of high-breast cancer strain, weekly injections of oestrogen increased the incidence and reduced the latent period of tumours, but it did not do so in mice of a low-breast cancer strain. Later, mammary cancer was also induced by prolonged oestrogen administration in male mice of the high-breast cancer strain, and, in 1938, Robson and Bonser‡ produced the tumours by using synthetic hormone.

Hormone Dependence.—Foulds§ reported studies on a certain strain of hybrid mice with a high-breast cancer incidence. In about one in three of the tumours, growth was progressive from the onset; these were 'unresponsive'. In the other two-thirds, the tumours advanced during pregnancy, but retreated, partially or completely, after parturition; these 'responsive' tumours were usually re-stimulated to growth during the next pregnancy. The degree of responsiveness varied, and, although the hormonal factors have not been defined, the inference that there is a varying grade of dependency upon the particular combination of hormones in pregnancy seems reasonable.

A concept of tumour progression based on these findings is noted in Chapter X, p. 113.

Hormone-dependence studies of breast tumours by Mühlbock,‖ using mice with and without Bittner's milk agent, led him to conclude that by the time tumours became palpable, they were independent. He confirmed the observation by Gardner¶ that hypophysectomy of animals with mammary tumours did not affect the development of tumours in the other mammary glands. Hyperplastic nodules in a premalignant stage, however, were found to be dependent.

Pullinger,** making a point of the high sensitivity of the milk agent to oestrogens, undertook tests of hormone-dependence on

* Loeb, L. (1919), 'Further Investigations on the Origin of Tumors in Mice. VI. Internal Secretion as a Factor in the Origin of Tumors', *J. med. Res.*, **40**, 477.

† Lacassagne, A. (1932), 'Apparition de Cancers de la Mamelle chez la Souris mâle, Soumise à des Injections de Folliculine', *C.r. hebd. Séanc. Acad. Sci., Paris*, **195**, 630.

‡ Robson, J. M., and Bonser, G. M. (1938), 'Production of Mammary Carcinomas in Mice in a Susceptible Strain by the Synthetic Oestrogen Triphenylethylene', *Nature, Lond.*, **142**, 846.

§ Foulds, L. (1949), 'Mammary Tumours in Hybrid Mice: Growth and Progression of Spontaneous Tumours', *Br. J. Cancer*, **3**, 345; and (1964), 'Tumour Progression and Neoplastic Development', in *Cellular Control Mechanisms and Cancer* (Ed. Emmelot, P., and Mühlbock, O.). Amsterdam: Elsevier.

‖ Mühlbock, O. (1958), 'Studies on the Hormone Dependence of Experimental Breast Tumours in Mice', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

¶ Gardner, W. U. (1942), 'Persistence and Growth of Spontaneous Mammary Tumours and Hyperplastic Nodules in Hypophysectomized Mice', *Cancer Res.*, **2**, 476.

** Pullinger, B. D. (1958), 'Hormone-dependent Tumours in Mice', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

mammary carcinomas of mice in which the milk agent had been excluded by cross-suckling. When grafts of such primary tumours into males had reached nodule formation, hypophysectomy, in two of three specimens, appeared to control the advance of the tumour.

The evidence is thus somewhat conflicting, and final judgement should be suspended.

Genetic Susceptibility.—Experiments with positive results, in terms of hormones and breast tumours, have been carried out in selectively bred strains of mice. The results in one strain are often not obtained in another, showing that specific susceptibilities play an important role. An example underlining this may be seen when ovaries are transplanted into male mice: in susceptible strains, male mice develop a significant incidence of breast cancer; in other strains, there is no such reaction. These results are repeated when male mice are given natural or synthetic oestrogens.

The definition of the ingredients composing susceptibility has been the objective of intensive investigation. Genetic constitution, association of other hormones, synergistic carcinogenesis by other agencies, chemical, viral, and physical, and various environmental influences, have all been found to have a place in the complex hormonal aetiological background of mammary cancer.

In some instances of particular strain behaviour, susceptibility is genetically determined, and the hormonal influence in these instances indicates that the hormone is so conditioned. In the strain of mice designated 'Strong A', breeding females carry a high incidence of spontaneous breast cancer, whereas virgins have a low incidence; a character which has been shown to be genetically transmitted. In this Strong A strain, pseudopregnancy by mating with vasectomized males has been found by Law,* and oestrogen administration has been shown by Gardner† to increase the incidence of mammary tumours. The suggestions emerging are that progesterone, produced in breeding females and possibly activated by pseudopregnancy or oestrogens, or some combination of both, in non-breeders, may provide the cancerigenic influence. Although the precise mechanism has not been determined, it does appear that it is some inherited hormonal factor that is involved.

Association with Hypophysis.—While oestrogens stimulate mammary hyperplasia, they do not, alone, initiate cancer (Lacassagne‡). Hypophysectomy gives rise to atrophy of the breast despite oestrogen administration. The hypophysial contribution to successful cancer induction by oestrogens is either prolactin (LTH) or growth hormone (STH), or both.

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* Law, L. W. (1941), 'Effect of Pseudopregnancy on Mammary Carcinoma Incidence in Mice of the A Stock', *Proc. Soc. exp. Biol. Med.*, **48**, 486.

† Gardner, W. U. (1938), 'Estrogens in Carcinogenesis', *Archs Path.*, **27**, 138.

‡ Lacassagne, A. (1955), 'Endocrine Factors concerned in the Genesis of Experimental Mammary Carcinoma', *J. Endocr.*, **13**, 9.

Association with Hypophysis, *continued.*

Furth and Clifton* report work which points to an anterior pituitary cell, called the mammotrope, as the *via media* by which most hormones acting on the breast exercise their influence. The main stimulus to the mammotrope is oestrogen. It seems that both radiation- and oestrogen-induced pituitary tumours are essentially the same, having mammotrophic properties which promote growth and mammary gland development.

Lyons and his colleagues† demonstrated that in rats, in whom gonadectomy and hypophysectomy were used in different combinations and with varying replacements by hormones, breast cancers were produced in the doubly operated animals only by the tetrad of hormones: oestrogen, progesterone, STH, and LTH.

Oestrogens and Adrenocortical Glands.—In some strains of mice, the usual reduction of breast cancer incidence by early ovariectomy does not occur. Gardner‡ reported that these exceptions were due to the advent of hyperplasia or neoplasia of the adrenal cortex, which replaced the oestrogen supply. Adrenocortical hyperactivity may thus substitute its oestrogenic secretion for that of the ovary.

The adrenal also possesses the potential for secretion of androgens which have been shown by Nathanson and Andervont§ and by Loeser|| to reduce the incidence of tumours in high-breast cancer strains of female mice.

Hormones and Bittner Milk Agent.—The discovery and elaboration of knowledge of the virus, Bittner's milk agent, are discussed in Chapter XVIII, p. 198 *et seq.* In this chapter it is also noted that male mice, developing breast cancer following prolonged oestrogen administration, are only from among those carrying the virus. The inference is that oestrogen activates, or releases control elements and thus frees viruses which have a carcinogenic power.

As has been mentioned above, under HORMONE DEPENDENCE, Mühlbock and Pullinger experimented with breast tumours in mice free of the milk factor. Breast carcinoma can be induced in milk agent-free mice by prolonged administration of oestrogen. It also appears (*see* following section on HORMONES AND CHEMICAL CARCINOGENS) that chemical carcinogens can replace Bittner's virus

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* Furth, J., and Clifton, K. H. (1958), 'Experimental Observations on Mammotropes and the Mammary Gland', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

† Lyons, W. R., Li, C. H., and Johnson, R. E. (1952), 'The Enhancing Effect of Somatotrophin on the Mammary Growth induced in Rats with Oestrogen, Progestin and Mammotropin', *J. clin. Endocrin.*, **12**, 937; and Lyons, W. R., Li, C. H., Cole, R. D., and Johnson, R. E. (1953), 'Some of the Hormones required by the Mammary Gland in its Development and Function', *Ibid.*, **13**, 836.

‡ Gardner, W. U. (1941), 'Estrogenic Effects of Adrenal Tumors of Ovariectomized Mice', *Cancer Res.*, **1**, 632.

§ Nathanson, I. T., and Andervont, H. B. (1939), 'Effect of Testosterone Propionate on Development and Growth of Mammary Carcinoma in Female Mice', *Proc. Soc. exp. Biol. Med.*, **40**, 421.

|| Loeser, A. A. (1941), 'Mammary Carcinoma Response to Implantation of Male Hormone and Progesterone', *Lancet*, **2**, 698.

in the sense that the additional carcinogenic influence of hormones is significantly augmented by the chemical agents. Other work has shown that X-irradiation may also act as a cocarcinogen with hormones in a similar manner. Thus, the three carcinogenic agents, Bittner's virus, chemicals, and ionizing radiation, may have their neoplastic potency enhanced by a suitable environment provided by hormonal imbalance. Some authorities (e.g., Gardner and others*) consider that this is, in essence, the fundamental cancerigenic property of hormones: they do not cause cancer directly; they modify environment, permitting or accelerating the operation of other neoplastic forces.

Hormones and Chemical Carcinogens.—In male mice treated with carcinogenic hydrocarbons, oestrogens increase the numbers of breast tumours (Dmochowski and Orr†). On the other hand, the development of tumours around subcutaneously implanted methylcholanthrene pellets is delayed by high doses of testosterone (Flaks‡).

Further examples of evidence of the influence of hormones in chemically induced breast cancer are to be found in the work of Howell,§ who reported a 75 per cent incidence of breast tumours in female rats, and none in males, following intranasal instillation of 9,10-dimethyl-1,2-benzanthracene. This author had established in 1960,|| that skin painting of rats by the same hydrocarbon induced both skin and breast tumours, but that only the breast tumour was prevented from developing by prior ovariectomy. In 1963,¶ his studies led him to conclude that the ovaries were essential throughout the whole period of carcinogenesis until the breast tumour actually appeared; thereafter ovariectomy had no effect, indicating tumour autonomy.

Haran-Ghera** reported investigations using a chemical carcinogen, methylcholanthrene (MC), a cocarcinogen, urethane, and a hormone, hypophysial implantation, in various combinations. The results in a low-breast cancer strain of mice were:—

1. MC alone failed to induce breast cancer.
2. Urethane was slightly carcinogenic.
3. Hypophysial implantation under the renal capsule induced breast cancer.

* Gardner, W. U., Pfeiffer, C. A., and Trentin, J. J. (1958), 'Hormonal Factors in Experimental Carcinogenesis', in *The Physiopathology of Cancer*. 2nd ed. (Ed. Homburger, F.). London: Cassell.

† Dmochowski, L., and Orr, J. W. (1949), 'Induction of Breast Cancer by Oestrogens and Methylcholanthrene in High and Low-breast Cancer Strain Mice', *Br. J. Cancer*, **3**, 376.

‡ Flaks, J. (1948), 'Influence of Testosterone Propionate on the Induction of Subcutaneous Tumours in Mice by 20-methylcholanthrene', *Ibid.*, **2**, 386.

§ Howell, J. S. (1961), 'Intranasal Administration of 9,10-dimethyl-1,2-benzanthracene to Rats: the Development of Breast and Lung Tumours', *Ibid.*, **15**, 263.

|| Howell, J. S. (1960), 'The Chemical Induction of Breast Tumours in the Rat: Hormonal Factors in Tumour Production', *Ibid.*, **14**, 657.

¶ Howell, J. S. (1963), 'Studies on Chemically Induced Breast Tumours in the Rat', *Acta Un. int. Cancr.*, **19**, 762.

** Haran-Ghera, N. (1963), 'The Mechanism of Carcinogenesis in Breast Tumour, Development in Mice', *Ibid.*, **19**, 765.

Hormones and Chemical Carcinogens, *continued.*

4. Hypophysial implantation plus MC or urethane resulted in augmented breast-cancer production, i.e., cocarcinogenesis.

An interesting feature is that hypophysial graft by itself, or graft followed by the chemical, produced adenocarcinomas; whereas concurrent exposure to combined agents resulted in adenoacanthomas and carcinosarcomatous tumours.

HORMONES AND HUMAN BREAST CANCER

Epidemiological Data.—The incidence of cancer of the breast is significantly higher in single than in married women, and this difference is as notable in the higher rate in childless married women as compared with women who have borne children (*see* Chapter VII, p. 77). Bogen* compared the birth-rate to breast-cancer rate and found a distinct relationship implying that a low birth-rate was accompanied by a high cancer rate. The social class in relation to breast cancer has been analysed by Stocks.† Women of higher social grading have an excess mortality-rate by comparison with those in lower grades. Social factors may explain the discrepancy; women in the higher social classes tend to marry later or have children later; they have fewer children; and they also, more frequently, either do not suckle their infants or wean them at an earlier age.

Among the possible explanations emanating from the data, deviations of hormonal balance, arising from low fertility or advanced age of childbearing, and absent or minimal breast-feeding, seem a reasonable theory. These circumstances lead to an hormonal status of relatively unopposed oestrogenic influence for protracted periods. The normal rhythmic alternation of a season of progesterone preponderance, as would be occasioned by pregnancy and lactation, is absent.

The effects of the lack of properly proportioned endocrine activity are manifest in the condition of fibro-adenosis, which, by a relationship to cancer prevalence, tends to support the contention that hormones have a role in breast-cancer causation.

Fibro-adenosis and Hormones.—In the complex of mammary conditions known as fibro-adenosis, among its many other names, and in which the cellular changes of adenosis with cyst formation, epitheliosis, fibrosis, and cellular infiltration, are found in varying combinations and proportions, Geschickter‡ has proposed a clinico-pathological subdivision into three groups. Their designations and characteristic clinical features are briefly summarized in *Table XII.*

The groups are not sharply defined or distinct entities: they not only grade into one another, but also, frequently, one condition, sufficiently dominant to be worthy of categorization, includes clinical and pathological features of another. However, despite the

* Bogen, E. (1935), 'The Cause of Breast Cancer', *Am. J. publ. Hlth,* **25**, 245.
† Stocks, P. (1963), 'The Association between Social Class and Susceptibility to Cancer', in *Cancer Progress,* 1963 (Ed. Raven, R. W.). London: Butterworths.
‡ Geschickter, C. F. (1945), *Diseases of the Breast.* Philadelphia: Lippincott.

Table XII.—Sub-groups of Fibro-adenosis

Clinical Feature	Mastodynia	Adenosis (Adenocystic)	Cystic (Solitary)
Peak age	30–39 years	35–44 years	40–50 years
Main symptom	Pain in indurated areas	Pain and tender lump	Lump. About half cases painless
Main signs	Tender, firm, granular feel between fingers	Tender, shotty nodularity between fingers. 'Saucer' sign	Distinct lump with flat hand. Solitary or several
Relation to menstrual cycle and pregnancy	Aggravation premenstrually. Improved by pregnancy	Aggravation premenstrually. Lump often disappears post-menstrually. Improved by pregnancy	Associated with menopause
Marital status and parity	Common in single women and in low-parity	Commoner in single and childless; and relatively common in low-parity	Childless and one-para women predominate
Other 'hormonal' features	Irregular menstruation. Breast-feeding difficult. Often undersized	Irregular menstrual cycles common. Breast-feeding difficult	—

Fibro-adenosis and Hormones, *continued.*

lack of border lines, the classification has proved of value in clinical and pathological correlation, particularly for comparative records of management and progress.

The pathological features of the three groups are closely associated with lobular and stromal elements. Mastodynia presents imperfect lobular formation, increase in periduct fibrous stroma, and irregular early epithelial proliferation in ductules. Adenosis is characterized by numerous small cysts, frank epitheliosis with 'spilling' mainly in lobular elements, and dense stromal fibrous tissue formation. In solitary cystic disease, involutionary changes are marked, with dense fibrous stroma replacement and progressive hyalinization affecting lobular structures, and leaving one or several large cysts at lobular or ductule terminations.

Hormonal associations are prominent, being notable in the clinical as well as the pathological pictures. The exact nature of the underlying disturbance is uncertain, but an imbalance of rhythmic hormonal change in oestrogen and progesterone interrelationship, with prolactin and growth hormone as probable participants, is strongly suggested. Persistent domination of oestrogenic influence, and absence of pituitary breast influence associated with low-level progesterone production, may be inferred as the pattern of imbalance. Geschickter quotes experimental support for the thesis that long-continued hyperoestrinism alone can bring about mastodynia and adenosis; and that unbalanced oestrogen-progesterone administration has similar effects.

Fibro-adenosis and Cancer.—An association between mammary fibro-adenosis and cancer has its advocates and opponents. The hormonal backgrounds of both lesions (*see below* for discussion of hormones and cancer) bear strong resemblances to one another. Geschickter found that the cancer incidence in breasts affected by fibro-adenosis was twice the expected number; the excess was most marked in adenosis, where it reached 7 times the rate in unaffected women. Copeland* arrives at a similar figure for his cases of adenosis.

Symington and Currie† present the contrary side of the debate. They quote the figure of 53 per cent as showing some lesion of fibro-adenosis in routine post-mortem histological examinations of the breasts of 157 women, in which there was no distinct correlation with cancer incidence. The findings reported by Sandison‡ also tend to cast doubt on the existence of a significant correlation. He stresses the variability of the picture in routine breast examinations. In about one-half of the postmenopausal breasts, there were signs of atrophy; in the other half, there were changes associated

* Copeland, M. M. (1963), 'Treatment of Mammary Dysplasia with Special Reference to Microcystic and Macrocystic Disease of the Breast', *Am. J. Surg.*, **106**, 382.

† Symington, T., and Currie, A. R. (1958), Pathology. Discussion and Summary, in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

‡ Sandison, A. T. (1958), 'A Postmortem Survey of the Adult Breast with some Observations on Gonad and Pituitary', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

with fibro-adenosis, occult carcinoma, or persisting lobular activity. The author finds no clear-cut hormonal correlation, only a very complex 'interrelationship of the endocrine system generally and the breast'.

Hormones and Breast Cancer.—In addition to the inferences emerging from epidemiological data and analogies that may be drawn from experimental findings, the effects of hormone administration and ablation of endocrine organs, as part of the treatment of human breast cancer, are important in assessing the relationship and influences of hormones in the initiation and progress of the disease.

Many hormones, singly and in combination, have been used as treatment for cancer of the breast. Because of general uncertainty of their effects and the possibility that they may worsen the cancerous lesions, their use is generally restricted to advanced cases. A survey of the status of hormone therapy by the A.M.A. Council* includes information on androgens and oestrogens which may be summarized as follows:—

ANDROGENS.—The preparations widely used were testosterone propionate and methyltestosterone. In advanced cases, both pre- and postmenopausal, there was subjective improvement in 80 per cent, and objective improvement, viz., regression of soft tissue and/or bony lesions, in 20 per cent. The mean duration of improvement was one year.

OESTROGENS.—Both synthetic and natural hormones were used. The rationale of usage is not clear; one idea was that it might disturb an hormonal environment that was unfavourable. Because of the danger of aggravation of the neoplasm, its use should be limited to cases with widespread dissemination and only after the passage of at least 3 years postmenopausally. About 40 per cent showed some improvement of symptoms; and those that did improve also had a longer survival time, 15 months as compared with the non-improvers' time of about 8 months.

Ovariectomy and Breast Cancer.—Beatson† first reported improvement in breast cancer consequent to ovariectomy; his recommendations for its use as palliation in inoperable carcinoma were not given a proper trial. Castration was revived for this purpose from time to time and with varying degrees of limited success. Adair‡ reported on irradiation and surgical extirpation of ovaries in a series of advanced cases: improvement was of short duration and occurred in about 13 to 15 per cent of cases. Treves and Finkbeiner§ reported a 37 per cent improvement in a survey of advanced cases.

* A.M.A. Council of Pharmacy and Chemistry, Subcommittee on Steroids and Cancer of the Committee on Research (1951), 'Current Status of Hormone Therapy of Advanced Mammary Cancer', *J. Am. med. Ass.*, **146**, 471.

† Beatson, G. T. (1896), 'On the Treatment of Inoperable Cases of Carcinoma of the Mamma', *Lancet*, **2**, 104 and 162.

‡ Adair, F. E. (1949), 'Surgical Problems involved in Breast Cancer', *Ann. R. Coll. Surg.*, **4**, 360.

§ Treves, N., and Finkbeiner, J. A. (1958), 'Evaluation of Therapeutic Surgical Castration in Treatment of Metastatic, Recurrent and Primary Inoperable Carcinoma in Women: Analysis of 191 Patients', *Cancer*, **11**, 421.

Ovariectomy and Breast Cancer, *continued.*

Oophorectomy has also been used as part of the treatment of the earlier stages of breast cancer. The trend of the reported results shows that this addition to the surgical treatment is of benefit. Horsley and Horsley,‡ in a series of cases submitted to standard radical procedures with concurrent surgical castration, report improved results in premenopausal and menopausal women with operable adenocarcinoma of the breast.

Bruce and Tough§ record that, in Stages I and II mammary cancer, similar local treatment consisting of simple mastectomy and X-ray therapy was followed by different survival rates in those whose ovaries were irradiated and those not so treated. Without ovarian irradiation, the survival rate was 63 per cent; with ovarian irradiation, the survival rate was 75 per cent. The authors point to the rather small numbers irradiated (52 cases) and make the reservation that their figures are therefore not conclusive.

Paterson* recorded the results of a controlled clinical trial of ovarian ablation with a 5-year follow-up, and this was extended by Cole† to a 7-year period of follow-up on the same clinical material. The trial was designed to evaluate artificially induced menopause in early breast-cancer cases which were premenopausal or within 2 years of the last menstrual flow. Apart from X-ray irradiation of the ovaries, the two randomly selected groups of cases received similar treatment—some form of standard radical mastectomy. The survival rates are tabulated in *Table XIII.*

Table XIII.—Survival Rates (per cent) after Ovarian Ablation in Mammary Carcinoma

	5-Year		7-Year	
	Ovariectomy	No ablation	Ovariectomy	No ablation
Stage I	87·8	79·0	83·1	73·8
Stage II	55·7	47·5	49·0	42·9
Overall	58·3	52·6	51·2	47·3

In more advanced cancer, viz., Stages III and IV, the 7-year records show virtually the same survival rates for the ovarian-irradiated as for the control series. Therefore, the earlier the stage at which menopause is induced, the greater the success of the treatment. Ovariectomy not only influences the survival rate, it is also beneficial in terms of the development of distant metastases: ovariectomy significantly delays their onset.

* Horsley, J. S., and Horsley, G. W. (1962), 'Twenty Years Experience with Prophylactic Bilateral Oophorectomy in Treatment of Carcinoma of Breast', *Ann. Surg.*, **155**, 939.

† Bruce, J., and Tough, I. (1964), 'Early Cancer of the Breast', *Br. J. Surg.*, **51**, 212.

‡ Paterson, R. (1962), 'Breast Cancer. A Report of Two Clinical Trials', *Jl R. Coll. Surg. Edinb.*, **7**, 243.

§ Cole, M. P. (1964), 'The Place of Radiotherapy in the Management of Early Breast Cancer. A Report of Two Clinical Trials', *Br. J. Surg.*, **51**, 216.

It is worthy of note that Huggins,* who was in the vanguard of research and applied work on the endocrinological attack on cancer, regards ovariectomy as 'a safe, sudden, and certain means of removing the ovarian component in mammary cancers. In our Clinic this operation is carried out routinely at the time of radical mastectomy in all women of menstrual age'.

The Adrenal and Breast Cancer.—The evidence for regression of breast cancer as a result of reducing oestrogen by ovariectomy, accompanied by the disappointing outcome of only partial and temporary beneficial effects, led to investigations into other sources of oestrogens. The adrenal cortex secretes androgens and oestrogens, and it was considered likely to be the major extra-ovarian source. As a consequence, the operation of bilateral adrenalectomy, made practicable by the availability of preparations of vitally necessary adrenal cortisone, has been added to ovariectomy as part of the treatment of breast cancer. Huggins and Bergenstal† were among the first to show that adrenalectomy could bring about profound changes.

Whereas ovariectomy, either by surgical removal or by X-irradiation, is often done at or about the same time as the definitive treatment of the tumour from its earliest stages, adrenalectomy has been generally reserved for the later stages with inoperable lymphatic and/or vascular dissemination. Reports show marked variations in results: some cases are apparently not affected; others show some degree of alleviation; no case has yet been cured.

It would be of obvious value to be able to forecast which case might benefit, so as to avoid an unnecessary major surgical procedure in those who do not. Cade‡ records the use of steroid hormones, either oestrogen or androgen, in cases where dissemination had occurred. Clinical aggravation might serve as an indication of hormone dependence, and therefore a probable good effect from an operation. Similarly, the effects of prednisone were tried: improvement indicating the possibility that deletion of organs supplying oestrogens might be effective. However, the tests did not confirm the theoretical expectations, and the results of surgery remained essentially unpredictable.

On the other hand, some promise of preoperative prediction is contained in the clinical investigation reported by Atkins.§ The steroid excretion by women prior to endocrine organ ablation was estimated. Postoperatively, the results of ablation were classified into successes, failures, and intermediates. This latter class was omitted, and only successes and failures were measured against the the preoperative findings of steriod excretion. A distinct pattern of correspondence was discovered: successful results of endocrine

* Huggins, C. B. (1959), 'On Hormone Dependent Cancers', *Jl R. Coll. Surg. Edinb.*, **4**, 191.

† Huggins, C., and Bergenstal, D. M. (1952), 'Inhibition of Human Mammary and Prostatic Cancers by Adrenalectomy', *Cancer Res.*, **12**, 134.

‡ Cade, Sir S. (1958), 'Adrenalectomy in Cancer of the Breast', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

§ Atkins, H. J. B. (1962), 'Preoperative Assessment of Response to the Operations of Adrenalectomy and Hypophysectomy', *Acta Un. int. Cancr.*, **18**, 885.

The Adrenal and Breast Cancer, *continued.*

organ ablation had a preoperative high level etiocholanolone relative to 17-hydroxycorticosteroid secretion, and postoperative failures were associated with the reverse relationship of steroid excretion.

The pattern of steroid excretion may provide more than a forecast of operative results. Bulbrook and others* suggest a possible link with causation; an aspect under investigation in many centres.

Cade† reports that following gonadectomy and adrenalectomy in 170 patients with disseminated lesions, 40 per cent showed no effects; in the remainder, improvement varied from disappearance of lesions to a period of quiescence in which the metastases remained *in statu quo*. The duration of improvement varied from several months to several years. In an evaluation of results, Hellström and Franksson‡ find that about 80 per cent of their patients had subjective and/or objective improvement; in some, subjective benefit coexisted with progression of the cancer. Improvement endured from 2 months in about half the cases, to 3 years in less than 7 per cent. In a number of their cases, adrenalectomy was done without oophorectomy: the results were not as good as in those submitted to both procedures.

Hyphophysectomy and Breast Cancer.—The explanations for the limited numbers and the transient nature of good results from extirpation of the organs secreting oestrogens are not known. It has been postulated that the reasons derive from the fact that gonadectomy and adrenalectomy do not necessarily remove all oestrogen-producing tissue, and that accessory adrenal tissue continues to produce steroids. Accessory adrenal gland tissues, the control exercised by anterior pituitary hormones over ovarian and adrenocortical secretions, and direct pituitary hormone influence upon breast tissues, form a triad of reasons which suggest that extirpation of the hypophysis, by itself or following oophorectomy and adrenalectomy, might be beneficial in breast cancer. *Fig.* 20 summarizes the therapeutic effects of ablation of the endocrine glands concerned with the production of oestrogen.

Luft and his colleagues§ were among the first to report results of hypophysectomy in women suffering from advanced cancer. In over 50 per cent, there was regression (decrease in metastases), or arrest (no new metastases nor progress of previous metastases).

The duration of improvement has a mean of just over 14 months. The authors make the point that their hypophysectomies were

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* Bulbrook, R. D., Hayward, J. L., and Spicer, C. C. (1962), 'Abnormal Excretion of Urinary Steroids by Women with Early Breast Cancer', *Lancet*, **2**, 1238.

† Cade, Sir S. (1958), 'Adrenalectomy in Cancer of the Breast', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

‡ Hellström, J., and Franksson, C. (1958), 'Adrenalectomy in Cancer of the Breast', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

§ Luft, R., and Olivecrona, H. (1953), 'Experiences with Hypophysectomy in Man', *J. Neurosurg.*, **10**, 301; (1955), 'Hypophysectomy in Man. Experiences in Metastatic Cancer of the Breast', *Cancer*, **8**, 261; and Luft, R., Olivecrona, H., and Sjogren, B. (1952), 'Hypophysektomie pa Mannika', *Nord. med.*, **47**, 351.

shown histologically to be incomplete in many instances, and that this may vitiate results.

Ray and Pearson* report that following surgical hypophysectomy in 109 women with metastatic spread from breast cancer there was regression in 42 and arrest in 10. In 25 women, hypophysectomy was done after castration. Seven of these, who had

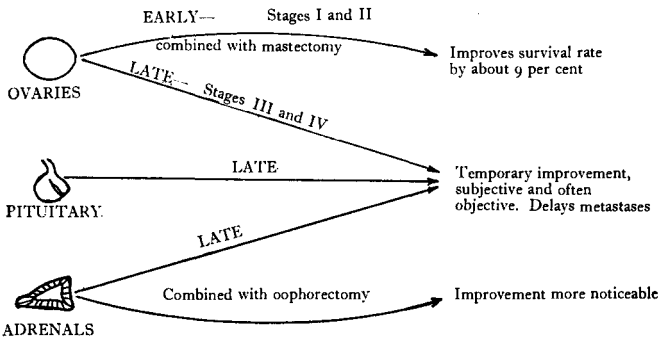

Fig. 20.—Breast cancer: therapeutic effects of ablation of oestrogen producers—following local treatment.

had temporary benefit from the first operation, derived a further period of remission from the hypophysectomy; some others were improved by the second operation after failure or undetermined effects of the ovariectomy.

Forrest,† in a symposium on advanced mammary cancer, described a method of local implantation of yttrium 90, so as to achieve an extensive, if not quite complete, ablation of the pituitary. Although this method did not accomplish better results than other ablative measures, its simplicity and safety gave it an advantage.

Atkins‡ reports studies of the comparative values of adrenalectomy and hypophysectomy. The investigation is incomplete and is, as yet, inconclusive. So far, the results of different treatments appear similar, and the author cannot go further than 'in hypophysectomy we have a therapeutic measure which is almost certainly as good as, and may be better than, adrenalectomy with oophorectomy'.

* Ray, B. S., and Pearson, O. H. (1958), 'Surgical Hypophysectomy in the Treatment of Advanced Cancer of the Breast', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

† Forrest, A. P. M. (1964), 'Symposium on Advanced Mammary Cancer', *Br. J. Surg.*, **51**, 795.

‡ Atkins, H. J. B. (1958), 'Comparisons and Results of Adrenalectomy and Hypophysectomy', in *Endocrine Aspects of Breast Cancer* (Ed. Currie, A. R.). Edinburgh: Livingstone.

CHAPTER XVI

HORMONES AND NEOPLASMS OF UTERUS

HUMAN PHYSIOLOGY

Endometrial Response to Hormonal Influence.—The histological picture of uterine endometrium is largely determined by its sensitivity to the influence of ovarian hormones. The changes may conveniently be considered in terms of preovulatory, postovulatory, and menstrual bleeding phases. *Figs.* 21, 22, and 23 show schematically the ovulatory cycle, its hormonal associations and relationships to endometrial changes.

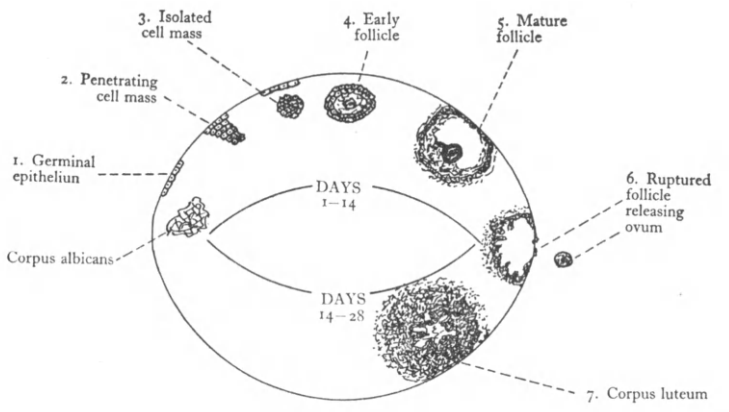

Fig. 21.—Schematic representation of ovulatory cycle.

PREOVULATION.—During this period the ovary secretes oestrogen only, and the general descriptive term of 'proliferation' is applied to its effects.

The endometrial glands are at first straight and tubular and their lining epithelium is composed of cuboidal cells. Regeneration, following loss at menstrual bleeding, progresses rapidly: the glands become increasingly tortuous; the cells become tall and columnar; and their nuclei begin to migrate part of the way towards the epithelial surface, leaving subnuclear vacuoles, which are regarded as presenting at or just after ovulation.

The stroma, at first compact with small cells and little vascularity, becomes less dense as epithelial proliferation advances.

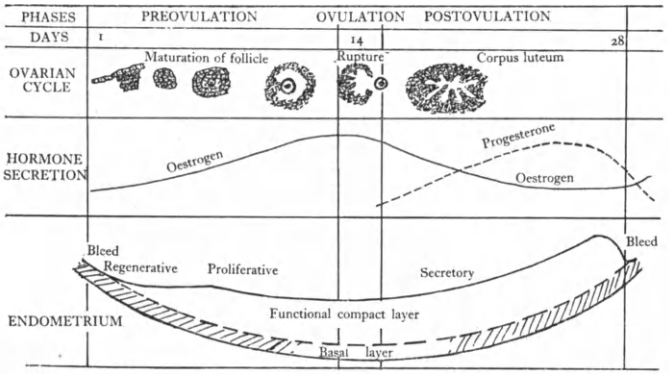

Fig. 22.—Ovulatory, hormonal, and endometrial cycles.

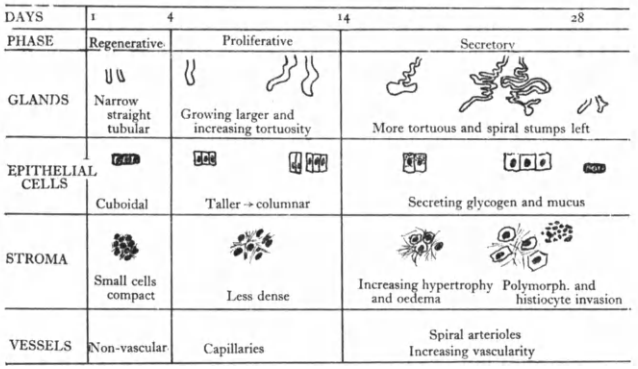

Fig. 23.—Endometrial changes in menstrual cycle.

POSTOVULATION.—The formation of a corpus luteum, which produces oestrogen and progesterone, introduces the 'secretory phase' of the cycle.

Endometrial glands undergo further convolution and tortuosity, thickening particularly into a mid spongy zone, and dividing a more compact superficial zone on one side, and a non-secretory basal zone in the depths. Glycogen and mucin are secreted into the glands.

Endometrial Response to Hormonal Influence, *continued.*

The stroma undergoes marked changes. Its cells hypertrophy, becoming polyhedral in shape; oedematous separation of cellular elements increases; and vascularity becomes richer, especially in regard to a bed of tortuous, spiral arterioles. The trend of change is towards a decidual reaction, which would flourish fully with the advent of pregnancy, but in its absence, and with the diminishing corpus luteal progesterone secretion in the premenstrual phase, the decidual trend regresses, showing degenerative changes in the superficial compact endometrial area, and leading to menstrual bleeding.

BLEEDING PHASE.—A complex of degenerative change, part constriction and part dilatation of stromal vessels giving rise to a plane of necrosis and infiltration by polymorphs and histiocytes, leads to loss of blood and desquamation of compact and spongy layers of the endometrium. The precise mechanism of hormonal influence in the bleeding phase is subject to debate, but it seems reasonably certain that the initial decline and then absence of progesterone, leaving relatively unopposed oestrogen activity, is closely associated with this phase of the cycle.

POSTMENOPAUSAL CHANGES.—Recession of oestrogen secretion at the menopause is followed by endometrial atrophy: the epithelium becomes cuboidal, glands are reduced, and the stroma undergoes fibrosis. Deviations from this usual series of changes are frequent because of the continuation of active oestrogenic influence.

PATHOLOGICAL DEVIATIONS FROM NORMAL HUMAN PATTERN

Divergencies and aberrations of endometrial patterns occur. Some are sequels of, or related to, hormonal disturbances.

Islands of endometrium may not participate concurrently with surrounding tissues. The cells may be immature, or they may persist in a proliferative, hyperplastic state and not react to progesterone. Such localized areas may heap up into polypi, and/or extend deeply into myometrium. On the other hand, abnormal continuation of progesterone may lead to sites of persistence of progestational endometrium. These abnormalities commonly give rise to irregular menstrual cycles, and they have been described by McKelvey,* McLennan,† and Novak and Woodruff‡ among others.

Endometrial Hyperplasia, commonly associated clinically with functional bleeding, is often a sequel of endocrine imbalance in which oestrogens predominate and the rhythmic influence of progesterone is reduced. The endometrium remains in a preovulation state for an unduly long period.

* McKelvey, J. L. (1942), 'Irregular Shedding of Endometrium', *Lancet*, **1**, 484.
† McLennan, C. E. (1952), 'Current Concepts of Prolonged or Irregular Menstrual Shedding', *Am. J. Obstet. Gynec.*, **64**, 988.
‡ Novak, E. R., and Woodruff, J. D. (1962), *Gynecologic and Obstetric Pathology*. Philadelphia: Saunders.

Absence of ovulation is a common underlying cause, occurring more especially at the extremes of cyclic life—menarche and menopause. The lack of development of a corpus luteum and the consequent absence of progesterone seems the obvious explanation.

Anovulation may be due to abnormal persistence of Graafian follicles because of pituitary dysfunction with relative or absolute lack of LH, or because of other hormonal disturbances, particularly hypothyroidism, or because of local ovarian pathology, e.g., cysts, hyperplasias, thecomas, and granulosa-cell tumours (Sherman and Wolff*). While the endometrium is subject to the proliferative effects of oestrogen, bleeding does not occur; when persistent high level oestrogens inhibit pituitary FSH, there may follow a sudden drop in oestrogen with uterine bleeding. If ovulation does occur, the secretory phase is provoked and menstrual bleeding follows.

Hormonal change and imbalance at menopause may well account for the frequent occurrence of endometrial hyperplasia at this time of life. Persistence of oestrogen, possibly of adrenocortical origin, and reduction or absence of corpus luteal formation can set the hormonal stage for persistent proliferative responses (Novak†).

Endometrial hyperplasia varies from a state which does not differ significantly histologically from that found normally during the proliferative phase of the cycle, to irregularity of gland and stroma patterns, gross hypertrophy and hyperplasia with polypoid excrescences on the surface, and/or deep penetration of glands through muscularis, with or without cystic dilatations.

ENDOMETRIAL CARCINOMA

Epidemiological Data.—*Table III*, p. 33, indicates that in New York State the probability of developing cancer of the body of the uterus was 1·6 per cent, i.e., about one-third of the figure for cancer of the cervix uteri. According to the next set of figures, in *Table IV*, the ratio was the same and the incidence of corpus cancer in London was 4·02 per cent of all female cancers. The peak age affected by corpus cancer is 8–10 years older than that of cervical cancer, and O'Donnell and others‡ report that in the U.S.A. over 75 per cent of cases occur between the ages of 50 to 80 years. There is an inverse relationship between corpus and cervix cancers and the marital state and parity. Whereas the cervix is involved much more commonly in married and parous women, than in single, nulliparous women (*see* Chapter VII, p. 76) the reverse is true for cancer of the body. Speert§ reported a series in which the proportions amounted to 18 per cent in single women and 41 per cent in nulliparous women.

The data suggest a possible interpretation involving hormonal derangement. The main incidence after menopause, when endocrine secretions change; and the more common occurrence in nulliparous

* Sherman, A., and Wolff, R. (1959), 'An Endocrine Basis for Endometrial Carcinoma', *Am. J. Obstet. Gynec.*, **77**, 233.

† Novak, D. (1956), 'Postmenopausal Endometrial Hyperplasia', *Ibid.*, **71**, 1312.

‡ O'Donnell, W. E., Day, E., and Venet, L. (1962), *Early Detection and Diagnosis of Cancer*. St. Louis: Mosby.

§ Speert, H. (1948), 'Corpus Cancer', *Cancer Res.*, **1**, 584.

Epidemiological Data, *continued.*

and low-parity women, implicating protracted oestrogen influence and absence of sufficient progesterone, constitute the suggestive evidences. The possibility of hormonal influence in cancer causation gains further support from the correlation of endometrial hyperplasia and cancer.

Endometrial Hyperplasia and Corpus Cancer.—The probable hormonal background of hyperplasia of the endometrium has been noted above. The range of cytological changes is such that in a small group of cases there is no definite dividing line between hyperplasia and adenocarcinoma. Novak and Woodruff* draw attention to the fact that some features usually associated with malignancy may be part of the picture of benign hyperplasia, and may not be seen in adenocarcinoma. The outstanding example is the fairly common penetration into myometrium of glandular elements in benign hyperplastic states (and named adenomyosis on account of it), as opposed to an appreciable proportion of adenocarcinomas without any evidence of break-through of the basement membrane. Novak and Woodruff put the point forcibly—'there is a whole series of gradations, a species of histologic stepping stones, between atypical hyperplasia and adenocarcinoma . . .'.

Further support for the belief that endometrial hyperplasia, particularly in postmenopausal years, may be a premalignant condition derives from: (*a*) Occasional malignant degeneration in a previously benign endometrial polyp. (*b*) While malignancy is seldom superimposed on a polyp, malignancy at another site on the endometrium, and associated with the presence of endometrial polyps, is not uncommon. Peterson and Novak† found the association in 15 per cent of adenocarcinomas in postmenopausal cases. (*c*) Speert,‡ among others, has reported a series of curettings showing endometrial hyperplasia in earlier examinations followed by ultimate adenocarcinoma. (*d*) Novak§ correlated clinical and pathological features. He found that women with postmenopausal hyperplasia and those with corpus carcinoma often have a common clinical story: delayed menopause, obesity, hypertension, diabetes, a low fertility, and evidence of previous anovulation. The correlation suggests that postmenopausal hyperplasia and adenocarcinoma are variants or stages of one pathological process, probably induced by an oestrogenic hormone. (*e*) Bilateral ovariectomy, an operation more commonly performed in the past than in recent years, is only exceptionally followed by corpus cancer. (*f*) Granulosa- and theca-cell tumours of the ovary, both of which produce excessive oestrogen, are frequently associated with endometrial hyperplasia, quite often with breast tumours, and, in 15–20 per cent (Mansell

* Novak, E. R., and Woodruff, J. D. (1962), *Gynecologic and Obstetric Pathology.* Philadelphia: Saunders.

† Peterson, W. F., and Novak, E. R. (1956), 'Endometrial Polyps', *Obstet. Gynec., N.Y.*, **8**, 40.

‡ Speert, H. (1952), 'The Premalignant Phase of Endometrial Cancer', *Cancer*, **5**, 927.

§ Novak, E. R. (1956), 'Postmenopausal Hyperplasia', *Am. J. Obstet. Gynec.*, **71**, 1312.

and Hertig*), with endometrial cancer. (*g*) Long-continued administration of oestrogen is becoming increasingly suspect as a causative influence (Gusberg and Hall†); it is noteworthy that there are examples of the effects of therapeutic oestrogen showing progressive stages, viz., cystic hyperplasia, proliferative adenomatous hyperplasia, and overt cancer.

While it is necessary to emphasize that not one piece of evidence noted above can be regarded as proof of a causative association between hormone derangement and cancer, the sum of all the factors constitutes a substantial foundation for the probable operation of hormonal carcinogenic influence.

EXPERIMENTAL WORK ON CORPUS CARCINOMA

Experimental investigation of uterine corpus carcinoma has lagged behind work in other types of cancer, and it has also trailed behind clinical and pathological observations in human patients. This is perhaps due to the infrequency of spontaneous uterine tumours in animals. Rabbits are an exception; they are particularly prone, and show strain variation, inculpating genetic susceptibility.

In the rabbit, progressive stages of evolution of adenocarcinoma from initial endometrial hyperplasia, to oestrogen-dependent adenoma and eventual autonomy, have been described by Greene and Newton.‡ Meissner and others§ described the different lesions, including adenocarcinoma, produced in the rabbit uterus by exogenous oestrogen administration. In mice, when prolonged administration of oestrogens does induce uterine neoplastic lesions, it almost always affects the cervix and not the uterine cornua (Gardner and others‖). These authors, too, point to the high incidence of uterine cancer in rabbits in association with a syndrome, apparently closely connected with hormonal upset; the syndrome consists of mammary hyperplasia and cancer, adrenal and pituitary hypertrophy, and toxaemia of pregnancy.

Another line of experimental work demonstrating the effect of excessive oestrogen influence upon uterine endometrium, and so indicating its probable carcinogenic power, is to be seen following the induction of feminizing hormone, i.e., oestrogen producing tumours of the ovary. Granulosa-cell tumours are the common and prominent examples. Furth and Butterworth¶ recorded a high incidence of such ovarian tumours in mice following whole-body X-ray irradiation, and

* Mansell, H., and Hertig, A. T. (1955), 'Granulosa-theca Cell Tumors', *Obstet. Gynec., N.Y.*, **6**, 385.

† Gusberg, S. B., and Hall, R. E. (1961), 'Precursors of Corpus Cancer. III. The Appearance of Cancer of the Endometrium in Estrogenically Conditioned Patients', *Ibid.*, **17**, 397.

‡ Greene, H. S. N., and Newton, B. L. (1948), 'Evolution of Cancer of the Uterine Fundus in the Rabbit', *Cancer*, **1**, 82.

§ Meissner, W. A., Sommers, S. C., and Sherman, G. (1957), 'Endometrial Hyperplasia, Endometrial Carcinoma and Endometriosis produced Experimentally by Estrogen', *Ibid.*, **10**, 500.

‖ Gardner, W. U., Pfeiffer, C. A., and Trentin, J. J. (1958), 'Hormonal Factors in Experimental Carcinogenesis', in *The Physiopathology of Cancer* (Ed. Homburger, F.). London: Cassell.

¶ Furth, J., and Butterworth, J. S. (1936), 'Neoplastic Diseases occurring among Mice subjected to General Irradiation with X-rays. II. Ovarian Tumors and Associated Lesions', *Am. J. Cancer*, **28**, 66.

Experimental Work on Corpus Carcinoma, *continued.*

the same tumour may also occur following intrasplenic ovarian transposition (*see* Chapter XIV, p. 152). Oestrogen production in excess is attested by many characteristic effects: increase in size of the uterus, proliferative changes in the endometrium of the castrate, cornification of the vagina, renal glomerular changes, and breast-tissue stimulation. (Progesterone effects do occur but are exceptional in granulosa-cell tumours.) The endometrial hyperplasia provides an experimental verification of the observations in similar circumstances in human subjects.

An interesting comparison arises between hormonal and chemical carcinogenesis in the rabbit uterus. Merriam and others* induced adenocarcinoma in one horn of a bicornuate uterus by the insertion of a pellet of methylcholanthrene. A series of progressive effects, comparable to the sequels to hormonal stimulation, occurred: endometrial cystic hyperplasia was followed by proliferative hyperplasia and ultimate adenocarcinoma.

EXPERIMENTAL WORK ON GUINEA-PIG UTERINE FIBROMYOMA

Lacassagne† reported fibromyomatous changes in the rabbit uterus, and Nelson,‡ using guinea-pigs, induced frank uterine fibroid tumours by long-continued oestrogen administration. Lipschütz,§ in a monograph, has given details of the very extensive experimental studies by himself and his colleagues on oestrogen fibromatogenesis in guinea-pigs. Not only were tumours induced in the uterus, but also almost everywhere in the abdominal cavity; a finding reported by Lipschütz and Iglesias.‖

The tumours varied in type, with different elements predominating: fibroids, fibromyomas, and myomas occurred. However, there were fundamental differences between these experimental tumours in guinea-pigs and fibroids in women. The most outstanding contrast was the invasive property of the tumours in the guinea-pig; direct spread involved smooth and striated muscle, the pancreas, and rarely the liver. Notwithstanding this malignant-like character, the oestrogen-induced tumours do not achieve an autonomous status, they do not 'take' on transplantation, and there are no metastases. If exogenous oestrogens are used for their production, the tumours are hormone-dependent, regressing when the hormone is withdrawn.

Progesterone, injected together with oestrogen, prevented the formation of fibroids. Similar antagonistic action occurred with testosterone

* Merriam, J. C., Easterday, C. L., McKay, D. G., and Hertig, A. J. (1960), 'Experimental Production of Endometrial Carcinoma in the Rabbit', *Obstet. Gynec., N.Y.*, **16**, 253.

† Lacassagne, A. (1935), 'Modifications Progressives de la Structure du Conduit Tubo-utérin chez des Lapines Soumises a Partir de la Naissance, à des Injections Répétées D'oestrone (Folliculine)', *C.r. Séanc Soc. Biol.*, **120**, 685.

‡ Nelson, W. O. (1937), 'Endometrial and Myometrial Changes, including Fibromyomatous Nodules, induced in the Uterus of the Guinea Pig by the Prolonged Administration of Estrogenic Hormone', *Anat. Rec.*, **68**, 99.

§ Lipschütz, A. (1950), *Steroid Hormones and Tumors.* Baltimore: Williams & Wilkins.

‖ Lipschütz, A., and Iglesias, R. (1938), 'Multiples Tumeurs Uterines et Extra-génitales Provoquées par le Benzoate D'oestradiol', *C.r. Séanc. Soc. Biol.*, **129**, 519.

and adrenocortical desoxycorticosterone. Lipschütz* reports controlled quantitative studies: each of the antagonists was found to have a distinct antifibromatogenic level; progesterone was the most potent in relation to oestrogen-induced fibroids in guinea-pigs, and it was successfully used to prevent tumour formation and also to speed regression when oestrogen was withdrawn.

HORMONE THERAPY IN HUMAN UTERINE FIBROIDS

The experimental use of antagonistic hormones has a counterpart in therapy for uterine fibroids in women. Such therapy falls short of complete success, but it holds the promise of further achievement. Loeser,† using testosterone propionate in pellets implanted subcutaneously, reported diminution in size of fibroids on clinical examination. Similar results with subcutaneous injections of progesterone were claimed by Goodman.‡ Ovariectomy has also brought about beneficial, although not curative results (Lacassagne§).

* Lipschütz, A. (1950), *Steroid Hormones and Tumors*. Baltimore: Williams & Wilkins.

† Loeser, A. A. (1938), 'The Action of Testosterone Propionate on the Uterus and Breast', *Lancet*, **1**, 373.

‡ Goodman, A. L. (1946), 'Progesterone Therapy in Uterine Fibromyoma', *J. clin. Endocrin.*, **6**, 402.

§ Lacassagne, A. (1957), 'Glandular Cancers of Hormonal Origin', in *Canadian Cancer Conference*. Vol. 2 (Ed. Begg, R. W.). New York: Academic Press.

CHAPTER XVII

HORMONES. PROSTATE AND OTHER TISSUE TARGETS

TUMOURS OF THE PROSTATE

John Hunter,* as early as 1786, published his observations on atrophy of the prostate following castration. In 1893, Romm† attempted treatment of prostatic hypertrophy by castration. This early, largely empirical, knowledge of the dependence of prostatic pathology upon the testis was succeeded by a more sophisticated elaboration of the hormonal associations. In 1926, Steinach and Kun‡ reported experiments demonstrating that ovarian extracts caused atrophy of the prostate; and so the stage was set for the formulation of the fundamental concept of hormone-dependence of certain malignant tumours. This idea arose first from the study of cancer of the prostate; a study in which Huggins and his colleagues have conducted much original research and have provided much basic knowledge.

EXPERIMENTAL WORK

The close association between the physiology and pathology of the prostate and the hormonal environment has extensive support from experimental observations. Androgens, oestrogens, and adrenocortical steroids, with hypophysial trophic secretions in the background, comprise the main hormonal influences. *Fig.* 24 A and B depicts a schematic summary of these influences.

Oestrogens and Androgens.—In 1937, Moore and his associates,§ having induced prostatic tumours in rats with 1,2-benzpyrene, used the technique of grafting portions of prostatic epithelium into the anterior chamber of the rabbit's eye so as to observe the sequels to hormonal derangements. Castration of the host led to rapid decrease in size of the graft; and massive doses of testosterone caused a marked increase in size.

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* Hunter, J. (1786), 'Observations on the Glands situated between the Rectum and Bladder called Vesicular Seminales', in *Observations on Certain Parts of the Animal Oeconomy*. London.

† Romm, F. (1893), 'Hypertrophia Prostatae Behandelt mit Kastration', *Centr. Chir.*, **20**, 759.

‡ Steinach, E., and Kun, H. (1926), 'Antagonistische Wirkungen der Keimdrusenhormone', *Biologia gen.*, **2**, 815.

§ Moore, R. A., and Melchionna, R. H. (1937), 'Production of Tumors of the Prostate in the White Rat with 1,2-benzpyrene', *Am. J. Cancer*, **30**, 731; and Moore, R. A., Rosenblum, H. B., Tolins, S. H., and Melchionna, R. H. (1937), 'Variation in Size of Transplants of Prostate and Seminal Vesicle in Anterior Chamber of Eye', *J. exp. Med.*, **66**, 273.

An interesting, but as yet unproven, theory was propounded by Hill and Strong.* Prostatic and seminal vesicle epithelium in rodents, which ordinarily atrophied after castration, was maintained at normal measure and in a normal cytological state by grafting ovaries to the host's ears. The explanation offered by the authors was that the lower temperature of the ear altered the ovarian secretion of oestrogens to androgens.

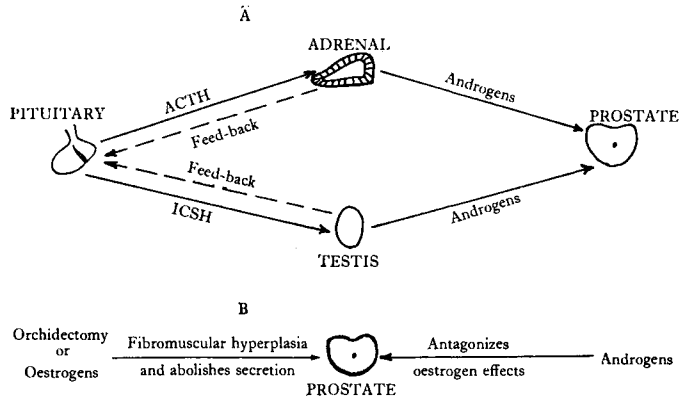

Fig. 24.—Hormonal influences on prostate. A, Pituitary-androgen-prostate axis; B, Antagonistic effects of androgens and oestrogens.

The fibromatogenic powers of oestrogens have been shown by Lipschütz (*see* p. 176) to extend to tissues beyond and remote from the uterus. The effect is also exercised upon the prostate. Zuckermann and Parkes† and Zuckermann‡ demonstrated fibromuscular hyperplasia of the prostate of monkeys as a result of oestrogen stimulation, and the inhibitory effect of testosterone on this reaction.

The prostate of the dog was found to undergo physiological and pathological changes, particularly spontaneous hypertrophy and neoplasm formation, similar to those in man. Zuckermann and McKeown§ noted prostatic alterations in the dog in relation to normal and abnormal testicular conditions, and Huggins and

* Hill, R. T., and Strong, M. T. (1938), 'Ovaries Secrete Male Hormone; Effect of Ovarian Androgens on Accessory Size in Mouse', *Endocrinology*, **22**, 663.

† Zuckermann, S., and Parkes, A. S. (1936), 'Effect of Sex Hormones on the Prostate of Monkeys', *Lancet*, **1**, 242; and (1936), 'Inhibitory Effect of Testosterone Propionate on Experimental Prostatic Enlargement', *Ibid.*, **2**, 1259.

‡ Zuckermann, S. (1938), 'The Effects of Prolonged Oestrogenic Stimulation on the Prostate of the Rhesus Monkey', *J. Anat.*, **72**, 264.

§ Zuckermann, S., and McKeown, T. (1938), 'The Canine Prostate in Relation to Normal and Abnormal Testicular Changes', *J. Path. Bact.*, **46**, 7.

Oestrogens and Androgens, *continued.*

others* gave further support to the evidence for the hormonal effect of steroids in their experiments showing that orchidectomy abolished prostatic secretion, that oestrogens achieved the same result and led to diminution in the size of the prostate gland, and that testosterone restored the secretion.

Horning† demonstrated that prostatic tumours, induced by methylcholanthrene, in mice, were transplantable only if the host was given testosterone. Prostatic grafts in rats, subjected to methylcholanthrene and stilboestrol, developed tumours; the incidence of such tumours was increased by coincident androgens. Most of the successful tumour grafts were found to undergo squamous metaplasia and progress to autonomy. If tumours were subtransplanted into mice castrated prior to puberty, most of the tumours regressed, but resumed growth if testosterone was given.

Burrows‡ reported that the direct action of massive oestrogen upon rodent prostatic tissue evoked benign hyperplasia with progressive metaplasia in most species, but advance to overt malignant neoplasia occurred only in hamsters.

Research using rodents has thus provided important knowledge of the biological effect of hormones on the prostate, but it has limitations on account of the absence of spontaneous hyperplastic and neoplastic lesions such as occur in man. As already noted, the dog has filled much of this gap in experimental work, and Huggins and his co-workers have exploited these circumstances.

Huggins and Clark§ found that spontaneous hyperplasia of the dog's prostate responded to anti-androgenic measures; the prostatic enlargement shrank when androgens were reduced, or when oestrogens were supplied. Testosterone restored hyperplasia and accelerated growth.

Adrenocortical secretion may replace gonadal androgens. Woolley and his associates|| reported the development of adrenocortical tumours following early gonadectomy. Some of these tumours produced oestrogens, others androgens, and some both. Frantz and Kirschbaum¶ demonstrated androgenic secretion by

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* Huggins, C., Masina, M. H., Eichelberger, L., and Wharton, J. D. (1939), 'Quantitative Studies of Prostatic Secretion; Characteristics of Normal Secretion; Influence of Thyroid, Suprarenal, and Testis Extirpation and Androgen Substitution on Prostatic Output', *J. exp. Med.*, **70**, 543.

† Horning, E. S. (1949), 'The Effects of Castration and Stilboestrol on Prostatic Tumours in Mice', *Br. J. Cancer*, **3**, 211; and (1952), 'The Local Action of 20-methylcholanthrene and Sex Hormones on Prostatic Grafts', *Ibid.*, **4**, 80.

‡ Burrows, H. (1949), *Biological Action of Sex Hormones.* 2nd ed. London: Cambridge Univ. Press.

§ Huggins, C., and Clark, P. J. (1940), 'Quantitative Studies of Prostatic Secretion; Effect of Castration and of Estrogen Injection on Normal and on Hyperplastic Prostate Glands of Dogs', *J. exp. Med.*, **72**, 747.

|| Woolley, G. W., Fekete, E., and Little, C. C. (1943), 'Gonadectomy and Adrenal Neoplasms', *Science*, **97**, 291; and Woolley, G. W., and Little, C. C. (1945), 'The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Male Mice of Extreme Dilution Strain', *Cancer Res.*, **5**, 211.

¶ Frantz, M. J., and Kirschbaum, A. (1948), 'Androgenic Secretion by Tumors of the Mouse Adrenal Cortex', *Proc. Soc. exp. Biol. Med.*, **69**, 357.

such tumours in mice, and, in 1949,* they showed that the particular sex hormone secreted varied with different strains. The androgens secreted in rat adrenocortical tumours have been shown by Price† to be capable of maintaining intra-ocular grafts of prostatic epithelium.

Human Prostatic Tumours

The findings and deductions from investigations of anti-androgenic effects in dogs were found to be applicable to man. Huggins and Hodges‡ and Huggins and others§ demonstrated definite hormonal influences: orchidectomy brought about marked regression of prostatic carcinoma; phenolic oestrogens similarly had a beneficial effect; and testosterone aggravated cancer extension. A series of reports followed this early work and culminated in the assertion by Huggins|| that about 95 per cent of cases suffering from widespread prostatic cancer were benefited by hormone therapy directed at reducing androgenic influences. The effects of hormone therapy are summarized schematically in *Fig.* 25.

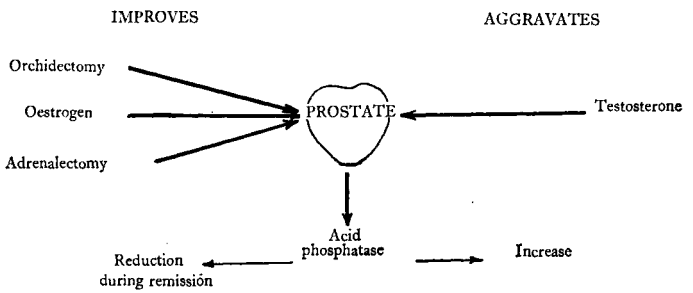

Fig. 25.—The effects of hormone therapy on prostatic cancer.

This form of therapy, based upon experimental findings on animals, and producing clinical results that have been widely confirmed, is the main basis for associating prostatic cancer with hormone dependence. It is only a partial dependence, and the addition of adrenalectomy in order to remove an extragonadal source of androgen (Huggins and

* Frantz, M. J., and Kirschbaum, A. (1949), 'Sex Hormone Secretion by Tumors of the Adrenal Cortex of Mice', *Cancer Res.*, **9**, 257.

† Price, D. (1941), 'Rat Prostate and Seminal Vesicle Grafts in Relation to Age and Sex of Hosts', *Physiol. Zoöl.*, **14**, 145.

‡ Huggins, C., and Hodges, C. V. (1941), 'Studies on Prostatic Cancer. I. The Effect of Castration, of Estrogen and Androgen Injection on Serum Phosphatases in Metastatic Cancer of the Prostate', *Cancer Res.*, **1**, 293.

§ Huggins, C., Stevens, R. E., and Hodges, C. V. (1941), 'Studies on Prostatic Cancer; Effects of Castration on Advanced Carcinoma of Prostate Gland', *Arch. Surg.*, **43**, 209.

|| Huggins, C. B. (1959), 'On Hormone-dependent Cancers', *Jl R. Coll. Surg. Edinb.*, **4**, 191.

Human Prostatic Tumours, *continued.*

Bergenstal*) has not provided a complete answer to the problem. Either there are other sources of androgens, or the tumour attains hormone-independence by some mechanism, or there are other causative factors that remain to be discovered before successful curative 'chemotherapy' can be attained.

Apart from the evidence of therapeutic effects, there are other indications of hormonal influences which are not so well based. The age of peak incidence may be adduced as a supporting factor. The peak is at the end of the seventh decade of life, a decade later than the peak age for cancer in males generally. The age may possibly be correlated with virtual complete disappearance of androgens.

The argument claiming a relationship between benign hypertrophy and cancer is insubstantial. Two sets of resemblances are usually cited in favour of the proposition: the two conditions occur in the same age-group; and both are caused by changes in hormonal balance. The evidence available on the question of an hormonal cause of cancer is strong enough to justify a reasonable inference, but it is far from proof.

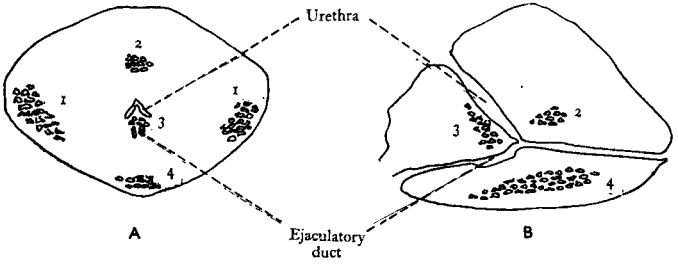

Fig. 26.—The situations of the lobes of the prostate. A, Coronal section; B, Sagittal section. 1, Lateral lobes (9 per cent); 2, Anterior lobe (15 per cent); 3, Mid-lobe (nil or exceptional); 4, Posterior lobe (76 per cent). The figures in brackets indicate the relative incidence of cancer in the different lobes. (Moore, R. A. (1935), 'The Morphology of Small Prostatic Carcinoma', *J. Urol.*, 33, 224.)

A prominent difference between the situations of the two lesions provides a difficult, and as yet unexplained, hurdle for the proponents of the idea. Benign hyperplasia affects mainly the lateral lobes of the prostate, and rarely, if ever, the posterior lobe; whereas about 75 per cent of cancers of the prostate are situated in the posterior lobe. Moreover, the different sites of the lesions accounts for the fact that conventional resection of benign hyperplastic lesions does not protect the individual from the development of prostatic cancer at a later stage. *Fig.* 26 indicates the morphology of the lobes and the relative incidence of carcinoma.

* Huggins, C., and Bergenstal, D. M. (1952), 'Inhibition of Human Mammary and Prostatic Cancers by Adrenalectomy', *Cancer Res.*, 12, 134.

LYMPHOID NEOPLASIA

Oestrogen and Androgen Influence.—In 1937, reports by Gardner* and Lacassagne† introduced the knowledge that oestrogens influenced the occurrence of lymphoid neoplasia in mice. The observation that spontaneous lymphoma was more common in female mice, also stirred inquiry into possible hormonal factors. Gardner and his associates,‡§ among others, elaborated on the influence of steroid hormones: in one strain of mice, the incidence of lymphomas, after treatment with oestrogens, was about 30 times that in a control group; and the higher the dose of oestrogen given, the greater was the incidence of lymphomas. Some strains of mice exhibited a high susceptibility to oestrogens, and others, very much less or none at all. Hybrid offspring of susceptible plus resistant strains exhibited an intermediate degree of response to oestrogens. Whether this was due to genetic or some other unknown factors is not settled.

The antagonistic influence of androgens also became clear from various forms of experiment. Murphy‖ showed that the incidence of leukaemia in a particular strain of male mice was raised by an additional 44 per cent as a result of castration. In a converse type of experiment, McEndy and others¶ showed that the incidence of spontaneous leukaemia in a strain of female mice was reduced from 74 to 45 per cent by ovariectomy. Dmochowski and Horning** reported that skin application of oestrogen in chloroform to castrated male mice resulted in an increased incidence of lymphoid hyperplasia. The experiments showing the stimulating leukaemic effect of oestrogens were complemented by work proving that testosterone negated the effect.

Hormones and Irradiation.—Leukaemogenesis by ionizing radiation is discussed in Chapter XI, p. 121 *et seq.* In the present chapter, some aspects of the association between irradiation and hormones in lymphoma are introduced. Kaplan and his colleagues†† and Gardner and Rygaard‡‡ have reported the influence of hormones on

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* Gardner, W. U. (1937), 'Influence of Estrogenic Hormones on Abnormal Growths', in Symposium, *Cancer Problem, Am. Ass. Adv. Sci.*, **4**, 67.

† Lacassagne, A. (1937), 'Sarcomes Lymphoides Apparus chez des Souris Longuement Traitées par des Hormones Oestrogénes', *C.r. Séanc. Soc. Biol.*, **126**, 193.

‡ Gardner, W. U., Kirschbaum, A., and Strong, L. C. (1940), 'Lymphoid Tumors in Mice receiving Estrogens', *Archs Path.*, **29**, 1.

§ Gardner, W. U., Dougherty, T. F., and Williams, W. L. (1944), 'Lymphoid Tumors in Mice receiving Steroid Hormones', *Cancer Res.*, **4**, 73.

‖ Murphy, J. B. (1944), 'The Effect of Castration, Theelin and Testosterone on the Incidence of Leukemia in a Rockefeller Institute Strain of Mice', *Ibid.*, **4**, 622.

¶ McEndy, D. P., Boon, M. C., and Furth, J. (1944), 'On the Role of Thymus, Spleen and Gonads in the Development of Leukemia in a High-leukemic Stock of Mice', *Ibid.*, **4**, 377.

** Dmochowski, L., and Horning, E. S. (1947), 'Influence of Oestrone on the Lymphoid Tissues of Male Mice', *J. Path. Bact.*, **59**, 307.

†† Kaplan, H. S., and Brown, M. B. (1951), 'Inhibition by Testosterone of Radiation-induced Lymphoid Tumor Development in Intact and Adult Male Mice', *Cancer Res.*, **11**, 706; Kaplan, H. S., Marder, S. N., and Brown, M. B. (1951), 'Adrenal Cortical Function and Radiation-induced Lymphoid Tumors of Mice', *Ibid.*, **11**, 629; and Kaplan, H. S., Nagreda, C. S., and Brown, M. B. (1954), 'Endocrine Factors and Radiation-induced Lymphoid Tumors of Mice', *Recent Prog. Horm. Res.*, **10**, 293.

‡‡ Gardner, W. U., and Rygaard, J. (1954), 'Further Studies on the Incidence of Lymphomas in Mice exposed to X-rays and given Sex Hormones', *Cancer Res.*, **14**, 205.

Hormones and Irradiation, *continued.*

radiation-induced lymphomas in mice. In most strains, with one significant exception, females were more susceptible and had a higher rate of lymphoma formation. Simultaneous oestrogen administration enhanced, and androgen diminished, the lymphoma reaction to irradiation. Cortisone also reduced the incidence of both spontaneous and radiation-induced leukaemia (Upton and Furth*).

Hormones and Chemical Carcinogens.—The leukaemogenic property of methylcholanthrene has an almost parallel association with hormones as has X-ray irradiation. A number of strains of female mice exhibited an increased incidence (Kirschbaum and Mixer†); castrated male mice have a higher incidence, and androgens eliminate the increase (Kirschbaum and others‡).

The Thymus in Experimental Leukaemia.—Leukaemogenesis by oestrogens, ionizing radiation, and polycyclic hydrocarbons; and synergistic, augmentative, or permissive powers of hormones upon the other carcinogens, apart from their provocation of a number of theories of mechanism of action, have also raised the question of a common *via media* through which their actions are directed. The thymus gland is involved and may provide a clue to this problem. In most strains of mice, but not all strains nor in other species, hormones as well as other agents appear to exercise their leukaemogenesis via the thymus (Kaplan§); thymic injury is a common effect, and is particularly liable to progress to malignant lymphoma formation. The place of hormones is positive but not fully understood. Kaplan‖ postulates a thesis on the following lines: thymic injury, occasioned by a lymphoid tumour-inducing agent, activates a compensatory repair mechanism; this mechanism depends upon two main desiderata: available suitable bone-marrow cells (which can be destroyed by irradiation), and secondly, an appropriate hormonal environment (which may be so altered as to obstruct repair). The situation that develops, viz., unsatisfied repair with persistence of the repair mechanism, is premalignant.

It is essential to maintain a proper perspective in regard to the experimental findings and the emerging theories of lymphoid tumour formation and hormones. The facts and hypotheses cannot be translocated to similar neoplasia in man. The remarkable variation of strain reaction, and the still greater differences among different species, is perhaps the most important of the signs

* Upton, A. C., and Furth, J. (1954), 'The Effects of Cortisone on the Development of Spontaneous Leukemia in Mice and on its Induction by Irradiation', *Blood*, **9**, 686.

† Kirschbaum, A., and Mixer, H. W. (1947), 'Induction of Leukemia in Eight Inbred Stocks of Mice Varying in Susceptibility to the Spontaneous Disease', *J. Lab. clin. Med.*, **32**, 720.

‡ Kirschbaum, A., Leibelt, A. G., and Falls, N. C. (1955), 'Influence of Gonadectomy and Androgenic Hormone on the Induction of Leukemia by Methylcholanthrene in DBA/2 Mice', *Cancer Res.*, **15**, 685.

§ Kaplan, H. S. (1954), 'On the Etiology and Pathogenesis of Leukemias: A Review', *Ibid*, **14**, 535.

‖ Kaplan, H. S. (1959), 'The Nature of the Neoplastic Transformation in Lymphoid Tumour Induction', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

directing attention to this warning. However, as knowledge of the genesis and biological behaviour of these tumours in animals increases, the chances of their application to the problems in man becomes more hopeful.

TUMOURS OF THE KIDNEY

Tumours in the Hamster.—Vasquez-Lopez* and Matthews and others† drew attention to the induction of renal tumours in the hamster after prolonged administration of oestrogen. The experimental aspects were subsequently elaborated by Kirkman and Bacon‡ and Horning and Whittick§ among others. Over 90 per cent of males were affected; intact females did not develop the lesion, but after ovariectomy, they did. In males, orchidectomy or simultaneous administration of testosterone negated the tumorigenesis of oestrogen. Once the tumour had developed, testosterone did not affect its subsequent growth and advance. Transplants of the tumour were maintained in an active state only in the presence of excess oestrogen in the host.

Several known carcinogenic agents, causing or influencing renal tumour development, are discussed in Chapter XIII, p. 140 *et seq.*, and the possible place of endocrine factors is included in the discussion.

Renal Tumours in Man.—With the background of established knowledge of hormonal influences upon human renal physiology and the experimental findings of hormonal factors in renal tumours in hamsters, treatment of human renal cancer by hormones has been attempted. Bloom and Wallace‖ report a case showing marked effects of such treatment. There were 3 other cases, from a total of 20 submitted to treatment, that showed some evidence of regression, but this was of limited character. The response in the one striking case was brought about by testosterone (after a progestational agent had accelerated cancer spread); regression endured for about 3 years; skeletal secondaries healed radiologically, and, in the case of a known deposit in the skull prior to treatment, healing was confirmed histologically.

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* Vasquez-Lopez, E. (1944), 'The Reaction of the Pituitary Gland and Related Hypothalamic Centres in the Hamster to Prolonged Treatment with Oestrogens', *J. Path. Bact.*, **56**, 1.

† Matthews, V. S., Kirkman, H., and Bacon, R. L. (1947), 'Kidney Damage in the Golden Hamster following Chronic Administration of Diethylstilboestrol and Sesame Oil', *Proc. Soc. exp. Biol. Med.*, **66**, 195.

‡ Kirkman, H., and Bacon, R. L. (1950), 'Malignant Renal Tumors in Male Hamsters (*Cricetus auratus*) treated with Estrogen', *Cancer Res.*, **10**, 122; and (1952), 'Estrogen-induced Tumors of the Kidney. I. Incidence of Renal Tumors in Intact and Gonadectomized Male Golden Hamsters treated with Diethylstilboestrol', *J. nat. Cancer Inst.*, **13**, 745.

§ Horning, E. S., and Whittick, J. W. (1954), 'The Histogenesis of Stilboestrol-induced Renal Tumours in the Golden Hamster', *Br. J. Cancer*, **8**, 451.

‖ Bloom, H. J. G., and Wallace, D. M. (1964), 'Hormones and the Kidney: Possible Therapeutic Role of Testosterone in a Patient with Regression of Metastases from Renal Adenocarcinoma', *Br. med. J.*, **2**, 476.

TUMOURS OF THE LIVER

The incidence of tumours of the rat liver and the duration of the latent period prior to their onset, depend not only on the carcinogen and its dose, but on the genetic constitution, diet, sex, and the hormonal environment. As with tumours affecting other tissue targets, the hormonal influence is probably more of a 'permissive' nature than a 'cocarcinogenic' one.

Following the initial observation by Bielschowsky* that 2-acetylaminofluorene induced more hepatic tumours in males than in females of a strain of rats, further studies revealed the effects of endocrine organ ablation and endocrine exhibition.

Griffin and his co-workers†‡ demonstrated that hypophysectomy prevented azo dye liver cancerigenesis, and that ACTH restored the property. Adrenalectomized rats were shown by Eversole§ to be protected from liver cancer ordinarily induced by azo dyes. It was later shown by Symeonides|| that the inhibitory effect operated during the early stages of liver tumour development, but once the tumour had started, adrenalectomy plus desoxycorticosterone acetate treatment had no influence.

In addition to the influence of adrenocortical hormones and the related pituitary trophins, androgens have an important place. Intact males and gonadectomized females have been shown by Morris and Firminger¶ to be more liable to 2-diacetylaminofluorene-induced hepatic tumours than intact females, castrate males and females, or castrate males given diethylstilboestrol.

TUMOURS OF BONE

Pybus and Miller** established a strain of mice with a high spontaneous bone-tumour incidence. They found a higher incidence in females, amounting to 2·5 times that in males. Oestrogen appears to favour the formation of bone tumours in some strains of mice, and androgen inhibits oestrogen activity when both hormones are given together.

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* Bielschowsky, F. (1944), 'Distant Tumours produced by 2-amino- and 2-acetoaminofluorene', *Br. J. exp. Path.*, **25**, 1.

† Griffin, A. C., Rinfret, A. P., and Corsigilia, V. F. (1953), 'The Inhibition of Liver Carcinogenesis with 3-methyl-4-dimethylaminoazobenzene in Hypophysectomized Rats', *Cancer Res.*, **13**, 77.

‡ Griffin, A. C., Richardson, H. C., Robertson, H. L., O'Neal, M. A., and Spain, J. D. (1955), 'The Role of Hormones in Liver Carcinogenesis', *J. natn. Cancer Inst.*, Suppl., **15**, 1623.

§ Eversole, W. J. (1957), 'Inhibition of Azo Dye Carcinogenesis by Adrenalectomy and Treatment with Desoxycorticosterone Trimethylacetate', *Proc. Soc. exp. Biol. Med.*, **96**, 643.

|| Symeonides, A. (1963), 'The Effect of Adrenalectomy and of Desoxycorticosterone Acetate during Early and Late Stages of Liver Carcinogenesis in Rats fed p-dimethylaminobenzene', *Acta Un. int. Cancr.*, **19**, 771.

¶ Morris, H. P., and Firminger, H. I. (1956), 'Influence of Sex and Sex Hormones on Development of Hepatomas and Other Hepatic Lesions in Strain AXC Rats ingesting 2-diacetylaminofluorene', *J. natn. Cancer Inst.*, **16**, 927.

** Pybus, F. C., and Miller, E. W. (1938), 'Hereditary Bone Tumours in Mice', *Br. med. J.*, **1**, 1300; (1938), 'Spontaneous Bone Tumours in Mice', *Am. J. Cancer*, **34**, 98; and (1938), 'A Sex Difference in the Incidence of Bone Tumours in Mice', *Ibid.*, **34**, 248.

CHAPTER XVIII

VIRUSES AND CARCINOGENESIS

It requires the prominence of the first sentence of the introduction to this subject to emphasize that although viruses are causatively related to many tumours among animals, no such cause has yet been demonstrated in any human cancer. Having stated this, it is justifiable to express the exciting prospects of possible viral association in human cancer; at present, only by analogy arising from comparison with animals, but with promise for identical findings in humans once some hidden obstacle has been overcome. A viral theory would explain much in human cancer, and, as has already been achieved in treatment and prevention of viral infective diseases, a break-through in the field of cancer might lead to equal success. There is admittedly much suggestive of wishful thinking here, but the situation is so charged with potential that numerous highly qualified investigators at expensively appointed laboratories in all corners of the world are spending most of their working lives in the pursuit of the possibilities.

CHARACTERS AND PROPERTIES OF VIRUSES

Definition.—Until the advent of the electron microscope, viruses were submicroscopic and could not be visualized; but, because cell-free filtrates transmitted a disease from one host to another, the deduction that this was due to organisms too minute to be seen by the devices of man had been generally accepted. Gross* defines almost all virus-caused neoplasms as those transmitted in the laboratory by inoculation of filtrates. The filterable agent can be recovered from the newly infected host and then transferred *ad infinitum* to successive hosts; indicating that the agent multiplies and reproduces itself.

More recently, electron microscopy has brought viruses into view. Morphological descriptions with photographs and diagrams have been published by Bernhard,† Howatson,‡ and many others.

Size.—The size varies from the smallest, e.g., polyoma virus and Shope papilloma agent in rabbits, where it is about 30 mμ or less, to the large types, e.g., Shope fibroma virus in rabbits and molluscum contagiosum in man, measuring up to 260 mμ or more. The rate of sedimentation produced by high-speed centrifugation, a method commonly employed to isolate viral bodies, gives a measure of the molecular weight of the virus.

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* Gross, L. (1961), *Oncogenic Viruses*. Oxford: Pergamon.

† Bernhard, N. (1960), 'The Detection and Study of Tumor Viruses with the Electron Microscope', *Cancer Res.*, **20**, 712.

‡ Howatson, A. F. (1964), 'Viruses, their Chemical Composition and Ultrastructure', in *Cellular Control Mechanisms and Cancer* (Ed. Emmelot, P., and Mühlbock, O.). Amsterdam: Elsevier.

Shape.—Spheroidal, rod, brick, cubical, and filamentous shapes occur.

Structure.—A central dense core of nuclear-like character is surrounded by an outer membrane-like enevelope of one, two, or more layers. The 'nucleus' probably consists of nucleic acid which carries the infective material, and the outer coat is a protein membrane, possibly contributed by the host. Sometimes there is no membrane. The 'nucleus' of a tumour virus consists of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), but not both (Andrewes*), as is the case with bacteria and some of the larger infective viruses.

Site within Host Cell.—Some tumour viruses reproduce within the nucleus of the host cell. The Shope papilloma agent lies close to the host nucleolus; polyoma virus is first found in chromatin tissue, then it increases rapidly and probably bursts through the nuclear membrane into the cytoplasm. The viruses of chicken tumours and leukaemias are found in host-cell cytoplasm. Bittner virus particles are found adjacent to the host-cell membrane, where they appear in the projecting microvilli prior to their being 'pinched off'. Some tumour viruses, e.g., Lucké's frog tumour, begin in the nucleus and continue in cytoplasm.

Infectivity.—It has been held for a long time that viruses require living cells for their reproduction, but this has recently become subject to some doubt. A certain specificity of selection of host cells is exhibited, some having a wider range than others, both as regards species and particular cells. Specificity may be affected by adaptation, which renders many viruses infective in a more extensive field. The maturity of host cells, too, may determine viral activity. This aspect has associations with immune mechanisms in viral infections, which are discussed in later sections of this chapter as well as in Chapter XXIV.

Origin.—The idea that cell constituents, e.g., mitochondria or genes, may replicate independently and become viruses or particulate agents, is not tenable. Whilst the status of viruses is not finally established, they are comparable to infective organisms in that they are specific pathogens causing recognizable disease entities, and they are produced from their own genetic material. Their multiplication, however, differs from the fission process of ordinary micro-organisms; viruses appear to utilize the nucleic acids of host cells, initially fusing or integrating, and subsequently giving rise to groups of newly constituted virus bodies.

Actions.—Viral infection causing cellular death is known as 'cellucidal'. If the cell survives, the virus is known as 'moderate' (comparable to 'temperate' in bacteriophage activity). Moderate viruses may unite with the cell forming a new system, a process known as 'integration' (comparable to lysogeny in bacteriophage association). When viral infection induces tumours, the virus is 'oncogenic'. The comparisons with bacteriophage activity are elaborated in Chapter XIX.

* Andrewes, Sir C. (1964), 'Tumour-viruses and Virus-tumours', *Br. med. J.*, **1**, 653.

Immunity and Anti-viral Mechanisms.—Immune phenomena in viral tumours are discussed in Chapter XXIV. It is necessary to add here that viral infected cells, under certain conditions, produce a protein, *interferon*, which may inhibit or restrain multiplication of viruses and bring about a state of stability and continuance of cellular activity compatible with normal life.

Masking and Latency.—Latency is a common state in virus infections just as it is with bacteria; and the two-stage mechanism (*see* Chapter X) of tumour formation appears to operate in many circumstances. A unique viral feature, which may be a part of latency, is the so-called 'masking' phenomenon. After invasion of a cell, a virus may integrate and lose its identity, becoming invisible on electron microscopy. It may remain in this masked state for some generations of the host cell and later reappear as an infective and recognizable particulate agent.

HISTORICAL MILESTONES.

Sanarelli,* in Uruguay, in 1898, interpreted an epidemic affecting domestic rabbits, and causing a fatal disease associated with multiple 'colloid' tumours, as due to a 'myxomatous virus'. This virus is probably the same as that described by Shope in 1932 as the fibroma-virus of cottontail rabbits.

Borrel† in France in 1903, on the basis of experimental observations in transplantation of tumour cells, first suggested the possibility of viruses as the cause of tumours. Actual demonstration of transmission of a malignant neoplastic condition (chicken leukaemia) by a cell-free filtrate was first achieved in Copenhagen by Ellermann and Bang‡ in 1908. This was followed by the experiments of Peyton Rous§ in New York, proving the transmission of chicken sarcoma by a cell-free filtrate. This was the first solid tumour in which a virus agent was shown as the cause. There have been many discoveries of virus tumours since then; they are summarized in *Table XIV*.

BIOLOGICAL SIGNIFICANCE

Table XIV pinpoints some outstanding events in the story of the investigation of viruses and their tumours. The items in the table represent but a tiny fraction of the total voluminous research on the subject. The work has produced highly significant knowledge in the field of the biology of tumours, and features of theoretical and practical importance are indicated in the following surveys of selected virus tumours and viral behaviour patterns.

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* Sanarelli, G. (1898), 'Das Myxomatogene Virus. Beitrage zum Stadium der Krankheitserreger Auserhalb des Sichtbaren', *Zentbl. Bakt. ParasitKde* (Abt. I), **23**, 865.

† Borrel, A. (1903), 'Épithelioses Infectieuses et Épithéliomas', *Annls Inst. Pasteur, Paris*, **17**, 81.

‡ Ellermann, V., and Bang, O. (1908), 'Experimentelle Leukämie bei Huhnern', *Zentbl. Bakt. ParasitKde* (Abt. I), **46**, 595.

§ Rous, P. (1910), 'A Transmissible Avian Neoplasm (Sarcoma of the Common Fowl)', *J. exp. Med.*, **12**, 696; and (1911), 'Transmission of a Malignant New Growth by Means of a Cell-free Filtrate', *J. Am. med. Ass.*, **56**, 198.

Biological Significance, *continued.*

Table XIV.—MILESTONES IN THE HISTORY OF VIRUS-NEOPLASIA

YEAR	AUTHOR	NEOPLASM	ANIMAL
1898	Sanarelli	Myxomatosis	Rabbit
1908	Ellermann and Bang	Leukaemia	Chicken
1910 and 1911	Rous	Muscle sarcoma	Chicken
1912	Rous, Murphy, and Tytler	Osteosarcoma	Chicken
1913	Rous and Lange	Angiosarcoma	Chicken
1932	Shope	Fibroma	Rabbit
1933	Shope	Papilloma	Rabbit
1933	Furth	Lymphomatosis	Chicken
1936	Bittner	Mammary carcinoma	Mouse
1938 and 1952	Lucké	Renal carcinoma	Frog
1940	Kidd	Brown-Pearce carcinoma	Rabbit
1946	Burmester, Prickett, and Belding	Lymphomatosis and osteopetrosis	Chicken
1949 and 1951	Lucas	Lymphomatosis	Chicken and dove
1951	Gross	Lymphatic leukaemia	Mouse
1953 1954 1955	Gross	Parotid tumour, mammary carcinoma, subcutaneous sarcomas, and adrenal medullary tumours	Mouse
1956	Graffi, Bielka, and Fey	Leukaemia, from cell-free extracts of transplants	Mouse
1956	Friend	Leukaemia	Mouse
1957	Stewart, Eddy, Gochenour, Borgese, and Grubbs	Parotid tumour and other tumours (polyoma virus)	Mouse, rat, and hamster

Bittner, J. J. (1936), 'Some Possible Effects of Nursing on the Mammary Gland Tumor Incidence in Mice', *Science*, **84**, 162.
Burmester, B. R., Prickett, C. O., and Belding, T. C. (1946), 'A Filtrable Agent producing Lymphoid Tumours and Osteopetrosis in Chickens', *Cancer Res.*, **6**, 189.
Ellermann, V., and Bang, O. (1908), 'Experimentelle Leukämie bei Huhnern', *Zentbl. Bakt. ParasitKde* (Abt. I), **46**, 595.
Friend, C. (1956), 'The Isolation of a Virus causing a Malignant Disease of the Hematopoietic System in Adult Swiss Mice' (Abstract), *Proc. Am. Ass. Cancer Res.*, **2**, 106.
Furth, J. (1933), 'Lymphomatosis, Myelomatosis and Endothelioma of Chickens caused by a Filterable Agent. I. Transmission Experiments', *J. exp. Med.*, **58**, 253.
Graffi, A., Bielka, H., and Fey, F. (1956), 'Leukämieerzeugung Durch ein Filtrierbares Agens aus Malignen Tumoren', *Acta haemat.*, **15**, 145.
Gross, L. (1951), '"Spontaneous" Leukemia developing in C3H Mice following Inoculation in Infancy, with Ak-leukemic Extracts, or Ak-embryos', *Proc. Soc. exp. Biol. Med.*, **76**, 27; (1953) 'A Filterable Agent, recovered from Ak Leukemic Extracts,

causing Salivary Gland Carcinomas in C3H Mice', *Ibid.*, **83**, 414; (1954), 'Is Leukemia caused by a Transmissible Virus? A Working Hypothesis', *Blood*, **9**, 557; and (1955), 'Induction of Parotid Carcinomas and/or Subcutaneous Sarcomas in C3H Mice with Normal C3H Organ Extracts', *Proc. Soc. exp. Biol. Med.*, **88**, 362.

Kidd, J. G. (1940), 'A Distinctive Substance associated with the Brown-Pearce Rabbit Carcinoma. I. Presence and Specificity of the Substance as determined by Serum Reactions', *J. exp. Med.*, **71**, 335; and (1940), 'A Distinctive Substance associated with the Brown-Pearce Rabbit Carcinoma. II. Properties of the Substance: Discussion', *Ibid.*, **71**, 351.

Lucas, A. M. (1949), 'Lymphoid Tissue and its Relation to So-called Normal Lymphoid Foci and to Lymphomatosis. I. Qualitative Study of Lymphoid Areas in the Pancreas of Chickens', *Am. J. Path.*, **25**, 1197; and (1951), 'Lymphoid Tissue and its Relation to So-called Normal Lymphoid Foci and to Lymphomatosis. VI. A Study of Lymphoid Areas in the Pancreas of Doves and Pigeons', *Poult. Sci.*, **30**, 116.

Lucké, B. (1938), 'Carcinoma in the Leopard Frog. Its Probable Causation by a Virus', *J. exp. Med.*, **68**, 457; and (1952), 'Kidney Carcinoma in the Leopard Frog: A Virus Tumour', *Ann. N.Y. Acad. Sci.*, **54**, 1093.

Rous, P. (1910), 'A Transmissible Avian Neoplasm (Sarcoma of the Common Fowl)', *J. exp. Med.*, **12**, 696; and (1911), 'Transmission of a Malignant New Growth by Means of a Cell-free Filtrate', *J. Am. med. Ass.*, **56**, 198.

Rous, P., and Lange, L. B. (1913), 'The Characters of a Third Transplantable Chicken Tumor due to a Filterable Cause: A Sarcoma of Intracanalicular Pattern', *J. exp. Med.*, **18**, 651.

Rous, P., Murphy, J. B., and Tytler, W. H. (1912) '"Transplantable Tumors of the Fowl: A Neglected Material for Cancer Research', *J. Am. med. Ass.*, **58**, 1682.

Sanarelli, G. (1898), 'Das Myxomatogene Virus. Beitrage zum Stadium der Krankheitserreger Ausserhalb des Sichtbaren', *Zentbl. Bakt. ParasitKde* (Abt. I), **23**, 865.

Shope, R. E. (1932), 'A Transmissible Tumor-like Condition in Rabbits', *J. exp. Med.*, **56**, 793; and (1933), 'Infectious Papillomatosis of Rabbits', *Ibid.*, **58**, 607.

Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., and Grubbs, G. E. (1957), 'The Induction of Neoplasms with a Substance released from Mouse Tumors by Tissue Culture', *Virology*, **3**, 380.

FOWL SARCOMAS

Of more than 40 fowl tumours studied by Rous and his associates, three were regarded as distinct and due to different virus agents (Rous and Murphy*). Their special characters of histology, latent interval, transplantability, and general behaviour, persisted throughout serial transmission by filtrate. Most of the other tumours were indistinguishable from one another. In certain apparently dissimilar tumours, transplantation gave rise to identical neoplasms; sometimes several transplantations were required to produce this result. Thus an aetiological pattern of several strains of one virus causing one tumour, or the adaptability of a virus, was suggested (Rous†).

The fowl sarcoma was successfully transplanted into ducks and back again to chickens (Fujinami‡), indicating a spread of viral infectivity to more than one species.

Transmissibility of the Rous sarcoma increases with successive filtrate transmissions as a general rule, but modifying factors exist: the older the host, the more slowly growing the tumour and the less potent the filtrate; the same applies to the age of the tumour, which produces its maximum activity during its early weeks of growth.

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* Rous, P., and Murphy, J. B. (1914), 'On the Causation by Filtrable Agents of Three Distinct Chicken Tumors', *J. exp. Med.*, **19**, 52.

† Rous, P. (1914), 'On Certain Spontaneous Chicken Tumors as Manifestations of a Single Disease. I. Spindle-cell Sarcoma rifted with Blood Sinuses', *Ibid.*, **19**, 570.

‡ Fujinami, A. (1930), 'Special Report. A Pathological Study in Chicken Sarcoma', *Trans. Jap. path. Soc.*, **20**, 3.

Fowl Sarcomas, *continued.*

Newly hatched chicks were more susceptible to infection by filtrate. Similarly, newly hatched ducklings readily accepted infection from both filtrate and transplant of Rous sarcoma. With increasing delay after birth, resistance became progressively more marked. These observations by Duran-Reynals* have an important bearing on immunity reactions in the neonatal period. The existence of immunological tolerance at this age means that viruses are accepted with resulting tumour formation. In later life, the challenge by virus is met by an immunological reaction with rejection of the virus, and consequent failure to develop tumours. 'Foreign' material introduced during embryonic life behaves in terms of the self-marker hypothesis of Burnet and Fenner.† This has been shown by Billingham and others‡ experimentally. In the embryo, a form of immunological tolerance is induced; with the result that after birth, the animal carries potentially active virus, but does not itself show the usual virus effects, nor does it exhibit an antiviral antibody reaction against further inoculation of the same virus (*see also* Chapter XXIV).

Some tumours fail to yield a potent filtrate. Bryan§ has shown that this is, in part at least, due to the dose of virus causing the tumour; the higher the dose, the shorter the latent interval and the greater the amount of extractable virus. Failure to yield an active filtrate sometimes persists for a period of months, despite concurrent successful tissue transplantation of the same tumour. This was held by some authorities to be part of the masking process. They postulated that this was the condition in human cancer which was due to viruses which were not biologically appreciable as they were in a masked condition. However, Gross‖ considers the phenomenon the result of low initial potency, and he has prepared tumour material which invariably produces an active filtrate.

Demonstrations that genetic factors play an important role have appeared in many investigations. Highly resistant as well as very susceptible breeds of chickens are well recognized, and inbreeding can increase the grades of susceptibility.

A further example of biological behaviour in chicken sarcomas is one of great significance, although its interpretation is still controversial. Spontaneous chicken tumours amenable to cellular transplantation, ultimately become transferable by a filtrate (Murphy and Sturm¶).

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* Duran-Reynals, F. (1942), 'The Reciprocal Infection of Ducks and Chickens with Tumor-inducing Viruses', *Cancer Res.*, **2**, 343.

† Burnet, F. M., and Fenner, F. (1949), *The Production of Antibodies.* Melbourne: Macmillan.

‡ Billingham, R. E., Brent, L., and Medawar, P. B. (1953), 'Actively acquired Tolerance of Foreign Cells', *Nature, Lond.*, **172**, 603; and (1956), 'Quantitative Studies on Tissue Transplantation Immunity. III. Actively acquired Tolerance', *Proc. R. Soc.* B, **239**, 357.

§ Bryan, W. R. (1955), 'Biological Studies on the Rous Sarcoma Virus. I. General Introduction. II. Review of Sources of Experimental Variation and of Methods for their Control', *J. natn. Cancer Inst.*, **16**, 285; and (1956), 'Biological Studies on the Rous Sarcoma. IV. Interpretation of Tumor-response Data involving one Inoculation Site per Chicken', *Ibid.*, **16**, 843.

‖ Gross, L. (1961), *Oncogenic Viruses.* Oxford: Pergamon.

¶ Murphy, J. B., and Sturm, E. (1941), 'Further Investigation on the Transmission of Induced Tumors in Fowls', *Cancer Res.*, **1**, 609.

More remarkable still, is the report by Oberling and Guerin,* confirming the work of others, that a methylcholanthrene-induced chicken sarcoma eventually produces a potent filtrate. The suggestion emerging from these findings is that virus infection is the fundamental cause of all chicken sarcomas, including spontaneous and chemically induced tumours, and that the chemical carcinogenic agent operates by activating virus mechanisms to produce the actual cancer.

Tissue Culture.—Tissue-culture growth of malignant cells was first reported in 1910 by Carrell and Burrows,† and by Volpino‡ in the same year. In 1926, Carrell§ reported the successful propagation of Rous sarcoma *in vitro*. The transformation of fibroblasts *in vitro* into malignant cells by the action of Rous sarcoma virus was first recorded by Halberstaedter and others|| in 1941. Since then many other reports on tissue-culture studies have appeared with regard to the effects upon oncogenic viruses and also upon the cells of the medium.

Contact Inhibition.—This subject is more fully considered in Chapter II, but here it is noted from the work of Temin and Rubin¶ that the addition of Rous sarcoma virus to cultures of chicken fibroblasts gives rise to rapid and uninhibited growth of infected fibroblasts, piling on top of one another, and indicating the loss of contact inhibition. This type of reaction also affects mouse embryo cells bearing polyoma virus, and it is noted later in this chapter.

'Defective' Virus.—Hanafusa and others,** following the discovery that Rous sarcoma virus (RSV) stocks were contaminated by another virus, named Rous-associated virus (RAV), report that RSV alone alters culture cells in the usual manner, but that these transformed cells do not produce or yield RSV as is usual, until RAV is added to the culture. The RSV ability to transform chick embryo cultures to malignancy, and RSV potential for producing further virus, are not lost in repeated serial transfers, as shown by eventual addition of RAV, which re-establishes RSV yield. The inference is that RSV requires a 'helper virus', e.g., RAV, for its continued propagation and release; or that Rous sarcoma virus is genetically defective by itself and needs additional genetic information for complete synthesis.

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* Oberling, C., and Guerin, M. (1950), 'Sarcome de la Poule par Methylcholanthrene Devenu Filtrable', *Bull. du Cancer*, **37**, 5.

† Carrell, A., and Burrows, M. T. (1910), 'Cultivation of Sarcoma outside of the Body', *J. Am. med. Ass.*, **55**, 1554.

‡ Volpino, G. (1910), 'Alcune Experience sul Cancro Traplantabili dei Topi', *Pathologica*, **2**, 495.

§ Carrell, A. (1926), 'Some Conditions of the Reproduction *in Vitro* of the Rous Virus', *J. exp. Med.*, **43**, 647.

|| Halberstaedter, L., Doljanski, L., and Tenenbaum, E. (1941), 'Experiments on the Cancerization of Cells *in Vitro* by Means of Rous Sarcoma Agent', *Br. J. exp. Path.*, **22**, 179.

¶ Temin, M. H., and Rubin, H. (1958), 'Characteristics of an Assay for Rous Sarcoma Virus and Rous Sarcoma Cells in Tissue Culture', *Virology*, **6**, 669.

** Hanafusa, H., Hanafusa, T., and Rubin, H. (1963), 'The Defectiveness of Rous Sarcoma Virus', *Proc. natn. Acad. Sci., U.S.A.*, **49**, 572.

CHICKEN LEUCOSES

This heading includes a number of types of leukaemia and neoplastic affections of cells of the haemopoietic system. The first one produced by a filtrate was that by Ellermann and Bang in 1908 (already referred to in the historical note p. 189); it was an erythro-myeloblastic leukaemia, in which excessive numbers of erythroblasts and myeloblasts appeared in the peripheral blood-stream. Another host reaction to inoculation of filtrate was the exhibition of leukaemic cell infiltration of visceral organs, known as lymphomatosis. Further passage of filtrates from this reproduced the typical blood-picture. The different manifestations were, in fact, part of one disease; a state rather reminiscent of Hodgkin's disease, lymphosarcoma, and lymphatic leukaemia in man.

Research on avian leucoses has been extended to other forms of the disease. Carr* reported the appearance of multiple renal adenocarcinomas following inoculation of a particular strain of virus of erythroblastic leukaemia. This only occurred in young chicks within a fortnight of birth, whereas the same inoculum in older fowls produced leukaemia. The range of different neoplastic effects has been the subject of a number of reports by Burmester and his colleagues.†‡§ Visceral lymphomatosis, erythroblastosis, myeloblastosis, osteopetrosis, fibrosarcomas, myxosarcomas, haemangiomas, and renal cancer, make up the spectrum of tumours induced by 'leukaemic' filtrates. A number of factors are known to influence the type of neoplastic reaction: the age of the host at the time of inoculation, the dose and latent interval, the influence of sequential passage on the potency of the virus, the route of infection, and genetically determined susceptibility—probably all play some part in the eventual manifestations; but there are many questions still unanswered. A crucial question is whether the whole spectrum of tumours is caused by one oncogenic virus, or whether there are two or several different viruses in chicken leukaemia filtrates. A similar problem was presented by the Gross mouse leukaemia, and its solution has stimulated interesting work and ideas.

MOUSE LEUKAEMIA

After many years of experimentation by different investigators, Gross‖ succeeded in transmitting leukaemia by centrifuged cell-free extracts and cell-free filtrates, taken from either spontaneous or transplanted leukaemia in the highly susceptible Ak strain of mice, and inoculated into newborn C3H mice, a strain with a very low spontaneous leukaemic rate.

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* Carr, J. G. (1956), 'Renal Adenocarcinoma induced by Fowl Leukaemia Virus', *Br. J. Cancer*, **10**, 379.

† Burmester, B. R., Prickett, C. O., and Belding, T. C. (1946), 'A Filtrable Agent producing Lymphoid Tumors and Osteopetrosis in Chickens', *Cancer Res.*, **6**, 189.

‡ Burmester, B. R., Walter, W. G., Gross, M. A., and Fontes, A. K. (1959), 'The Oncogenic Spectrum of Two "Pure" Strains of Avian Leukosis', *J. natn. Cancer Inst.*, **23**, 277.

§ Burmester, B. R., Fontes, A. K., and Walter, W. G. (1960), 'Pathogenicity of a Viral Strain (RPL 12) causing Avian Visceral Lymphomatosis and Related Neoplasms. III. Influence of Host Age and Route of Inoculation', *Ibid.*, **24**, 1423.

‖ Gross, L. (1951), '"Spontaneous" Leukemia developing in C3H Mice following Inoculation in Infancy, with Ak-leukemic Extracts, or Ak-embryos', *Proc. Soc. exp. Biol. Med.*, **76**, 27.

Hormonal Influences upon spontaneous leukaemia in high-leukaemic strains of mice are suggested by the higher incidence of leukaemia in females (Cole and Furth*), and from the effects of experimental ovariectomy and orchidectomy. McEndy and others† report that removal of the ovaries reduced the incidence by more than one-third, and orchidectomy brought an increase of about 10 per cent.

Thymus.—McEndy and others† noted the common involvement of the thymus in spontaneous leukaemia in high-leukaemic strains. The authors found that thymectomy, between the ages of 1 and 2 months, considerably reduced the leukaemia incidence. Experiments by Gross‡ showed that descendents of thymectomized mice, after 4 successive generations had been operated upon, preserved the same incidence of spontaneous leukaemia as in non-operated mice. Thus, whilst thymectomy reduced the incidence, the protective effect was not hereditable and was not transmitted to descendent generations.

Two-stage Mechanisms.—The combined effects of leukaemic-inducing virus and other leukaemogenic agents, viz., X-ray irradiation, polycyclic hydrocarbons and hormones, have demonstrated several varieties of cocarcinogenic activity. Salaman and Roe§ review the various combinations of all types of viruses and second agents giving rise to enhanced neoplasia; the effect being real augmentation and not simply additive. Oncogenic and non-oncogenic viruses plus chemical carcinogens, cocarcinogens, and hormones, as well as the combination of virus and virus, are reported. Urethane (noted in Chapter X, p. 111, as possessing an initiating action in skin cancer) is not leukaemogenic by itself, but it has a marked augmentation effect on the incidence of leukaemia when applied with X-rays or oestrogens (Kawamoto and others‖).

Immunological Tolerance.—Gross¶ has made extensive use of neonatal mice for experiments in transmitting leukaemia. Neonates are more susceptible, accepting cell transmissions even from foreign tissues, e.g., rats and mice reciprocally 'take' the others' tumours for a limited time. The circumstances are the same as those referred to in an earlier section on chicken sarcoma and the work of Duran-Reynals. Neonatal susceptibility is comparable to 'acquired tolerance'; there is no immunological recognition, and thus an absence of rejection of foreign cells. The result is the development of

* Cole, R. K., and Furth, J. (1941), 'Experimental Studies on the Genetics of Spontaneous Leukaemia in Mice', *Cancer Res.*, **1**, 957.

† McEndy, D. P., Boon, M. C., and Furth, J. (1944), 'On the Role of Thymus, Spleen and Gonads in the Development of Leukemia in a High-leukemia Stock of Mice', *Ibid.*, **4**, 377.

‡ Gross, L. (1961), *Oncogenic Viruses.* Oxford: Pergamon.

§ Salaman, M. H., and Roe, F. J. C. (1964), 'Cocarcinogenesis', *Br. med. Bull.*, **20**, 139.

‖ Kawamoto, S., Ida, N., Kirschbaum, A., and Tayler, G. (1958), 'Urethan and Leukemogenesis in Mice', *Cancer Res.*, **18**, 725.

¶ Gross, L. (1950), 'Susceptibility of Newborn Mice of an Otherwise Apparently "Resistant" Strain to Inoculation with Leukemia', *Proc. Soc. exp. Biol. Med.*, **73**, 246; (1950), 'Susceptibility of Suckling-infant and Resistance of Adult Mice of the C3H and of the C57 Lines to Inoculation with Ak Leukemia', *Cancer*, **3**, 1073; and (1951), '"Spontaneous" Leukemia developing in C3H Mice following Inoculation in Infancy with Ak Leukemic Extracts, or Ak-embryos', *Proc. Soc. exp. Biol. Med.*, **76**, 27.

Immunological Tolerance, *continued.*

neoplasia. With increasing age, there is a rapid and progressive loss of tolerance; foreign cells are 'recognized'; immunological reaction follows with rejection of the challenging material. Acquired tolerance is not always permanent, tending to weaken with time. Tolerance is also deleted by lymph-node cell inoculation: either with cells from lymph-nodes from other mice of the same strain, which act slowly; or with rapid effects, by using cells from previously sensitized mice of the same strain.

The intimate association of virus-tumours in experimental animals with immunological mechanisms is clearly established, and is providing a means of probing fundamental biological mechanisms.

Radiation Leukaemia.—The possibility that ionizing radiation-induced leukaemia in mice may be the result of activation of a latent virus has been discussed in Chapter XI. The suggestion emerged from experimental work on the thymus. In the context of the present section on mouse leukaemia, it should be noted that X-ray irradiation of C3H mice, a strain of low spontaneous leukaemia rate, readily produces lymphatic leukaemia, from which transmission by cell-free filtrate inoculation into neonates of the same strain has been achieved (Gross*). This was also found by Lieberman and Kaplan† working on another low leukaemic strain of mice. These findings are of a highly significant and provocative nature; they are distinct pointers, though not proven supports, of the theory of viruses as *the* cause of cancer.

POLYOMA VIRUS

In experiments to extend the results of his earlier work in 1950 and 1951, Gross‡ discovered that cell-free filtrates from leukaemic Ak mice caused, in addition to leukaemia in C3H mice, some cases of parotid adenocarcinoma. Mice with these tumours quite often developed further lesions: subcutaneous fibrosarcomas or fibromyxosarcomas; peritoneal and fascial sarcoma; uterine rhabdomyosarcomas; mammary adenocarcinomas; and adrenal medullary tumours. Later, still other tumours due to this virus were discovered, not only in mice, but in other rodents, rabbits, and in the ferret. Gross§ differentiated and identified the parotid-tumour virus as distinct from the leukaemogenic virus by their different sizes (by filtration and ultracentrifugation), and by their different sensitivities to heat. Stewart and others‖ applied the name 'polyoma virus', and promoted and transmitted the virus via tissue cultures.

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* Gross, L. (1959), 'Serial Cell-free Passage of a Radiation-activated Mouse Leukemia Agent', *Proc. Soc. exp. Biol. Med.*, **100**, 102.

† Lieberman, M., and Kaplan, H. S. (1959), 'Leukemogenic Activity of Filtrates from Radiation-induced Lymphoid Tumors of Mice', *Science*, **130**, 387.

‡ Gross, L. (1953), 'A Filterable Agent, recovered from Ak Leukemic Extracts, causing Salivary Gland Carcinomas in C3H Mice', *Proc. Soc. exp. Biol. Med.*, **83**, 414.

§ Gross, L. (1953), 'Neck Tumors, or Leukemia, developing in Adult C3H Mice following Inoculation, in Early Infancy, with Filtered (Berkefeld N), or Centrifuged (144,000 × g) Ak-leukemic Extracts', *Cancer*, **6**, 948.

‖ Stewart, S. E., Eddy, B. E., and Borgese, N. G. (1958), 'Neoplasms in Mice inoculated with a Tumor Agent carried in Tissue Culture', *J. natn. Cancer Inst.*, **20**, 1223.

The parotid tumour virus (or the polyoma virus) has been submitted to intensive research. The facts that it caused at least 23 different neoplasms and is pathogenic to a wide host range have provoked many ideas and explorations for biological models upon which to fashion theories of the nature of cancer in man. In view of the necessary limitations imposed by the scope and balance of this synopsis, it is possible to indicate only some of the outstanding disclosures of these studies.

Pathogenicity.—The spontaneous development of parotid gland tumours, or other tumours induced by polyoma virus, is exceedingly rare, despite the fact that the virus is commonly carried by normal, healthy mice of various strains, not only among adults but also in embryos. Polyoma viral pathogenicity is promoted by special conditions. Inoculation of cell-free filtrates and extracts into neonatal susceptible animals, and inoculation at later ages postnatally with tissue-culture-grown virus are effective; prolonged administration of cortisone has been shown by Woolley* as an activator; and total-body fractionated X-ray irradiation has been demonstrated by Gross† and Gross and others‡ to be a promoting cause.

Oncogenic potential is augmented by culture on tissues *in vitro*. Stewart and her colleagues§‖ reported a much increased potency of the virus harvested from tissue cultures. An increased number and a greater variety of tumours were produced. The range of host susceptibility was broadened, and tumours developed when transplanted at more advanced ages than the early neonatal period.

Cytopathogenic Effect on Cells of Culture Media.—Experiments on different cultures also revealed notable phenomena. Tissue-culture growth on monkey kidney cells, while productive of rich and potent virus, did not apparently alter the kidney cells; whereas, on mouse embryo cells (a culture medium used at a later stage and found to be more suitable), cytopathogenic effects were noted. The embryo cells gradually changed, manifesting abnormal mitoses and separation from the main culture plaque. It seems that the virus is non-infective in some, and infective in other cells, although it replicates in both.

SIMIAN VIRUS 40 OR VACUOLATING VIRUS

Simian virus 40, or SV40, a naturally occurring virus in kidney tissues of rhesus monkeys, was separated from a large number of such 'natural' viruses by a group of research workers in the Division of Virus and Tissue Culture of the Merck Institute for Therapeutic Research, West Point,

* Woolley, G. W. (1964), 'Occurrence of "Neck Tumors" in Cortisone-treated Leukemic-strain Mice', *Proc. Am. Ass. Cancer Res.*, **1**, 53.

† Gross, L. (1958), 'Attempt to recover Filterable Agent from X-ray-induced Leukemia', *Acta haemat.*, **19**, 353.

‡ Gross, L., Roswit, B., Mada, E. R., Dreyfuss, Y., and Moore, L. A. (1959), 'Studies on Radiation-induced Leukemia in Mice', *Cancer Res.*, **19**, 316.

§ Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., and Grubbs, G. E. (1957), 'The Induction of Neoplasms with a Substrate released from Mouse Tumors by Tissue Culture', *Virology*, **3**, 380.

‖ Stewart, S. E., Eddy, B. E., and Borgese, N. G. (1958), 'Neoplasms in Mice inoculated with a Tumor Agent carried in Tissue Culture', *J. natn. Cancer Inst.*, **20**, 1223.

Simian Virus 40 or Vacuolating Virus, *continued.*

Pa. Sweet and Hilleman,* members of the research group, summarized the information on SV40 in 1960. The virus grew but did not cause any cytopathological changes in rhesus kidney-cell cultures; however, it grew and evoked cellular changes in cultures of kidney cells from other species of monkeys, viz., the green or grivet monkey and the vervet monkey. The prominent change was vacuolation of the cells, hence the alternative name of 'vacuolating virus'.†

Eddy and others‡ showed that SV40 in extracts from rhesus monkey kidney-cell cultures were oncogenic in newborn hamsters. The tumours differed in distribution and in some cellular characters from those induced by polyoma virus. However, resemblances in immunological reaction have appeared in further research. Like the polyoma virus, SV40 gives rise to a tumour antigen. Hamsters inoculated with SV40 reject transplants of tissue from SV40-induced tumours, and the antibodies exhibit specificity.

Oral administration infects man, who excretes virus in his faeces for a considerable time. Koprowski and others§ have shown that the virus can be maintained and propagated on cultures of human buccal mucosa and skin, and that it is cytopathogenic to these cells, evoking chromosomal deviations. Furthermore, it has been shown that these altered culture cells induce subcutaneous nodules when injected into man (volunteers among 'terminal' cancer patients).

The properties and host range of SV40 are stimulating intensive current research. Its cytopathogenicity in human culture cells and its tumorigenicity in human subcutaneous tissues are of great moment. These properties are also giving rise to anxiety and apprehension as many of the earlier anti-poliomyelitis 'vaccinations' probably contained SV40 which was undetected prior to 1960.

MOUSE MAMMARY CANCER

A strain of mice, known as C3H, produced by selective inbreeding, exhibits a high incidence of spontaneous mammary carcinoma among females, both breeding and virgin. A strain, designated A, has a high spontaneous incidence among breeding females and a low rate among virgins. A number of other strains (e.g., Ak, C58, and CBA), also reared by inbreeding, display a very low breast-tumour rate. The

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* Sweet, B. H., and Hilleman, M. R. (1960), 'The Vacuolating Virus SV40', *Proc. Soc. exp. Biol. Med.*, **105**, 420.

† A new name for a group of viruses has been coined from three viruses of similar size, distinctive fine structure with cubic symmetry, biochemical constitution, and replicative behaviour. They are the viruses causing papillomas in man and various animals; polyoma virus; and the vacuolating virus, SV40. The first two letters of each of the three are combined to produce *papova virus.* (Melnick, J. L. (1962), 'Papova Virus Group', *Science*, **135**, 1128.)

‡ Eddy, B. E., Borman, G. S., Berkley, W. H., and Young, R. D. (1961), 'Tumors induced in Hamsters by Injection of Rhesus Monkey Kidney Cell Extracts', *Proc. Soc. exp. Biol. Med.*, **107**, 191.

§ Koprowski, H., Ponten, J. A., Jensen, F., Ravdin, R. G., Moorhead, P., and Saksela, E. (1962), 'Transformation of Cultures of Human Tissues infected with Simian Virus, SV40', *J. cell. comp. Physiol.*, **59**, 281.

presence or absence of breast cancer in these mice might have been considered a purely genetically heritable factor, were it not for the observation that in hybrid mice from high and low susceptible parents, the incidence of breast cancer tended to follow the maternal and not the paternal pattern. The results of cross-breeding may be expressed by the formulae:—

1. High ♀ + Low ♂ → High ♀;
2. Low ♀ + High ♂ → Low ♀.

If susceptibility was genetically transmitted, the two cross-breedings would produce the same results. These fundamental observations, proving a non-hereditary factor, emanated from the work of the staff of the Roscoe B. Jackson Memorial Laboratory in 1933.*

The Milk Factor.—Bittner investigated the possibility that the cancer was transmitted to suckling offspring by some agent in maternal milk. High breast-cancer strain A mice were taken soon after birth from their own mothers to be suckled by mice of CBA, i.e., a low breast-cancer strain. The A mice so fed developed very much fewer breast cancers than expected. Similar observations on another strain of low incidence led to the tentative suggestion by Bittner in 1936,† that nursing might offer the explanation for the extra-chromosomal factor in transmission of breast-cancer in mice. Experiments were designed to prevent the passage of even a few drops of milk, as this was found to transmit the tumorigenic agent. Andervont‡ ensured this objective by Caesarean section removal of mice of high spontaneous tumour strain, and then giving them to foster mothers of low tumour strain. The result was a reduction of breast-cancer incidence to that of the low tumour strain. Apart from demonstrating transmission in milk, the procedure proved that transplacental passage did not occur. Bittner§ continued his experiments, confirming the findings with larger numbers of mice, and using the reverse factors of foster-nursing, i.e., foster-nursing low tumour strains by mothers of high tumour lines, eventuating in a high tumour incidence, far beyond the usual anticipated low rates for this strain. Other investigators repeated the work in various forms and confirmed the findings. Andervont‖ and

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* Roscoe B. Jackson Memorial Laboratory Staff (1933), 'The Existence of Non-chromosomal Influence in the Incidence of Mammary Tumors in Mice', *Science*, **78**, 465. ("Staff" consisted of Little, C. C., Murray, W. S., Bittner, J. J., and Green, C. V., quoted by Gross, L. (1961), *Oncogenic Virus*. Oxford: Pergamon.)

† Bittner, J. J. (1936), 'Some Possible Effects of Nursing on the Mammary Gland Tumor Incidence in Mice', *Science*, **84**, 162.

‡ Andervont, H. B. (1941), 'Effect of Ingestion of Strain C3H Milk in the Production of Mammary Tumors in Strain C3H Mice of Different Ages', *J. natn. Cancer Inst.*, **2**. 13.

§ Bittner, J. J. (1939), 'Relation of Nursing to the Extra-chromosomal Theory of Breast Cancer in Mice', *Am. J. Cancer*, **35**, 90; (1940), 'Breast Cancer in Mice as influenced by Nursing', *J. natn. Cancer Inst.*, **1**, 155; and (1940), 'Further Studies on Active Milk Influence in Breast Cancer Production in Mice', *Proc. Soc. exp. Biol. Med.*, **45**, 805.

‖ Andervont, H. B. (1945), 'The Milk Influence in the Genesis of Mammary Tumors', in Symposium on *Mammary Tumors in Mice*, *Amer. Ass. Adv. Sci.*, No. 22.

The Milk Factor, *continued.*

Dmochowski,* two among many others, reviewed these earlier experiments. Mouse breast cancer was proved to be transmissible by an agent in milk. This was later shown to be a filterable virus, commonly known as the Bittner virus.

Endocrine Factors.—The evidence for endocrine influence is seen in the following: Females, but not males, of susceptible strains developed breast cancer. Lacassagne† reported that long-continued oestrogen administration to male mice led to the formation of breast cancers.

It is of interest to note that only those males carrying the virus, having received it from infected mothers via breast milk, were so affected; leading to the inference that the hormone was influential but not determinant in the development of the breast tumour.

Breeding females of strain A have a 90 per cent rate of occurrence but only 5 per cent of virgins of the same strain are affected; a comparative incidence which is at variance with the over 90 per cent incidence in both breeding and virgin females of the C3H strain. The findings appear related to hormonal factors, but the reason for the difference between A and C3H virgins is not clear (*see* Chapter XV).

Organs infected by Virus.—Apart from breast milk, transmissible oncogenic virus was also found in the blood, thymus, and spleen. This wide distribution served to explain the puzzling finding that mating of males of a high breast-tumour strain with females of a low breast-tumour strain, sometimes resulted in progeny with a relatively high tumour susceptibility, i.e., not as high as the rate for the high-strain parent, but higher than that for the low-strain female partner. It was established by Andervont and Dunn,‡ Foulds,§ and others, that the females were infected by virus during coitus. However, many unsolved problems still exist in interpretation of a number of results of high and low mating experiments.

Chemical Carcinogenic Influences.—Female mice belonging to a high tumour strain but foster-nursed by low tumour strain mothers have a very low incidence of spontaneous breast cancer. Notwithstanding the presumption that they are free of Bittner virus, it has been established by Dmochowski and Orr‖ that the application of chemical carcinogens, like methylcholanthrene, could induce breast cancer. The virus agent was not demonstrable in the tumours

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* Dmochowski, L. (1953), 'The Milk Agent in the Origin of Mammary Tumors in Mice', in *Advances in Cancer Research*, **1**, 103. New York: Academic Press.

† Lacassagne, A. (1932), 'Apparition de Cancers de la Mammelle chez la Souris Mâle, Soumise a des Injections de Folliculine', *C.r. hebd. Séanc. Acad. Sci., Paris*, **195**, 630.

‡ Andervont, H. B., and Dunn, T. B. (1948), 'Mammary Tumors in Mice Presumably Free of the Mammary Tumor Agent', *J. natn. Cancer Inst.*, **8**, 227.

§ Foulds, L. (1949), 'Mammary Tumours in Hybrid Mice: the Presence and Transmission of the Mammary Tumour Agent', *Br. J. Cancer*, **3**, 230.

‖ Dmochowski, L., and Orr, J. W. (1949), 'Chemically Induced Breast Tumours and the Mammary Tumour Agent', *Ibid.*, **3**, 520.

produced, and the explanation of the experimental findings is not yet clarified, although a number of explanations are conceivable. The agent may be 'masked'; available techniques, including ultra-thin sections, may not yet be sufficiently refined and sophisticated to reveal the virus; or there may be some other cause.

Some of the outstanding findings and experimental results concerning a number of different oncogenic viruses have been described in this chapter. The impact on the theoretical evaluation of the causes of human cancer has been profound. Theories of viral mechanisms in the production of tumours in experimental animals, and speculation as to their possible operation in man are the subjects of the following chapter.

CHAPTER XIX

VIRAL NEOPLASIA. MECHANISMS AND ROLE IN MAN

GENETIC CONCEPT OF VIRUS ACTION

The place of oncogenic viruses in the mutation theory of cancer is discussed in Chapter XXIII. Appreciation of the concept of the mechanism and effects of mutagenesis by viruses is facilitated by reference to the model of microbial genetics and the interactions of bacteria and phages.

Microbial Genetics.—Variation in the genetic constitution of bacteria may occur by mutation and by several forms of sexual mechanism, wherein genes from one cell combine with genes from another, producing a new type of bacterium. Combinations of genes are of three varieties: *Transformation* implies that deoxyribonucleic acid or DNA (*see* Chapter XXII) from a donor confers on the recipient cell the characteristics of the donor.* *Transduction* is the mechanism whereby a bacterial virus (i.e., bacteriophage), whilst multiplying in a host cell, takes up some of the chromosomal elements of the disintegrating host, and transfers the genetic material to new host cells. *Conjugation* is the third variety of combination, in which many genes of one cell are transferred to a recipient cell during a period of fusion between the two cells.

Bacterial viruses, or bacteriophages, bear special genetic relationships to host cells. One type of phage is known as 'virulent'. Its DNA transforms the host bacterium, which undergoes lysis; subsequently phage DNA is replicated. The other type of phage is 'temperate'. Its DNA integrates with that of a sensitive host cell which appears normal and reproduces itself together with the integrated phage DNA. Thus the infected cell acquires the power to transmit the virus to its progeny. The relationship between the temperate phage and host cell is known as lysogeny. The subject has been reviewed by Lwoff† and more briefly by Fuerst.‡ The phage, which seems to have disappeared by integrating with the host DNA, is known in this state as 'prophage'. Its presence in the host cell is confirmed by the existence of its specific antigen, and because free temperate phage particles are released in certain circumstances. Lwoff and others§ demonstrated the release of

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* Work on this mechanism of genetic influence provided the first indication that genes were composed of DNA.

† Lwoff, A. (1953), 'Lysogeny', *Bact. Rev.*, **17**, 269.

‡ Fuerst, C. R. (1959), 'Lysogeny', in *Third Canadian Cancer Conference*. New York: Academic Press.

§ Lwoff, A., Siminovitch, L., and Kjeldgaard, N. (1950), 'Induction de la Production de Bactériophages chez une Bactérie Lysogène', *Annls Inst. Pasteur, Paris*, **79**, 815.

phage from a lysogenous state by the action of ultra-violet light; Latarjet* recorded a similar effect by ionizing radiation from X-rays; and such phage activation has also been reported by Lwoff† with other inducing agents. These are all highly suggestive findings, giving point to the possibility that certain carcinogenic agents may act through their effect in releasing viruses from a state of integration.

Most bacteria are combined with temperate phages, so that lysogeny is probably the normal state. The combination is effected by attachment of the phage's tail to the bacterium; followed by injection of phage DNA from its head, via the attached tail into the bacterium, where it integrates with the host nucleic acid and comes to occupy a particular site in the chromosomal pattern.

Recently, Zinder‡ has reported the discovery of a bacteriophage containing RNA, possessing powers of replication and carrying infective potential. The viral membrane contains a specific amino-acid with antigenic properties; it is synthesized by RNA in a cell-free system. Viral RNA seems to act like cell RNA as a messenger carrying genetic information to ribosomes (*see* Chapter XXII).

It has not been definitely established that animal viruses behave in the same manner as bacterial phages, but an analogous process seems probable. This has led to the theory of virus-induced mutation as *the* basic carcinogenic effect. It is supposed that oncogenic viruses react with host cells in one or other form of combination of their nucleic acid components. In particular, the mechanism of temperate bacterial phage in forming a lysogenic state with the host bacterium is offered as the model of oncogenic viral conversion of host cell to neoplastic cell. In this integrated form, the oncogenic virus apparently disappears and may remain 'masked' for several generations. The hypothesis thus explains tumour formation and provides an answer to the common puzzling finding of apparent disappearance of the virus from the tumour which it has caused.

The latter feature of viral oncogenesis is cited in reconciling the theory of viruses as *the* cause of all tumours, with the absence of evidence of the existence of viruses in any form of human cancer. There are considerations, however, which tend to negate this idea. Gross§ thinks that 'masking' in animals is due to low viral potency; a view that has been noted in the preceding chapter. Recent work on 'helping' viruses may also throw some light on the question; it is discussed later in this chapter.

The concept that viruses are essentially genetic elements, composed of DNA or RNA, which are endowed with the properties of replication and control of biosynthesis, is widely advocated. In fact Luria‖ defines viruses as 'elements of genetic material of cells

* Latarjet, R. (1951), 'Induction, par les Rayons X, de la Production d'un Bactériophage chez B. Megatherium Lysogène', *Annls Inst. Pasteur, Paris*, **81**, 389.

† Lwoff, A. (1953), 'Le Bactériophage; L'induction', *Ibid.*, **84**, 225.

‡ Zinder, N. D. (1963), 'The Functions of the RNA Bacteriophage f2', in *Viruses, Nucleic Acids, and Cancer*. Baltimore: Williams & Wilkins.

§ Gross, L. (1961), *Oncogenic Viruses*. Oxford: Pergamon.

‖ Luria, S. E. (1959), 'Viruses as Determinants of Cellular Function', in *Third Canadian Cancer Conference*. New York: Academic Press.

Microbial Genetics, *continued.*

which, in the cells where they reproduce, can determine the biosynthesis of a specific apparatus for their own transfer to other cells'. It seems that the DNA and RNA (oncogenic viruses have one or the other) not only carry genetic properties and information, but are the infective moieties. If this assumption is correct, the theory that virus tumorigenesis arises by a genetic mechanism has a strong basis.

PARASITIC CONCEPT OF VIRUS ACTION

This was an earlier theory of the mechanism of action of oncogenic viruses, preceding the more recent genetic theory, and taking as its model the infectious character of non-tumorigenic viruses which are compared to bacteria.

Furth* emphasizes that many aspects of the theory are entirely speculative. The conception involves several alternative or complementary suppositions. In summary, the theory takes the following rationale: The viral effects are not those of a cytopathological alteration of host genetic material; there is no mutagenesis and no production of a new genome. The parasitic virus preserves its own identity, replicating from its own genetic template. Viral growth may possibly progress at the expense of host synthesized products, but, by definition, it necessarily interferes with host-cell function and/or reaction to normal controls. Host-cell homeostatic regulation is upset, either by the addition of growth-stimulating factors or by the deletion of certain enzymes or other substances, so that host cells become endowed with malignant properties.

Parasitic activity may be combined with, or followed by, mutagenic effects. Some viruses induce tumours which are virus dependent for a time. The Shope papilloma virus, for example, gives rise to a benign tumour initially; when malignancy supervenes, the virus 'disappears' from the tumour, i.e., it is no longer recoverable and infectious by cell-free filtrate. The malignant tumour is autonomous and apparently independent of the original viral infection, its altered nature being regarded as due to somatic mutation.

Latarjet† reported an acceleration phenomenon which is relevant to the discussion of the theories of mechanisms of viral tumour formation. In the high-leukaemic Ak strain of mice, injection of leukaemic extract into neonates resulted in an increased incidence and a reduction of the latent interval of leukaemia. Controls exposed to non-leukaemic extracts did not show the same results. Latarjet argues that if the virus-host cell relationship was comparable to lysogeny, in which the bacteria are known to be immune to superinfection by further phage, the high spontaneous rate of leukaemia would result from transmission of the altered genome, containing the 'provirus' (i.e., similar to 'prophage'), to embryonic cells, which, on maturing, would release active virus and lead to obvious neoplasia. But, in fact, the accleration

* Furth, J. (1959), 'Mechanisms of Carcinogenesis by Viruses'. In Ciba Symposium on *Carcinogenesis*. London: Churchill.

† Latarjet, R. (1959), 'Carcinogenesis by Leukaemic Cell-free Extracts in Mice' in Ciba Symposium on *Carcinogenesis*. London: Churchill.

phenomenon proves viral leukaemogenesis to be different from phage activity. The host cells are shown to be sensitive to superinfection, and to react by increased and earlier incidence. This reaction is comparable to that found with many organismal infections acting by parasitic mechanisms. The comparison, therefore, lends colour to the parasitic theory.

VIRUSES AND TWO-STAGE MECHANISMS

The theories of mutagenic and parasitic mechanisms of virus oncogenesis do not imply that either of them is necessarily the complete carcinogenic agent. The theories may be applied to the concept of the two-stage mechanism. This aspect is discussed in Chapter X, and further references to possible cocarcinogenic mechanisms appear in Chapter XVIII. Although these references are very far from being exhaustive, it may prove instructive to abstract and discuss some examples that are pertinent to two-stage carcinogenesis.

The polyoma, Rous sarcoma, and Shope papilloma viruses appear to be complete carcinogens. As with examples among chemical carcinogenic agents, it is possible that these viruses, given in subthreshold doses, will act as initiators. This seems clear for the Shope fibroma virus, which can act as an initiator, the promoter being tar. Methylcholanthrene-induced chicken sarcomas and irradiation-induced leukaemias, which ultimately become transferable by cell-free agents, are strongly suggestive of the action of cocarcinogenic agents. It appears patent that the action of Bittner's milk agent is consonant with initiation; the promoting agent being oestrogen. Breast cancers may pass through a phase of hormone-dependency before reaching malignant autonomy, thus generating the idea of progressive stages in tumour development. Progression does not occupy a circumscribed independent status among theories of mechanisms, it is a variant of the two-stage theory: stated in simple terms it implies that between the stages of initiation and promotion, a phase of responsiveness is interposed.

It appears, however, that variants of two-stage carcinogenesis may, in fact, be of a more profound nature; and the question presents as to whether these variants should be called 'two-stage' or 'multifactorial' mechanisms. This is not merely a problem in semantics; more fundamental issues are suggested by the following examples.

Spontaneous polyoma virus tumours are exceedingly rare in their natural hosts, in whom leukaemia has a high incidence. Experimental oncogenesis requires special circumstances and preparations; and then, when polyoma tumours are induced, it is very exceptional for leukaemia, in an otherwise high spontaneous leukaemic strain, to co-exist with parotid tumours, and it is unknown for leukaemia to coexist with the other polyoma-induced tumours. Is there some form of interaction between the leukaemogenic and polyoma viruses, with one reciprocally preventing the neoplastic effect of the other? The polyoma virus appears to require separation from its co-boarder and dominating partner before it can induce neoplasia, which then assumes the dominant role *vis-à-vis* leukaemia.

This example of interaction between viruses representing a form of mutual antagonism can be matched by an instance of virtual contrary effect, where one virus is necessary for maturation and full evolution of

Viruses and Two-stage Mechanisms, *continued.*

its partner. Rous sarcoma virus alone produces sarcomas in tissue cultures, but does not yield infective virus; the addition of Rous associated virus acts like a catalyst and brings this about. An association of viruses thus enters the field of carcinogenesis: some prevent, others help, viral activity.

VIRUSES AND HUMAN TUMOURS

At present, virus infection can be blamed for causing several benign tumorous conditions in man. It is salutary to keep in mind that the proven range of virus-cause and tumour-effect is so limited. Several intellectually attractive speculations and many inferences by analogy from virus tumours in animals are possible and may provoke rewarding research. But, notwithstanding their value as plausible, or even workable, hypotheses, facts are still lacking. It is against this background that mental reservations are essential in regard to the following notes and considerations of the assessment of the place of viruses in the aetiology of neoplasia in man. With this degree of intellectual discipline, it is permissible to take hope from such statements as those of Gross,* who claims that in view of the facts that many, or most, malignant conditions in chickens, mice, and rats have an established viral cause, and that neoplasia in man is so similar in morphology and progress, 'it would be rather difficult to assume a fundamentally different etiology for human tumors'. Andrewes† writes in similar but more cautious vein, 'It would seem unlikely that cancer should have a different cause in different areas of the animal kingdom. . . .'

The following notes briefly describe the four known virus-induced tumours in man.

Human Common Wart.—This is a benign tumorous condition occasioned by virus infection. It is contagious by contact, spreading from one part of the skin to another and to other individuals. It is also transmissible to other humans by subcutaneous inoculation, infection varying considerably with host susceptibility. It is unsightly, but otherwise harmless. The 'tumour' may regress spontaneously or clear following ablative therapy. Its viral cause is visible microscopically in the form of inclusion bodies; electron-microscopic studies have revealed its shape, finer architecture, and measurements; and, more recently, its numbers and densities have been enumerated (Barrera-Oro and others‡). Attempts to transmit this papilloma 'tumour' or its causative agent to experimental animals, as well as attempts to maintain and propagate it on tissue culture, have been largely unsuccessful and non-productive of meaningful observations. Its significance in the field of virus-neoplasia is at present virtually non-existent.

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* Gross, L. (1961), *Oncogenic Viruses.* Oxford: Pergamon.
† Andrewes, Sir C. (1964), 'Tumour-viruses and Virus-tumours', *Br. med. J.*, **1**, 653.
‡ Barrera-Oro, J. G., Smith, K. O., and Melnick, J. L. (1963), 'Quantitation of Papova Virus Particles in Human Warts', *J. natn. Cancer Inst.*, **29**, 583.

Condyloma Acuminatum, a non-syphilitic lesion on the external genitalia, is another human wart probably due to a virus. It is transferable by inoculation. Despite one or two reports of carcinomatous degeneration, its status does not contribute to the question of whether viruses are the cause of human cancer.

Laryngeal Papilloma.—There may be some significance in this human papilloma, which has been reported as transferable to human skin (Ullmann* and Dahmann†), because it has been successfully transplanted into the anterior chambers of monkey's eyes (Green and others‡). But it requires much further study before its place, if any, in the general aetiology of human tumours can be appraised.

Molluscum Contagiosum is another of the proven virus tumour conditions in man. It is contagious by contact and affects children particularly. Small, white, waxy skin nodules are typical; cheesy matter may be squeezed from them; spontaneous regression or large lobulated heaping may occur. Molluscum bodies, squeezed or discharged from the lesions, contain virus inclusions.

None of these virus-induced benign conditions offers, up to the present, any evidence on the general problem of whether viruses are a cause of one or more of the important neoplastic conditions in man. Inferences and associations are being submitted to tests and experiments; and evidence may be gleaned from the following lines of investigation and reasoning.

Widened Specificity of Tumour-viruses.—Before the discoveries of the wider range of susceptible hosts to tumorigenesis by polyoma virus and SV40, it was argued that the highly specific character of tumour-viruses, attacking a particular cell in a particular animal host, and productive of a special type of neoplasm, rendered it unlikely that numerous undiscovered specific tumour-viruses existed to account for all the different tumours in man. The wider spectrum of tumours (polyoma gives rise to 23 different neoplastic conditions) raises the possibility of one or a limited number of viruses, acting under special circumstances and in conjunction with other contributory factors, in man. Furthermore, the extension of oncogenesis by a virus from one species of animals to others has opened the door to possible transmission of human tumours to animal hosts.

The possibilities have been heightened by a number of discoveries. Stewart and others§ report that polyoma virus excites neutralizing antibodies, not only in mice with tumours, but in uninoculated and control mice living in the same environment, and in personnel working with the laboratory animals. The fact that SV40, removed from its natural host, becomes tumorigenic to other primates is also suggestive. Another recent laboratory finding, carrying some weight

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* Ullmann, E. V. (1924), 'On the Aetiology of the Laryngeal Papilloma', *Acta otolar., Stockh.*, **5**, 317.

† Dahmann, H. (1929), 'Die Larynxpapillomatose', *Z. Lar. Rhinol. Otol.*, **18**, 383.

‡ Green, R. G., Goodlow, R. J., Evans, C. A., Peyton, W. T., and Titrud, L. A. (1940), 'Transmission of a Human Papilloma to Monkeys', *Am. J. Cancer*, **39**, 161.

§ Stewart, S. E., Eddy, B. E., and Stanton, M. F. (1959), 'Induction of Neoplasms in Mice and other Mammals by a Tumor Agent carried in Tissue Culture', in *Third Canadian Cancer Conference*. New York: Academic Press.

Widened Specificity of Tumour-viruses, *continued.*

in the controversy, is the successful transmission of certain types of human adenovirus to hamsters, in which animals tumours are produced. (Trentin and others,* and Pereira and MacCallum†).

Virus Activation.—Serious doubts about the validity of the theory of viruses as *the* cause of cancer arise from the apparently indubitable cancerigenic properties of ionizing radiation and certain chemicals in animals and man. However, the possibility of their action being mediated by viruses has been raised to a practical level by the findings that both hydrocarbon-induced and radiation-induced neoplasms in experimental animals eventually become transmissible by cell-free filtrates.

Experimental evidence of the special circumstances and modifying influences commonly associated with viral tumorigenesis is also apposite to the question of viral activation. The fact that polyoma virus is harmless to its natural host (Furth,‡ among many other investigators, had never seen spontaneous neoplastic changes in tens of thousands of Ak strain of mice), and has an astounding potential for producing many different forms of cancer in a number of species, has led to a search for some special modifying factor which provokes its tumour-forming properties. Some factors have been discovered: in its natural host, the Ak strain, its tumour-inducing capacity can be released by cortisone, total body X-ray irradiation, and by passage through several hosts or through tissue culture and back to host. A comparable finding is that in the low spontaneous leukaemic strain of C3H mice, the existence of ordinary dormant leukaemogenic virus is proved by its activation by irradiation, hormones, and chemical carcinogens.

It seems reasonable to infer that viruses may be *the* cause of cancer in animals, and, by analogy, in man, requiring some special circumstances or accessory agent to start it on its course of oncogenesis.

Electron Microscopy.—Electron microscopy of viruses was given additional practical value by the technique of ultra-thin sections, elaborated by Palade§ and Porter and Blum.|| The special microtome was able to cut through a virus, so that its internal architecture could be visualized and distinguished from other non-viral particles. This has led to a new field of investigation in which much hope has been invested. The new science is in the process of gradual and laborious construction. As yet, its contribution to the problem

* Trentin, J. J., Yabe, Y., and Taylor, G. (1962), 'The Quest for Human Cancer Viruses', *Science*, **137**, 835.

† Pereira, M. S., and MacCallum, F. D. (1964), 'Infection with Adenovirus Type 12', *Lancet*, **1**, 198.

‡ Furth, J. (1959), 'Mechanism of Carcinogenesis by Viruses', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

§ Palade, G. E. (1952), 'A Study of Fixation for Electron Microscopy', *J. exp. Med.*, **95**, 285.

|| Porter, K. R., and Blum, J. (1953), 'A Study in Microtomy for Electron Microscopy', *Anat. Rec.*, **117**, 685.

of viruses in human cancer has not proved positive. Among the many difficulties is the presence of so-called 'passenger viruses' in tumours, i.e., resident but not pathogenic viruses.

Immunological Studies.—The scope of this line of research is suggested in Chapter XXIV. Immune phenomena in viral neoplasms in animals are established and recognized, and they have played an important part in the study of such tumours. The application of the study and techniques to human cancer is, of course, considerably restricted by the impossibility of using any form of potentially harmful experiment. However, immunology is a fundamental discipline in the study of human cancer, and its role in research is likely to increase.

In Vivo Studies.—Girardi and others* report their attempts to transfer human malignant material to laboratory animals. Transplantation experiments, using minced primary tumour or 'cell culture enriched' extracts from humans to mice and hamsters, did not reveal significant oncogenic properties.

Tissue-culture Studies.—The first successful transformation by virus action upon normal human cells, converting them into neoplastic cell types in tissue culture, was described by Koprowski and others in 1962.† SV40 infected tissue-culture grown human fibroblasts, which then assumed sarcomatous features. Viral cytopathogenicity was attested by the formation of inclusion bodies, nuclear enlargement, and localized cytolysis at an early stage; later, mitosis was increased and altered cells appeared; this was followed by loss of contact inhibition (*see* Chapter II), with cellular and nuclear pleomorphism, and marked chromosomal deviations.

The difficulties and frustrations attending efforts to discover oncogenic viruses in human neoplastic material in tissue-culture systems are as great in *in vitro* investigations as they are in *in vivo* studies. McAllister and others‡ contrast the ease in 'unmasking' adenovirus from human tonsils by tissue-culture techniques, with the failure to reveal any virus from more than 100 culture systems of the same type, with neoplastic, abnormal, and normal tissues from children.

Of all the many efforts to assist 'viruses in search of disease', the one example of success, with adenovirus types 12 and 18, concerns a virus which is not carcinogenic in man. This should not be interpreted as evidence in favour of the negative side of the argument, i.e., it does not signify that viruses are not the cause of human cancer. The subject is relatively young, and wider areas of exploration may lead to more finite results.

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* Girardi, A. J., Hilleman, M. R., and Zwickey, R. E. (1962), 'Search for Virus in Human Malignancies. 2. *In vivo* Studies', *Proc. Soc. exp. Biol. Med.*, **111**, 84.

† Koprowski, H., Ponten, J. A., Jansen, F., Ravdin, R. G., Moorhead, P., and Saksela, E. (1962), 'Transformation of Cultures of Human Tissues infected with Simian Virus, SV40', *J. cell. comp. Physiol.*, **59**, 281.

‡ McAllister, R. M., Mikenas, M., Straw, R. M., and Landing, B. H. (1963), 'Cytologic and Virologic Studies of Cultures derived from Neoplastic and Non-neoplastic Tissues of Children', *Lab. Invest.*, **12**, 343.

Epidemiology.—Epidemiological studies may add evidence of viral agencies. Infectivity may be suggested by examples of hereditary transmission and familial incidence of certain malignant conditions in man. These are referred to in Chapter XXI. There are other suggestive examples of direct inoculation of cancer cells, but the extreme rarity of these cases makes general inferences unjustifiable.

It has been suggested, on the basis of geographical and epidemiological data, that Burkitt's tumour is due to an arbovirus, i.e., an arthropod-borne virus. This is discussed in Chapter V. It is important to repeat here that an oncogenic virus has not been demonstrated, and the whole subject of the aetiology of Central African lymphoma is still a matter of conjecture.

CHAPTER XX

TRAUMA. PARASITES. TISSUE CULTURE. FILMS

TRAUMA

Cancer following Single Trauma.—Reviews of cases of cancer claimed as due to previous single traumatic episodes, when set against strict criteria for diagnosis such as those laid down by Warren,* have reduced the numbers of authentic cases to small proportions.

An analysis by Hauser† of 262 cases purporting to have followed single trauma reduced this figure to 13 acceptable examples. Leighton and Schmidtke‡ surveyed the records of considerable numbers of cases seen at the Barnard Free Skin and Cancer Hospital, and found 42 instances where single trauma may have initiated a series of changes eventually leading to cancer. The changes were nearly all associated with unhealed wounds, so that 'single trauma' was not a complete or accurate description of the supposed aetiological factor, and 'repeated' or 'continuous' irritation might have been more correct.

If these figures are conceded, the number of cancers caused by single injuries is infinitesimal by comparison with single trauma not followed by cancer, and the whole thesis becomes very nebulous.

Cancer following Burns, Scars, and Chronic Ulcers.—Cancer superimposed on scar tissue, or on the edge of long-standing and poorly healing ulcers, is not rare. Cases following burns, frostbite, especially oft-repeated injuries, and chronic ulceration have been diagnosed after applying proper criteria. Treves and Pack§ estimated that, of all scar cancers, about 80 per cent followed burns or freezing injuries. The Kangri burn cancer (*see* Chapter VII) is a probable example of malignancy following repeated burns of the skin.

Malignant epitheliomas have been recorded as complications of long-standing varicose ulceration, of fistula-in-ano, and of the sinuses of chronic osteomyelitis. Chronic ulcerative colitis is associated with an excess incidence of carcinoma amounting to about 30 times that which ordinarily occurs.

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* Warren, S. (1943), 'Minimal Criteria to prove Causation of Traumatic or Occupational Neoplasms', *Ann. Surg.*, **117**, 585.

† Hauser, W. (1933), 'Krebs und Umfall', *Mschr. Krebsbekämpf.*, **1**, 241.

‡ Leighton, W. E., and Schmidtke, E. C. (1940), 'A Single Trauma as an Etiological Factor in Carcinoma', *J. Mo. med. Ass.*, **37**, 267.

§ Treves, N., and Pack, G. T. (1930), 'The Development of Cancer in Burn Scars' *Surgery Gynec. Obstet.*, **51**, 749.

Cancer following Burns, Scars, and Chronic Ulcers, *continued.*

The example of epidermoid cancer of the tongue or buccal lining, following prolonged irritation by an irregular tooth or denture, is similarly well established. While some authorities deny that this exemplifies chronic mechanical irritation as the pathogenic agent, and hold that the associated sepsis has an important role, the fact remains that it is a chronic inflammatory, and thus irritative, process.

These cancers may be regarded as developing from chronic inflammation or chronic irritation, by a process of incomplete and continuous healing, ultimately evolving beyond normal regeneration to uncontrolled autonomy, i.e., neoplasia.

The Theory of Chronic Irritation.—Broussais (1826)* appears to have been the first to ascribe cancer to chronic inflammation or irritation; and Virchow renewed the theory in 1863.† There are, in current times, a number of proponents of the theory, holding it as *the* causative mechanism. Berglas‡ puts the case that all the known carcinogenic agents act by chronic irritation which prevents completion of healing until ordinary regulating mechanisms fail. Menkin§ does not adopt such a sweeping generalization, but he considers that chronic inflammation is a cocarcinogen. Its action is effected by a growth-promoting factor, which is part of the exudate of inflammation, and which induces irreversible cellular changes. In this respect, Menkin claims, chronic irritation is comparable to virus infection and hormone imbalance. He conceives of inflammation and neoplasm as different manifestations of cellular injury: inflammation arising from severe injury; and neoplasm from milder, more chronic injuries, which induce proliferation in genetically susceptible individuals.

There is some experimental support for the view that trauma may act as a cocarcinogen in the form of an initiator of the first stage of cancer induction. The work of Rous and Kidd,‖ which contributed towards the concept of cocarcinogenesis with the stages of initiation and promotion, originated in experiments with trauma and tar. This work, and other references to trauma as a cancerigenic agent, are discussed in greater detail in Chapter X.

Most authorities do not accept chronic irritation as *the* cause, nor as a common cause, of cancer. The discovery of chemical carcinogenesis, with the varying ability of chemical substances to excite neoplastic transformation, brought the view that much more was involved than mere irritation. In fact, as is noted in Chapter XII, carcinogenic potency was found to be inversely related to the irritative properties of certain chemical agents.

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* Broussais, F. J. V. (1826), *Histoire des Phlegmasies ou Inflammations Chroniques.* 4th ed. Paris.

† Virchow, R. (1863), *Die Krankhaften Geschwülsten.* 3rd ed. Berlin: Hirschwald.

‡ Berglas, A. (1959), 'The Genesis of Cancer and Possible Interference with its Growth', *Trans. N.Y. Acad. Sci.*, **22**, 83.

§ Menkin, V. (1960), 'Role of Inflammation in Carcinogenesis', *Br. med. J.*, **1**, 1585.

‖ Rous, P., and Kidd, J. G. (1941), 'Conditional Neoplasms and Subthreshold Neoplastic States: Study of Tar Tumors of Rabbits', *J. exp. Med.*, **73**, 365.

The induction of malignant tumours by plastic and other films (noted later in this chapter) provides an illustration of irritation as a most unlikely mechanism in the carcinogenic process. The tumours following film embedding bear no relation to the thickness of the film, whereas this might be expected if irritation were a factor. The qualities of smoothness and inertness of the film cannot be correlated with tumour production; if irritation were a factor, the less smooth and the more chemically active films would induce more tumours. Perhaps the most crucial of the arguments against irritation as a cause is that malignancy follows the implantation of a sheet of film, but it does not when the same material, in the form of powders, fibres, or pellets, is embedded.

METAZOON PARASITES

In a number of instances, carcinogenic activity of a metazoon parasite is strongly suggested by the evidence; in other examples, the premises are flimsy and there is much doubt.

Schistosoma Haematobium.—The attributability of schistosoma as a cause of carcinoma of the bladder has a reasonable, but not certain, validity, based upon the sequences of pathological changes, geographical pathology, and statistical associations. The subject is discussed in some detail in Chapter V.

Spiroptera Neoplasticum.—This was the first metazoon parasite for which there seemed to be good grounds for the label of an experimental carcinogenic agent. Fibiger* originally described a rat stomach carcinoma supposedly due to *Spiroptera neoplasticum*. The parasite larvae infest cockroaches, and when eaten by rats, mature to adult forms which, Fibiger held, caused primary carcinoma of the stomach with metastatic spread to the lungs. This work was later questioned; it appears that the lesions described, including those in the lungs, were probably not neoplastic but arose from dietary deficiencies.

Taenia Crassicollis and Cysticercus Fasciolaris.—The latter is the larval form of the former, which is the cat tapeworm. Rats eat the tapeworm which is passed with the cat's faeces; tapeworm ova pass via portal veins to the liver, where they grow to larvae and cause cysts; sarcomas develop in the tissue adjacent to the connective-tissue cyst wall. Bullock and Curtis† fed *Taenia crassicollis* ova to rats, proving this sequence of events. Later Dunning and Curtis‡ induced sarcomas by intraperitoneal injection of ground-up parasites.

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* Fibiger, J. (1913), 'Untersuchungen über eine Nematode und daren Fähigkeit, papillomatose und carcinomatose Geschwulstbildungen in Magen der Ratte herforzurufen', *Z. Krebsforch.*, **13**, 217.

† Bullock, F. D., and Curtis, M. R. (1924), 'Reactions of Tissues of Rat's Liver to Larvae of *Taenia crassicollis* and Histogenesis of Cysticercus Sarcoma', *J. Cancer Res.*, **8**, 446; and (1925), 'Types of Cysticercus Tumors', *Ibid.*, **9**, 425.

‡ Dunning, W. F., and Curtis, M. R. (1946), 'Multiple Peritoneal Sarcoma in Rats from Intraperitoneal Injection of Washed, Ground Taenia Larvae', *Cancer Res.*, **6**, 668.

Opisthorchis Felineus is a trematode infesting the muscle of fish. Passage to man occurs when he eats raw fish. The worm reaches the bile-ducts of the liver, where it may induce biliary cirrhosis and ultimate cholangiocarcinoma (Strong*). As with the next variety, the evidence is strongly suggestive, but not proven, that the parasite is carcinogenic.

Opisthorchis (Clonorchis) Sinensis is also a trematode which infests man. An encysted form of immature worm occurs in fish; in parts of Japan and China the fish is eaten raw; the worms mature in the bile-duct system; cirrhosis, adenomata, and carcinomata follow.

The mechanism of action is not known. Chronic irritation, a chemical carcinogen with the parasite, and a virus introduced with the parasite, have been invoked at one or other time.

FUNGI

Aspergillus Flavus.—Blount† traced a fatal condition in turkeys to toxic products of strains of *Aspergillus flavus*, which contaminated some lots of Brazilian ground-nut meal. The toxic effects were mainly on liver cells, and they were found to cause similar damage in rats, where primary hepatomas and bile-duct cystadenomas developed. Dickens,‡ in a review of carcinogenic lactones, records that several toxic components come from the fungus. Aflatoxin B, the most potent, proved to be a lactone.

Other fungal toxins also produce liver necrosis and ultimate hepatomas in rodents. *Penicillium islandicum*, a fungus infecting rice in parts of Japan, and *Penicillium griseofulvum* are quoted by Roe and Lancaster§ as having some supporting evidence for causing hepatoma.

These authors, Roe and Lancaster, refer to work showing the similarity of hepatotoxic and carcinogenic activity by *Aspergillus flavus* toxins and *Senecio* alkaloids. The latter substances had been shown by Cook and others‖ as a cause of hepatic cell necrosis followed by primary liver tumours in rats. Consequently, the question had been raised as to whether the excessively high incidence of primary cancer of the liver in some parts of Africa (*see* Chapter V) might not be related, especially as *Senecio* plants were known to be used as 'tribal' medicinal remedies in certain geographical areas. A simple and direct relationship has not been discovered in the case of *Senecio* alkaloids (although associated factors like diet, alcohol, etc., might still prove its contributory culpability). However, the toxins from *Aspergillus flavus*, named 'aflatoxins',

* Strong, R. P. (1931), 'The Role played by Helminths in the Production of Tumours in Man and Animals', *Int. Clin.*, **4**, 68.

† Blount, W. P. (1961), *Turkeys*, **9**, 57. Quoted by Roe, F. J. C., and Lancaster, M. C. (1964), 'Natural, Metallic and other Substances, as Carcinogens', *Br. med. Bull.*, **20**, 127.

‡ Dickens, F. (1964), 'Carcinogenic Lactones and Related Substances', *Ibid.*, **20**, 96.

§ Roe, F. J. C., and Lancaster, M. C. (1964), 'Natural, Metallic and Other Substances, as Carcinogens', *Ibid.*, **20**, 127.

‖ Cook, J. W., Duffy, E., and Schöntal, R. (1950), 'Primary Liver Tumours in Rats following Feeding with Alkaloids of *Senecio Jacobaea C*', *Br. J. Cancer*, **4**, 405.

and possibly toxins from other moulds as well, now enter the field of suspect hepatic cancerigenic agents; and they are being submitted to further investigation.

TISSUE CULTURE

Since 1910, when Carrel and Burrows* reported the successful culture of human sarcoma cells *in vitro*, extensive investigation into different aspects of cells grown in tissue cultures has contributed appreciably to an understanding of many biological phenomena of normal and abnormal cells. Reference to the use of tissue cultures appear in Chapter XVIII and Chapter II. Its value and relevance to the study of cancer have barely been exploited, and the added new technique of electron microscopy holds much promise for its future potential. The discussion in the context of the present chapter concerns the changes, particularly in the direction of development and regression of carcinogenic properties of cells grown *in vitro*.

Changes affecting Culture-grown Cells.—Changes may be effected by the exhibition of external agents, or the cells may undergo spontaneous transformation. Whether induced or spontaneous, the changes may be temporary or reversible, when they are known as 'modulations', or the transformations may be irreversible and are then known as 'alterations'. Alterations may involve genetic mutation but are not necessarily of this nature, as differentiation may occur on the lines of cytological development in embryonic growth and maturation, or along the reverse course from differentiated cells to immature ones. The actual nature of alteration is still an unsettled problem, and its solution may provide fundamental information on malignant cell genesis and behaviour.

Microscopic features of tissue-culture cells undergoing modulation and, more particularly, alteration may suggest malignant change; but morphological characters have so far proved inaccurate in providing a basis for diagnosis of malignancy; the crucial test is retransplantation of the cultured tissue into a normal host: here, the presence or absence of autonomous growth is the important criterion.

Experimentation includes purposive variation of the *in vitro* conditions, e.g., in terms of the media, general environmental circumstances, confrontation by different agents, time programmes, and sub- and serial cultivation; and it also involves selection and preparation of hosts to receive retransplants, e.g., species, age, and genetic constitution of the animal, pretreatment with cortisone and X-irradiation to overcome rejection mechanisms, and serial transplantation, i.e., passage of tumour cells through a series of susceptible animals, with the object of possible augmentation of certain potentialities which may have been weakened by culture variations.

* Carrel, A., and Burrows, M. T. (1910), 'Human Sarcoma cultivated outside the Body', *J. Am. med. Ass.*, **55**, 1732.

Malignant Cells in Culture.—Lewis* observed that tumour cells retained their malignant properties during cultivation *in vitro*. This was based on the production of tumours on injection of the cells, even after heterologous culture, into species of the same strain that had originally provided the tumour cells. Malignancy can also be induced in cells in culture; e.g., normal fibroblasts from mice, explanted on cultures, were shown by Earle† to undergo sarcomatous transformation after exposure to 20-methylcholanthrene.

Earle and others‡ later reported that in the 6 strains of cells so rendered malignant there was a progressive decrease in sarcoma production on reinjection of the cultured cells into the original strain of C3H mice. The measure of the diminution of malignant potency may be judged from the behaviour of one of the cell strains, designated strain L, in which there was a drop from an incidence of 63 per cent in 1943 to 1 per cent in 1946. Since these experiments made use of mixed strains of cells, and a fortuitous selection of a special type may have occurred and so vitiated the results, pure strains were developed by Sanford and others.§ Single cells were isolated from strain L, and the distinct clones produced by their descendants were used to test tumour-producing potency and other properties. Sanford and colleagues‖¶ reported the findings which included: sarcoma production occurred in 15 per cent, i.e., lower than the original mixed strain, but higher than the reduced power after long serial cultivation; potency was enhanced by increased dosage of culture cells; pretreatment of host-to-be C3H mice by total body X-ray irradiation raised the sarcoma tumour production to 68 per cent, indicating the probability that irradiation reduced host immunity; and, according to the 1954 report, the supposition emerged that spontaneous alterations were occurring in the cultures, and that these were probably of a genetic character.

Burnet** supports the interpretation of these findings as indicative of somatic mutation and adds the remark, '. . . but would require a fantastic series of secondary *ad hoc* assumptions to be interpreted as manifestations of virus infection'.

Variation of the tumour-producing properties of cultivated cells extends to involve the type of tumour produced. Sanford and others†† report the induction of sarcomas in mice on reinjection of culture-grown cells from hepatomas, melanomas, and thyroid tumours.

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* Lewis, W. H. (1935), 'Normal and Malignant Cells', *Science*, **81**, 545.

† Earle, W. R. (1943), 'Changes induced in a Strain of Fibroblasts from a Strain of C3H Mouse by the Action of 20-methylcholanthrene', *J. natn. Cancer Inst.*, **3**, 555.

‡ Earle, W. R., Shelton, E., and Schilling, E. L. (1950), 'Production of Malignancy in Vitro: XI. Further Results from Reinjection of in Vitro Cell Strains into Strain C3H Mice', *Ibid.*, **10**, 1105.

§ Sanford, K. K., Earle, W. R., and Likely, G. D. (1949), 'The Growth in Vitro of Single Isolated Tissue Cells', *Ibid.*, **9**, 229.

‖ Sanford, K. K., Likely, G. D., and Earle, W. R. (1954), 'The Development of Variations in Transplantability and Morphology within a Clone of Mouse Fibroblasts transformed to Sarcoma-producing Cells in Vitro', *Ibid.*, **15**, 215.

¶ Sanford, K. K., Hobbs, G. L., and Earle, W. R. (1956), 'The Tumor Producing Capacity of Strain L Mouse Cells after 10 Years in Vitro', *Cancer Res.*, **16**, 162.

** Burnet, Sir M. (1957), 'Cancer—a Biological Approach', *Br. med. J.*, **1**, 779.

†† Sanford, K. K., Likely, G. D., Evans, V. J., Mackey, C. J., and Earle, W. R. (1952), 'The Production of Sarcomas from Cultured Tissues of Hepatoma, Melanoma, and Thyroid Tumours', *J. natn. Cancer Inst.*, **12**, 1057.

Spontaneous Transformation of Normal to Malignant Cells.—During the course of experiments on cultured cells which had been transformed to malignant ones by methylcholanthrene, Earle and his co-workers noted spontaneous malignant alterations in some of the control cultures. This was reported by Earle* and Earle and Nettleship.† The possibility that the control cultures may have been contaminated by methylcholanthrene was eliminated by adequate separation, and it was confirmed that, occasionally, normal mouse fibroblasts cultured for long periods and by serial transfer in heterologous media were irreversibly transformed into sarcoma-inducing cells, as proved on reinjection into mice of the original strain. Firor and Gey‡ and Gey and others§ report a similar alteration, of infrequent occurrence, in untreated rat fibroblasts in culture.

The observations on malignant transformation in culture-grown cells from experimental animals carries provocative possibilities, and, although experimentation is necessarily limited in humans, a considerable volume of work is being conducted. Illustrations may be seen in the report of Puck and Fisher|| on spontaneous mutants in HeLa cells, and that of Leighton and associates¶** on histologically malignant changes in cultivations of human normal fibroblasts.

FILMS AND SHEETS

During the conduct of experiments using cellophane wrapping around rat kidneys in order to produce hypertension, Oppenheimer and others†† accidentally discovered that the plastic sheet induced sarcomas of the kidney. Soon after this discovery it was shown that embedding films in the abdominal wall also produced sarcoma. It was then recalled that Turner‡‡ had reported sarcomatous tumour formation at the sites of subcutaneously embedded bakelite disks. It appeared that as this was a similar carcinogenic process, it should occupy pride of place in being the first record of a new mode of tumour induction.

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* Earle, W. R. (1943), 'Production of Malignancy in Vitro. IV. The Mouse Fibroblast Cultures and Changes in Living Cells', *J. natn. Cancer Inst.*, **4**, 165.

† Earle, W. R., and Nettleship, A. (1943), 'Production of Malignancy in Vitro. V. Results of Injection of Cultures in Mice', *Ibid.*, **4**, 213.

‡ Firor, W. M., and Gey, G. O. (1945), 'Observations on the Conversion of Normal into Malignant Cells', *Ann. Surg.*, **121**, 700.

§ Gey, G. O., Gey, M. K., Firor, W. M., and Self, W. O. (1949), 'Cultural and Cytological Studies on Autologous Normal and Malignant Cells of Specific in Vitro Origin. Conversion of Normal into Malignant Cell', *Acta Un. int. Cancr.*, **6**, 706.

|| Puck, T. T., and Fisher, H. W. (1956), 'Genetics of Somatic Mammalian Cells. I. Demonstration of Existence of Mutants with Different Growth Requirements in a Human Cancer Cell (HeLa)', *J. exp. Med.*, **104**, 427.

¶ Leighton, J., Kline, I., and Orr, H. C. (1956), 'Transformation of Normal Human Fibroblasts into Histologically Malignant Tissue in Vitro', *Science*, **123**, 502.

** Leighton, J., Kline, I., Belkin, M., Legallais, F., and Orr, H. C. (1957), 'The Similarity in Histological Appearance of Some Human "Cancer" and "Normal" Cell Strains in Sponge-matrix Tissue Culture', *Cancer Res.*, **17**, 359.

†† Oppenheimer, B. S., Oppenheimer, E. T., and Stout, A. P. (1948), 'Sarcomas Induced in Rats by implanting Cellophane', *Proc. Soc. exp. Biol. Med.*, **67**, 33.

‡‡ Turner, F. C. (1941), 'Sarcomas at Sites of Subcutaneously implanted Bakelite Discs', *J. natn. Cancer Inst.*, **2**, 81.

Films and Sheets, *continued.*

A series of publications by Oppenheimer and associates*†‡§‖ disclosed a notable range of phenomena associated with carcinogenesis by polymer, plastic, glass, quartz, silk, and metal films and foils. Certain qualities appeared to be essential for tumour induction. 'Purity' was not important: impure 'commercial' and specially prepared purified forms were compared; sometimes the pure product was more productive of tumours. Continuity of the sheet was significant; the use of perforated films reduced tumour formation, and implantation of fragments, fibres, or powders of the material did not induce tumours.

The size of the sheet has an important bearing on tumorigenic capacities. Alexander and Horning¶ showed that when films 2 cm. × 2 cm., 1 cm. × 1 cm., and ½ cm. × ½ cm. were embedded, the largest size produced most tumours and the latent interval was shortest; the smallest size produced only 1 tumour in 24 rats, and the latent period was more than 20 months. A significant finding in experiments with larger films in which tumours failed to develop was that the sheets had become folded in their subcutaneous position; a finding which reinforces the importance of the extent of surface area.

Histological Changes following Embedding.—Within 2–3 weeks, the film usually becomes encapsulated by a sheath of connective tissue of varying thickness. This forms a pocket for the film which remains free and unattached within it. The generalized fibroblastic activity of the sheath endures for a time, and is followed first by reduced activity and then almost complete inactivity. In some, localized proliferative areas may be found. About 14–24 months later, the time varying with the type and size of the film and with other unknown factors, sarcomatous cells appear. The tumour cells are usually in subcutaneous tissues, mostly on both sides of the film, sometimes only on one aspect; muscle is occasionally invaded, and more rarely, peritoneum. In a small proportion, about 5 per cent, the lesion is an osteogenic sarcoma.

Both the film and the reactive sac of connective tissue affect the ultimate occurrence of malignancy. In the case of polystyrene, if the film is removed within 6 months, despite the presence of a well-formed sheath, a tumour does not develop; if it remains in situ for longer, a tumour appears; if the sheath and film are removed after the critical 6-month period, tumours are prevented.

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* Oppenheimer, B. S., Oppenheimer, E. T., and Stout, A. P. (1952), 'Sarcomas induced in Rodents by embedding Various Plastic Films', *Proc. Soc. exp. Biol. Med.*, **79**, 366.

† Oppenheimer, B. S., Oppenheimer, E. T., Danishefsky, I., and Stout, A. P. (1953), 'Malignant Tumors Resulting from imbedding Plastics in Rodents', *Science*, **118**, 305.

‡ Oppenheimer, B. S., Oppenheimer, E. T., Danishefsky, I., Stout, A. P., and Eirich, F. R. (1955), 'Further Studies of Polymers as Carcinogenic Agents in Animals', *Cancer Res.*, **15**, 333.

§ Oppenheimer, B. S., Oppenheimer, E. T., Danishefsky, I., and Stout, A. P. (1956), 'Carcinogenic Effects of Metals in Rodents', *Ibid.*, **16**, 439.

‖ Oppenheimer, B. S., Oppenheimer, E. T., Stout, A. P., Willhite, M., and Danishefsky, I. (1958), 'The Latent Period in Carcinogenesis by Plastics in Rats and its Relation to the Presarcomatous Stage', *Cancer*, **11**, 204.

¶ Alexander, P., and Horning, E. S. (1959), 'Observations on the Oppenheimer Method of inducing Tumours by Subcutaneous Implantation of Plastic Films', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

Mechanism of Action.—The theory of chronic irritation in relation to plastic sheets has been noted earlier in this chapter, and the point was brought out that the evidence from film-carcinogenesis runs counter to the theory.

Although there are authoritative opinions that the mechanism of carcinogenesis is essentially chemically triggered, the weight of evidence seems to oppose this hypothesis. The wide range of sarcoma-inducing substances would involve numerous separate hypothetical chemical interactions. Many of the films are inert, or nearly so, and it is difficult to visualize the liberation from them of free radicals into the tissues; in any event, measurements of free radicals in polymers do not indicate that those with more radicals cause more tumours. A final, and apparently powerful, piece of evidence against a direct chemical carcinogenic action is that fragmented, powdered, or filamentous material does not induce tumours.

The physical form is so outstanding a feature that it must be fundamental to any explanation of the mechanism. An analogy has been drawn with certain tissue-culture experiments, in which the transformation of normal fibroblasts to sarcomatous tissue was facilitated by exposing the culture to intermittent anoxia (Goldblatt and Cameron*). Alexander† suggests the possibility that the film creates an unnatural environment of deficient oxygen, and defects in other nutrient substances, so that, as in the malignant transformation observed by Goldblatt and Cameron, some normal growth-control is deleted. Kaplan‡ accepts the view of physical interference by the film, but not the idea that this operates via anoxia. The interference, he thinks, is with normal growth: connective tissue is disconnected; it is prevented from normal repair activity; the result of persistent stimulus to growth together with sustained prevention of normal growth leads to cancer.

The final answer is not yet in sight, but the similarities between spontaneous malignant transformation of normal cells in cultures and that following film implantation seem striking; almost as if the embedded sheet creates an *in vitro* set of conditions *in vivo*.

* Goldblatt, H., and Cameron, G. (1953), 'Induced Malignancy in Cells from Rat Myocardium subjected to Intermittent Anaerobiosis during Long Propagation in Vitro', *J. exp. Med.*, **97**, 525.

† Alexander, P. (1954), 'The Reactions of Carcinogens with Macromolecules', *Adv. Cancer Res.*, **2**, 1.

‡ Kaplan, H. S. (1959), Discussion on Tumour Induction with Plastic Films, in Ciba Symposium on *Carcinogenesis*. London: Churchill.

CHAPTER XXI

HEREDITY IN CANCER

Appraisal of the place of heredity in cancer has to be viewed against the background of the prevalence of the disease. When cancer is as common as to affect 1 in 4 or 5 people, the evidence for heredity as an aetiological entity requires the most critical analysis.

In the experimental field, the concept of hereditary susceptibility to cancer, or hereditary diminution of tissue resistance to it, has found considerable support. Whether deductions derived from laboratory experiments on animals can be applied to humans is a matter of doubt. A salutary example of an apparently logical concept being partly undermined as doubtful derives from two great pieces of research by workers whose names will for ever be milestones in the story of the investigation of cancer.

In 1914, Maud Slye,* following protracted and well-controlled experiments on the inbreeding of mice, proved, apparently conclusively, that susceptibility to the development of spontaneous tumours, including breast cancer, was passed on as an heritable factor in successive generations. In fact, a strain of inbred mice was evolved in which there occurred an 80 per cent spontaneous breast-cancer rate.

Twenty-five years later, on another page of the history of cancer research, Bittner† showed that mice of a low breast-cancer strain nursed by mothers of a high breast-cancer strain developed breast cancer with a frequency approaching the maternal rate; and further, that mice of a high breast-cancer strain nursed by mothers of a low breast-cancer strain showed a reduced cancer rate. This research points to the passage of a cancerigenic agent in maternal milk to offspring. This Bittner milk agent may be a virus, but whatever its nature, it undermines the full acceptance of heritable susceptibility to breast cancer in inbred mice.

Whilst maintaining the mental discipline imposed by critical appreciation, it is possible to evaluate and accept with reservation probable or possible evidence of hereditary factors in neoplasia.

Congenital Tumours.—Several records of congenitally transmitted tumours are extant. Wells‡ summarizes the evidence and quotes cases of intra-uterine transmission of tumours: 1 bronchogenic carcinoma, 1 lymphosarcoma, and 2 melanomas. Darjeon and

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* Slye, Maud (1914), 'The Incidence and Inheritability of Spontaneous Tumours in Mice'. Second report. *J. med. Res.*, **25**, 281.

† Bittner, J. J. (1939), 'Relation of Nursing to Extrachromosomal Theory of Breast Cancer in Mice', *Am. J. Cancer*, **35**, 90.

‡ Wells, H. G. (1940), 'Occurrence and Significance of Congenital Malignant Neoplasms', *Archs Path.*, **30**, 535.

others* describe a melanoma in an infant. The great rarity of such tumours suggests some bizarre form of metastasization across the placenta.

Other congenital tumours, of which rhabdomyosarcoma, neuroblastoma, nephroblastoma, retinal tumours, medulloblastoma, and teratoma are examples, occur. Most of them arise during embryonic life from embryonal tissues, but there is no evidence that they are hereditary in origin.

Congenital leukaemia is of interest in that some cases suggest a strong genetic factor, e.g., those reported by Anderson† in a family in which 5 of 8 children were affected. However, there are other congenital leukaemias which, as has been shown by Russell and Russell,‡ can reasonably be ascribed to an environmental agent, viz., ionizing radiation.

Judgement must be suspended, but it is possible that in leukaemia, as well as in a number of other neoplastic conditions, a genetic susceptibility of varying strength is combined with an environmental agent which promotes the malignant change. This type of combined mechanism for the induction of leukaemia is supported by Kolmeier and Bayrd,§ who report on a leukaemic mother and son, and review the literature, which, they claim, contains at least 100 authentic cases of familial leukaemia.

Court Brown‖ compares the dual mechanism to the two-stage hypothesis of chemical carcinogenesis (*see* Chapter X), describing genetic predisposition as an inducing agent and the environmental trigger as the promoting agent.

Carcinoma in Twins.—Tumours affecting different organs in a pair of twins do not provide evidence of a probable congenital or hereditary factor in their causation; tumours of the same organ are, however, very suggestive. Such examples are extremely rare.

Koller¶ noted one recorded case of gastric cancer in identical male twins, cancer of the cervix in 17 pairs of twins, and leukaemia in 4 pairs of twins. Even this rare occurrence, especially in monochorial twins, is deemed greater than might be expected from chance, and is thus regarded as offering some support for the theory of genetic predisposition.

Inherited Cancerous and Precancerous Conditions.—There are a number of conditions, initially either frankly tumorous or predisposing to the formation of tumour, that are clearly and definitely

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* Darjeon, H. W., Eversloe, J. W., and Del Duca, V. (1950), 'Malignant Melanoma in an Infant', *Cancer*, **3**, 299.

† Anderson, R. C. (1951), 'Familial Leukemia: A Report of Leukemia in Five Siblings, with a Brief Review of the Genetic Aspects of this Disease', *Am. J. Dis. Child.*, **81**, 313.

‡ Russell, L. B., and Russell, W. L. (1952), 'Radiation Hazards to the Embryo and Fetus', *Radiology*, **58**, 369.

§ Kolmeier, R. H., and Bayrd, E. D. (1963), 'Familial Leukemia: Report of Instance and Review of Literature', *Proc. Staff Meet. Mayo Clin.*, **38**, 523.

‖ Court Brown, W. M. (1962), 'Role of Genetic Change in Neoplasia', *Br. med. J.*, **1**, 961.

¶ Koller, P. C. (1952), 'The Genetic Component of Cancer', in *Cancer*, Vol. 1 (Ed. Raven, R. W.). London: Butterworths.

Inherited Cancerous and Precancerous Conditions, *continued.*

inherited. The aetiology of these conditions is unequivocally linked with genetically determined factors.

RETINOBLASTOMA.—The condition is rare, being estimated at 1 case per 34,000 live births. The tumour arises from undifferentiated, embryonal optic retinal cells; it grows rapidly, destroying the bulb of the eye and forming a fungating mass. Two-thirds of the cases present before the age of 3 years. The sexes are equally affected. It appears to be multifocal in origin, bilateral tumours occurring in about 25 per cent. As the tumour cells differentiate, they form rod and cone cells in rosette pattern; less often there is further differentiation to fibrillary tissue and still more rarely to retinal layers.

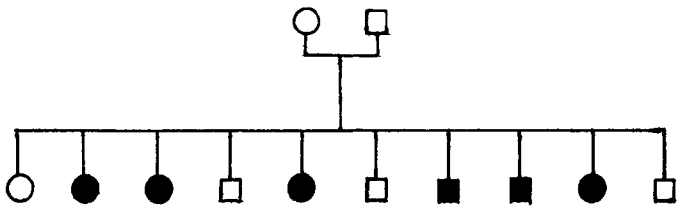

Fig. 27.—Pedigree of retinoblastoma inherited via a dominant gene in one generation. Neither parent affected clinically; 6 of 10 offspring affected. □ Normal male; ○ Normal female; ■ ● Affected offspring.

Inheritance of the condition is well documented, among others by Weller.* *Fig.* 27 is taken from one of his records. It is determined by a single dominant gene passed by either parent. It has also been known to occur by chance, being due to an occasional mutation, which then initiates new familial lines of inheritance.

XERODERMA PIGMENTOSUM.—A disease manifesting in early childhood; the exposed skin becomes red, pigmented, and atrophic. These features are aggravated by sunlight, which, at young ages, causes more marked changes resulting in multiple cancers of squamous- and basal-cell types; sometimes both types are present and they may be mixed.

Inheritance is dependent upon a recessive autosomal gene, and there is a high incidence in first-cousin marriages. *Fig.* 28 shows such a pedigree recorded by Koller.†

In this condition, the heritable factor is not cancer itself, but a much heightened sensitivity to sunlight which promotes the cancer.

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* Weller, C. V. (1941), 'The Inheritance of Retinoblastoma and its Relationship to Practical Eugenics', *Cancer Res.*, **1**, 517.

† Koller, P. C. (1948), 'Inheritance of Xeroderma and its Chromosome Mechanism', *Br. J. Cancer*, **2**, 149.

NEUROFIBROMATOSIS OR VON RECKLINGHAUSEN'S DISEASE.—The disease presents with multiple small hard nodules in the skin, often symmetrically disposed along cutaneous nerves. The nodules are neurofibromas, most or all of which are benign although they lack encapsulation. Cranial and visceral nerves may also be involved. The proliferation of cellular elements may be roughly confined within nerve-sheaths and present as plexiform neuromas, a form of the condition most

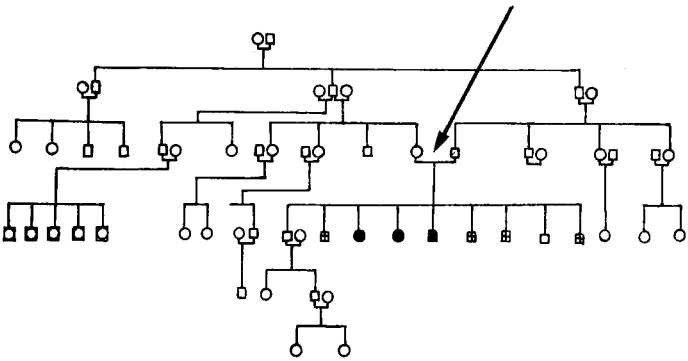

Fig. 28.—Pedigree of inheritance of xeroderma pigmentosum by a recessive autosomal gene. The first-cousin marriage (marked by an arrow) produced the 3 clinical cases. □ Normal male; ○ Normal female; ⊞ Abortions or neonatal deaths; ◘ Sex unknown; ■ ● Affected persons.

commonly found in the 5th cranial and upper cervical nerves. The skin is customarily pigmented in irregular patches. Soft overgrowths of skin and connective tissue are not uncommon, causing enlarged, irregular overhanging folds and producing a form of elephantiasis of a limb. The tumours often remain unchanged for a long time.

Histologically, there are variations in appearance: cellularity (mainly of spindle shapes) varies in quantity and arrangement; it is often set in whorled fibroblastic tissue, and is also often associated with nerve-fibres traversing the tumour mass.

The multiple tumours may remain benign for many years or even throughout life, but there is a tendency for one or more to undergo sarcomatous degeneration. The sarcoma presents in different forms: fibrosarcoma, myxosarcoma, highly cellular in either spindle shapes or mixed and pleomorphic forms, have all been reported.

The condition is genetically transmitted by a dominant gene. A pedigree of an affected family is shown in *Fig.* 29, which is adapted from the records of Gardner and Frazier.*

* Gardner, W. J., and Frazier, C. H. (1930), quoted by Wright, G. Payling (1954), *An Introduction to Pathology*. London: Longmans.

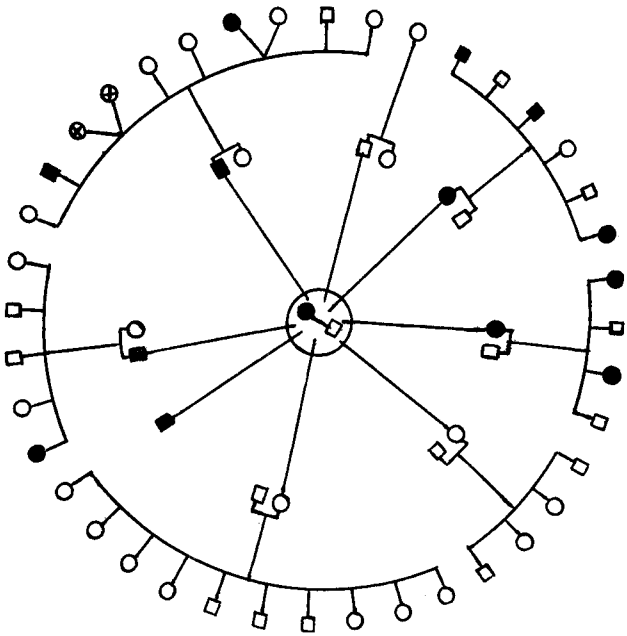

Fig. 29.—Pedigree of inheritance of the dominant gene of neurofibromatosis. □ Normal male; ○ Normal female; ⊕ Abortions or neonatal deaths; ■ ● Affected persons.

POLYPOSIS OF THE COLON.—Multiple adenomatous polypi, studding colonic and often rectal mucosa, appear most commonly during the second and third decades of life, affecting males and females equally. The polypi are highly susceptible to cancerous transformation which affects one or several of the polypi. Dukes,* in tracing the pedigrees of 41 affected families, found that 70–75 per cent of those with polypi developed cancer. Most of the cancers appeared in from 10 to 20 years after the initial diagnosis of the presence of the polypi.

The nature of the disease is considered by Lockhart-Mummery and Dukes† to be an unduly rapid growth of large-bowel epithelium, leading first to hyperplasia, then to adenomata, and eventually to adenocarcinoma.

* Dukes, C. E. (1952), 'Familial Intestinal Polyposis', *Ann. Eugen.*, **17**, 1.
† Lockhart-Mummery, J. P., and Dukes, C. E. (1939), 'Familial Adenomatosis of Colon and Rectum, its Relation to Cancer', *Lancet*, **2**, 586.

Inheritance of multiple polyposis of the colon is via the passage of a dominant gene, transmitted by either sex, and affecting the sexes equally. There are records of apparently spontaneous and sporadic origin of multiple polyposis which also has a proclivity to the formation of cancer. *Figs.* 30, 31, and 32, showing pedigrees recorded by Dukes* and Lockhart-Mummery and others,† demonstrate several important aspects of the inherited condition.

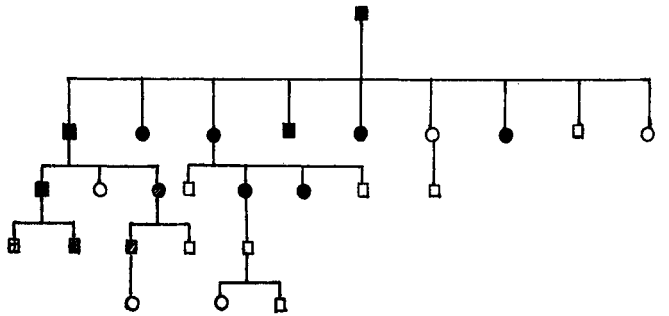

Fig. 30.—Inheritance of polyposis coli. Pedigree of family with longest period of follow-up in Dukes (1952) series. During the 26 years of observation, from the first report by Lockhart-Mummery in 1925‡ to 1951, shown here, it appears that only those who inherit the disease pass it on; half the children are affected; and the distribution in the sexes is equal. □ Normal male; ○ Normal female; ■ ◒ Polyposis; ■ ● Cancer.

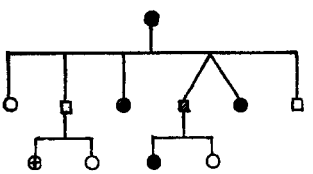

Fig. 31.—Transmission of polyposis coli. Both members of a twin were affected. The daughter of the male member of the twin was charted as 'polyposis' in the 1952 paper and as having developed cancer in the 1956 paper.

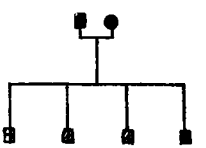

Fig. 32.—Transmission of polyposis coli. Both parents affected. One child died in childhood, the other 3 had polyposis, 1 of whom died of cancer. This is an example of increased liability to inherit the abnormality. It should also be noted that severity and liability vary from family to family even when one parent is affected.

□ Normal male; ○ Normal female; ■ ◒ Polyposis; ■ ● Cancer; ⊞ ⊕ Died young.

* Dukes, C. E. (1952), 'Familial Intestinal Polyposis', *Ann. Eugen.*, **17**, 1.
† Lockhart-Mummery, H. E., Dukes, C. E., and Bussey, H. J. R. (1956), 'The Surgical Treatment of Familial Polyposis of the Colon', *Br. J. Surg.*, **43**, 476.
‡ Lockhart-Mummery, J. P. (1925), 'Cancer and Heredity', *Lancet*, **1**, 427.

Inherited Cancerous and Precancerous Conditions, *continued.*

DIAPHYSIAL ACLASIS.—This condition is characterized by the formation of multiple osteochondromata mainly near the epiphyses of long bones in children and adolescents. These benign tumours, which grow and ossify *pari passu* with the child, have a tendency, estimated by Jaffe* at more than 20 per cent, to the supervention of chondrosarcoma.

The findings of Stocks and Barrington† that about two-thirds of cases were transmitted by an affected father, and that, although unaffected fathers seldom transmitted the disease, unaffected mothers frequently did, have been brought into question by recent work of Solomon.‡ His investigations included radiographical studies, which showed that females transmitting the disease were just as often affected as male parents. Therefore, inheritance was in fact carried by a dominant gene, and not, as might be suggested by the work of Stocks and Barrington, via a 'relative' dominant gene.

Family Pedigrees.—In the genetically determined conditions noted above, the plotting of family pedigrees provides basic evidence for the fact of genetic transmission. The study by Dukes§ of familial intestinal polyposis is a classic in this regard. However, the whole group of conditions in which such proven inheritance of cancer, or marked susceptibility to cancer, is present accounts for but a very small proportion of human cancers. It follows that deductions which are valid in this small segment cannot, on the basis of available evidence, be applied with anything approaching the same certainty to the much larger cancer area as a whole.

In order to test this aspect, a number of pedigree studies in fields of cancer which are much more common have been conducted. Whilst firm inferences are not in order from these studies, there may be some suggestive evidence of a positive influence of heredity as a factor in several malignant tumours. Some authorities, for example Berenblum,‖ consider that it is not the cancer which is inherited, but host susceptibility or lack of resistance to cancer. Whatever the precise nature of the factor transmitted, the following studies are of interest and significance.

BREAST CANCER.—Jacobsen¶ studied the incidence of cancer in relatives of 200 cases of proven breast cancer and compared the figures to those found in a control series of similar size.

Female relatives of women suffering from breast cancer showed a notably increased breast-cancer rate, amounting to more than double that found in the control group. In a similar study,

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* Jaffe, H. L. (1943), 'Hereditary Multiple Exostosis', *Archs Path.*, **36**, 335.

† Stocks, P., and Barrington, A. (1925), *Hereditary Disorders of Bone Development.* Eugenics Lab. Memoirs, No. 22. London: Cambridge Univ. Press.

‡ Solomon, L. (1964), 'Hereditary Multiple Exostosis', *Am. J. Human Genetics*, **16**, 351.

§ Dukes, C. E. (1952), 'Familial Intestinal Polyposis', *Ann. Eugen.*, **17**, 1.

‖ Berenblum, I. (1952), *Man Against Cancer. The Story of Cancer Research.* Baltimore: Johns Hopkins Press.

¶ Jacobsen, O. (1946), *Heredity in Breast Cancer.* London: Lewis.

Penrose and others* found a significantly increased incidence of breast cancer in sisters and mothers of known breast-cancer cases. Further confirmatory evidence is reported by Smithers and others.† Markedly more cases of breast cancer appeared in maternal grandmothers and aunts than in such relatives on the paternal side. The latter set of figures may be regarded as a control group. However, the fact that inheritance appears to be mainly maternal raises the possibility of the passage of a milk agent. Positive evidence for this is lacking, but it remains a plausible supposition.

Other circumstances enter the field of evaluation of heredity in the aetiology of breast cancer. Social customs, especially marital age, size of family, and breast-feeding, have an influence on breast cancer (*see* Chapter VII). It is reasonable to assume that fairly uniform social circumstances and customs prevail in one family group, and this may contribute to a particular rate of breast cancer in the members of that family.

An associated circumstance challenging the validity of heredity as part of the causation of breast cancer arises from the known hormonal influences in its aetiology. Hormones may be affected by customs, e.g., age of marriage and age of childbearing as well as by hereditary factors, and so there is an intrusion of an additional aetiological factor.

STOMACH CANCER.—Videbaek and Mosbech‡ surveyed over 3200 relatives of 302 gastric cancer patients and also a like number in a control series including persons of the same age and sex distribution. In summary, their findings show:—

1. The incidence of gastric cancer in relatives of patients with gastric cancer is 4 times higher than in the control series.
2. The incidence of cancer in other sites did not differ significantly in the gastric cancer and control groups.
3. In the gastric cancer group, 41 per cent of all cancers affected the stomach; among the controls, only 17 per cent were so situated.

These findings are confirmed in an analysis by Macklin§ of the incidence of gastro-intestinal cancer in descendants and siblings of gastric cancer patients.

The presumption that the increased rate is due to a greater familial susceptibility appears reasonable. But a number of environmental influences, such as eating and drinking habits and the nature of the water supplies of the geographic area in which the surveys were conducted, has to be taken into consideration before firm deductions become justifiable.

Other collateral evidence supporting heredity as part of the aetiological framework of gastric cancer require scrutiny.

* Penrose, L. S., MacKenzie, H. J., and Kern, M. N. (1948), 'A Genetical Study of Human Mammary Cancer', *Br. J. Cancer*, **2**, 168.

† Smithers, D. W., Rigby-Jones, P., Galton, D. A. G., and Payne, P. M. (1952), 'Cancer of the Breast', *Br. J. Radiol.*, Suppl. 4.

‡ Videbaek, A. A., and Mosbech, J. (1954), 'The Etiology of Gastric Carcinoma elucidated by a Study of 302 Pedigrees', *Acta med. scand.*, **149**, 137.

§ Macklin, M. J. (1955), 'The Role of Heredity in Gastric and Intestinal Cancer', *Gastroenterology*, **29**, 507.

Inherited Cancerous and Precancerous Conditions, *continued.*

PERNICIOUS ANAEMIA arises from a deficiency of an intrinsic factor in the stomach which limits absorption of vitamin B_{12}. This deficiency has a high familial incidence of 30 per cent, indicating heredity as a factor in causation. This has been correlated with reports of increased frequency of gastric cancer in pernicious anaemia. This observation was first reported by Quincke in 1876,* and subsequently repeated by many authors. Kaplan and Rigler† found a threefold greater than expected incidence of gastric cancer in 293 autopsy examinations of pernicious anaemia cases. Mosbech‡ explored the pedigrees of pernicious anaemia and stomach cancer patients. In 2881 relatives of 234 patients with pernicious anaemia, there were 105 stomach cancers and 147 cancers in other sites; whereas in a control group of 2956 persons with similar sex and age distribution, there were 33 gastric cancers and 123 others. The numbers of pernicious anaemia cases in relatives of gastric cancer patients was also higher, although not so markedly so, than the figures in a control series.

Wilkinson§ has thrown some doubt on these findings by his own observations over a period of 21 years of 1820 cases of pernicious anaemia. The 34 instances of gastric cancer and the 47 cancers at other sites are regarded by him as falling within the range of chance expectation.

Schell and others‖ also weaken the validity of an hereditary role by their discovery that the gastric cancers occurring in patients with pernicious anaemia are usually of a special type, namely polypoid, multicentric, and fundal in position; i.e., a type pointing to causation by an exogenous influence.

GASTRIC CANCER AND BLOOD GROUPS.—Aird and others¶ record the relationship between gastric cancer and blood groups; the condition being found more often in group A than in the other blood groups.

Since A, B, and O groups are inherited qualities, it is argued that a genetic factor is concerned with the aetiology of gastric cancer. However, A, B, and O substances are present in salivary and mucoid secretions, making it possible that they exercise

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* Quincke (1876), quoted by Mosbech, J. (1961), 'Genetic Considerations in Polyps and Cancer of the Gastro-intestinal Tract', *Acta Un. int. Cancr.*, **17**, 302.

† Kaplan, H. S., and Rigler, L. G. (1945), 'Pernicious Anaemia and Carcinoma of the Stomach—Autopsy Studies concerning their Interrelationship, *Am. J. med. Sci.*, **209**, 339.

‡ Mosbech, J. (1953), 'Heredity in Pernicious Anaemia. A Proband Study of the Heredity and Relationship to Cancer of the Stomach', M.D. Thesis, University of Copenhagen.

§ Wilkinson, J. F. (1945), 'Pernicious Anaemia and Cancer' (correspondence), *Br. med. J.*, **2**, 664; and (1950), 'Pernicious Anaemia and Gastric Carcinoma' (correspondence), *Ibid.*, **2**, 576.

‖ Schell, R. F., Dockerty, M. B., and Comfort, M. W. (1954), 'Carcinoma of the Stomach associated with Pernicious Anaemia', *Surgery Gynec. Obstet.*, **95**, 710.

¶ Aird, I., Bentall, H. H., and Fraser-Roberts, J. A. (1953), 'A Relationship between Cancer of the Stomach and the A B O Blood Groups', *Br. med. J.*, **1**, 799.

different combinations and reactions with extraneously introduced carcinogens, which may thus occupy a main place in causing gastric cancer.

UTERINE CANCER.—Murphy* reports a higher incidence of uterine cancer in relatives of known uterine cancer patients than in a control series. This familial predisposition does not extend to cancer of other organs.

A possible fallacy in reading into these figures an hereditary influence arises in a similar manner to that discussed in breast cancer. Social class confers different risks of uterine cervical cancer (*see* Chapter VII); this is probably correlated with different rates of childbearing which are known to affect the rate of uterine cervical cancer. Customs of living and family planning tend to be similar within family groups, or in particular districts, and also different economic groups; and thus vitiate the relevance of pedigree studies as evidence for inherited factors.

Direct and simple deductions from pedigree studies are fraught with yet additional complications. The low incidence of uterine cervical carcinoma in Jewish women has been correlated with the circumcision of their husbands in infancy, and has been explained on the basis of the absence of smegma which is thought to contain a carcinogenic substance. Whatever the degree of validity of such an hypothesis, it illustrates further the difficulties and possible fallacies of inferring the operation of genetic factors from pedigree studies in uterine cancer.

RACIAL INCIDENCE

Racial incidence has often been quoted as supporting evidence for an hereditary influence in the origin of cancer. However, this line of reasoning is subject to considerable criticism.

There are many instances of racial distribution of cancer which arise from extraneous or environmental influences. An example of such a set of circumstances is in cancer of the penis, which is rare in Jews and common in Chinese, and for which the racial distribution is incidental to an exogenous factor, namely the ritual circumcision practised on infant Jews and thus the removal of smegma and balanitis as causative agents of neoplasm. Similarly, primary carcinoma of the liver, which is common in the South African Bantu and rare in the South African White races, is probably not caused by any genetic factor but by deficiencies or harmful ingredients in the diet of the Bantu.

Many other cancers arising from the action of exogenous agents introduced in the course of different racial customs and habits are discussed in Chapter VII.

* Murphy, D. P. (1952), *Heredity in Uterine Cancer*. Cambridge: Harvard Univ. Press.

CHAPTER XXII

GENETIC APPARATUS AND PROCESSES

Genetic processes are probably fundamental in a number of aspects of aetiology and pathology of cancer. This is self-evident in the consideration of the place of heredity in aetiology, but it is also of importance in current ideas of the nature, cause, and mechanism of development of cancer.

The following general considerations of genetic material and its biological behaviour will help clarify certain theories of cancer pathology.

Genes.—Inheritance of normal and abnormal features is dependent upon the replication of genes and their transmission to descendent cells. That is, genes determine the hereditary characters of an individual.

Chromosomes.—Within each cell is a nucleus, or nuclear material, composed mainly of chromosomes, which are made up of genes, numbering many thousands, and arranged in consistently regular situations or loci along the length of the curled spring-like chromosome.

Chromosomes—Replication and Reduction.—Each human somatic cell contains 46 chromosomes. These are derived from the union of female and male germ cells, each of which contributes 23 to form pairs with like chromosomes, i.e., in which gene loci are correspondingly arranged.

Multiplication, by a process of division of the cell so as to form two daughter cells from a somatic cell, is accompanied by early formation of a replica of each of the 46 chromosomes, so that descendant cells resemble parent cells. The mechanism of such cell division is called *mitosis* (*see* Chapter I).

By contrast, the formation of germ cells, or gametes, is accompanied by reduction of the number of chromosomes to half, i.e., 23, so that each ovum or sperm contains only 1 member of the paired chromosomes of the somatic cells. This type of cell reduction is known as *meiosis*.

Chromosomes and Sex.—Of the 46 chromosomes present in each somatic cell, the female has 44 neutral, i.e., non-sexual, named autosomes, and 2 specifically female or X chromosomes. The male has 44 autosomes, 1 X chromosome, and 1 specifically male or Y chromosome.

Reduction division in an ovum produces 22 autosomes and 1 X chromosome; in a spermatozoon, 22 autosomes and, in one-half of the spermatozoa, 1 X, in the other half 1 Y chromosome.

Fertilization of an ovum by a sperm containing 22 autosomes and 1 X chromosome produces a female. In the case of the presence of 1 Y chromosome in the spermatozoon, the result of fertilization will be a male. This is represented diagrammatically in *Fig.* 33.

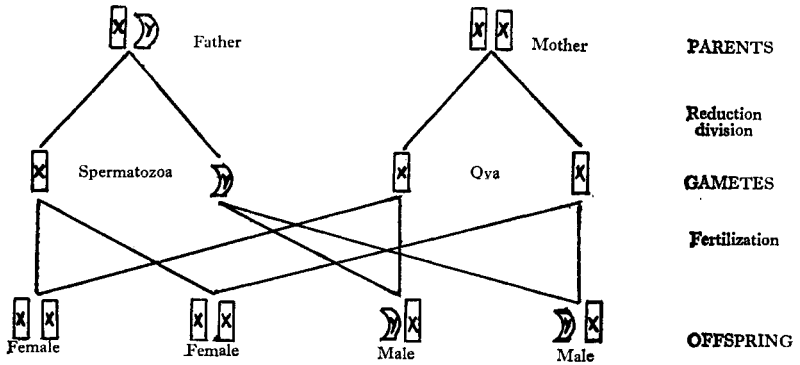

Fig. 33.—Normal pattern of sex chromosomes in reproduction.

Changes in Genes and Chromosomes.—Three types of changes may occur:—

1. Change or mutation of a gene. The mutant, also known as a variant, altered gene, or allele (short form of allelomorph), is reproduced and passed on to subsequent generations. Multiple and single alleles may occur, and they come to occupy particular loci in the chromosome. It is estimated that the average human carries 5–10 mutants in a total of more than 20,000 genes.
2. Structural change of chromosomes may occur as a result of exposure to a number of known agents. If the chromosomes survive, abnormalities comprising skeletal and other deformities may be transmitted to further generations.
3. Variation in number of chromosomes may occur. An example of an increase in number of autosomal chromosomes is that in mongolism. Klinefelter's syndrome is a condition arising from the presence of an extra sex chromosome. The following terms are used in connexion with chromosomal patterns and indicate the varieties found in their numbers.

HAPLOID.—The chromosomal number is singly represented, viz. 23 in the human; gametes are haploid cells, all other normal cells, i.e., somatic cells, are diploid.

DIPLOID cells have a paired set of homologous chromosomes, viz. numbering 46 in the human; each member of the pair being derived from one parent.

Changes in Genes and Chromosomes, *continued.*

ANEUPLOIDY connotes the occurrence of a chromosome number different from the normal and not a multiple of the normal haploid number.

POLYPLOIDY.—An increased number of chromosomes as a multiple of more than twice the haploid number.

KARYOTYPE is the complement of chromosomes in an individual; it refers to their number, relative sizes, and morphology. It is usually illustrated diagrammatically in an *idiogram*, or often arranged in sizes as a karyotype.

MONOSOMY.—Presence of only 1 member instead of the normal pair of chromosomes.

TRISOMY.—Presence of an extra chromosome at a particular locus.

MOSAIC.—Different chromosomal numbers in different cells from the one individual (exclusive of the normal difference between gametes and somatic cells).

Homozygotes and Heterozygotes.—If similar alleles derived from female and male forebears are paired in a descendant, the result is a homozygous condition at the particular locus affected. Pairing of 2 different alleles results in a heterozygous state.

A homozygote will pass the variant on to all descendants; a heterozygote will pass 1 of the 2 alleles to half the offspring and the other to the second half. This reassortment of genes results from reduction division during the formation of germ cells and is known as segregation. The processes are represented diagrammatically in *Fig.* 34, A and B, and *Fig.* 35.

Recessive Genes.—Mutants that produce noticeable effects only in homozygous individuals, i.e., where the determining gene has been inherited from both parents, are recessive. Such homozygotes are relatively rare, with the result that it is usual for the effects of a recessive gene to be hidden, and for the affected individual to be a carrier without outward evidence of the abnormality.

Marriages of first cousins and inbreeding in 'closed' communities (closed because of geographic, social, or religious limitations) emphasize recessive genetic effects because of the greater numbers of homozygous individuals produced under such circumstances. Examples of inheritance of recessive characters are colour-blindness in Quakers, xeroderma pigmentosum, and albinism. *Fig.* 34 A gives the pattern of recessive autosomal inheritance when both parents carry the variant, as this has the same pattern as the formation of a homozygote, and results in progeny in the proportion of: 1 normal; 2 carriers without clinical effects; and 1 clinically affected.

Dominant Genes.—Alleles producing an obvious effect in heterozygotes are dominant. If it is inherited from one parent, the effect will be apparent in every generation in about half the offspring and equally distributed in the sexes. The pattern of such inheritance is represented diagrammatically in *Fig.* 36 A.

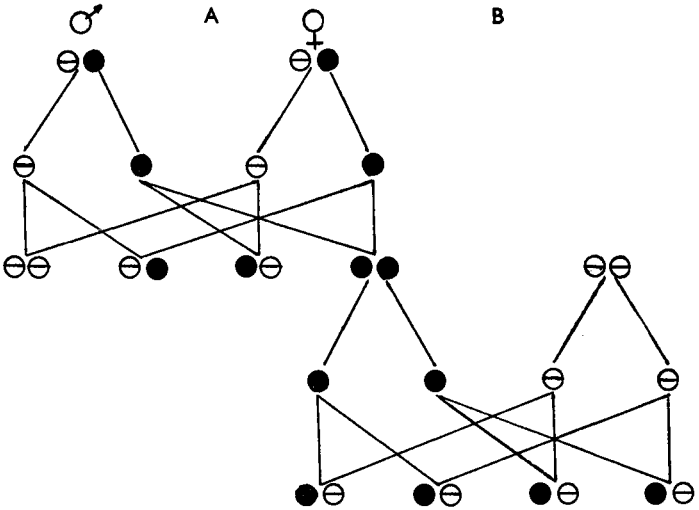

Fig. 34.—A, Formation of homozygote. Similar alleles from each parent result in offspring in proportion of: 1 normal; 2 affected; 1 homozygote. B, Homozygote transmits allele to all descendants. ⊖ Normal gene; ● Allele.

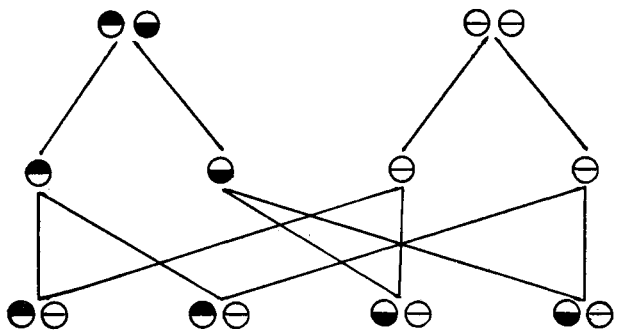

Fig. 35.—Segregation in heterozygote. ⊖ Normal; ◓ Allele 1; ◒ Allele 2. Heterozygote parent, containing 2 different alleles, mated with normal parent, produces 1 allele in half the offspring and the second allele in the other half.

Dominant Genes, *continued.*

Corresponding dominant genes may be inherited from both parents. The transmission in such an event is represented in *Fig.* 36 B.

Recessiveness and dominance are not absolute qualities: they are relative, producing many gradations between extreme recessive alleles and fully dominant ones.

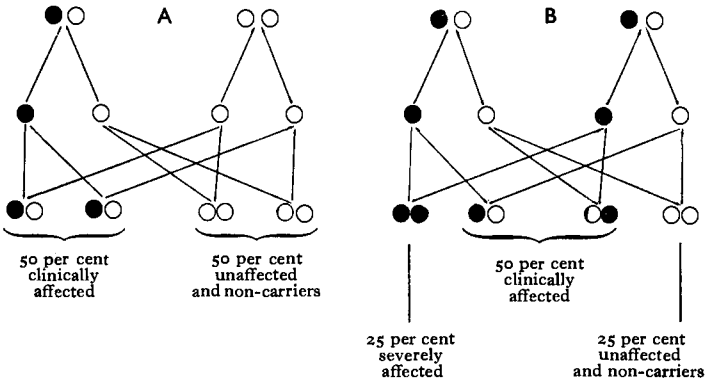

Fig. 36.—A, Inheritance of a dominant gene from one parent. B, Inheritance of similar dominant genes from both parents.

Detrimental dominant characters tend to die out by a process of natural selection because the progeny either die before reaching sexual maturity or because the condition prevents procreation. Recessive features usually persist although they may remain hidden for several successive generations.

Sex-linked Genes.—These are located on sex chromosomes. They are not themselves concerned with sex characters but are transmitted together with determinants of sex.

The Y chromosomal component of the male bears few gene loci and has little influence on most hereditary characters. On the other hand, the X chromosome in the male produces obvious effects from any abnormal mutant connected with it even if it is recessive in the maternal sex chromosome. In the female, such a recessive gene will only produce outward effects when paired with a like gene, i.e., in a homozygous female. This is a rare occurrence; but the female with only 1 X chromosome bearing the variant will transmit it to the X chromosome of male progeny, where it will result in clinical effects.

Haemophilia is the classic example of a sex-linked mutant gene transmitted by an apparently unaffected female to the male. The patterns of transmission of sex-linked genes are illustrated in *Fig.* 37 A, B, and C.

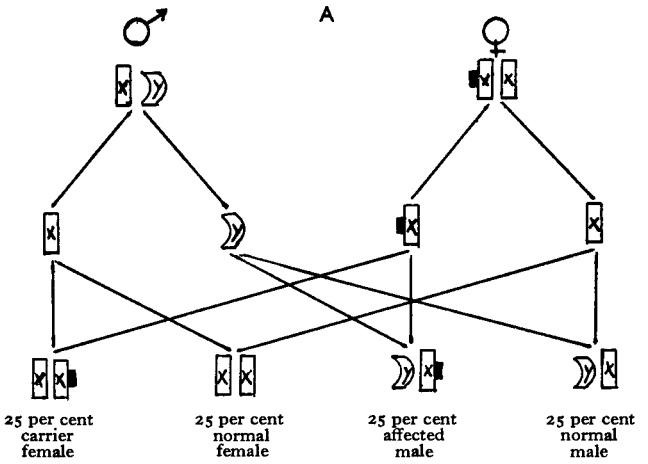

Fig. 37.—A, Pattern of transmission of sex-linked gene, maternal carrier.

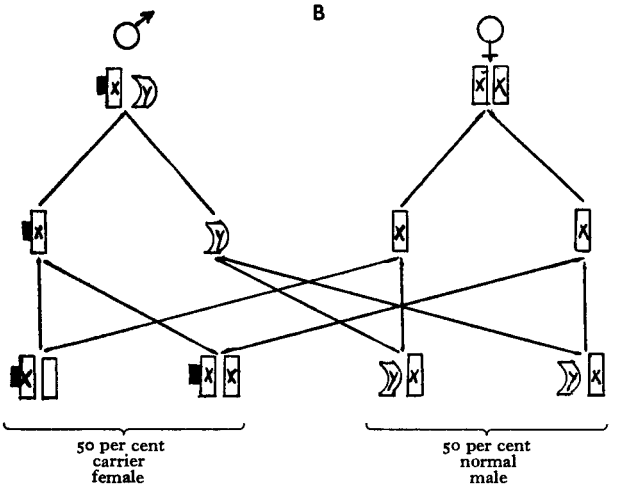

Fig. 37.—B, Pattern of transmission of sex-linked gene, affected father.

Sex-linked Genes, *continued.*

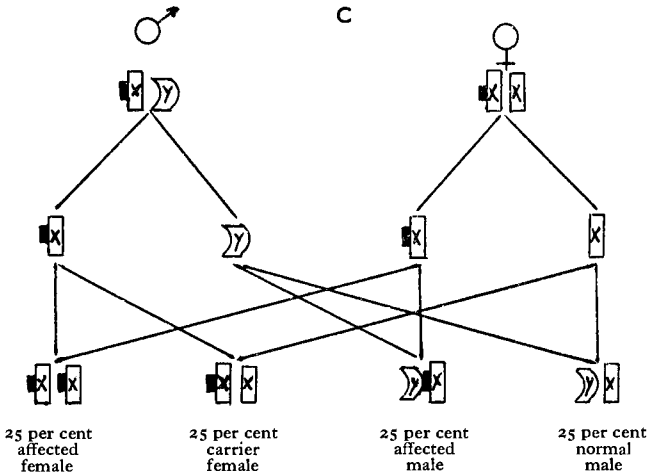

Fig. 37.—C, Pattern of transmission of sex-linked genes, both parents affected.

Genetic Transmission of Blood Groups.—Blood groups A and B are dominant genes in relation to group O which is recessive. Combinations of inheritance thus result in the following possible categories:—

A plus A, and A plus O, produce group A.
B plus B, and B plus O, produce group B.
A plus B remains combined in a group AB.
O plus O is the only combination regularly producing group O.

These computations are of the simple groupings of blood, but there are more complex factors arising from other genes transmitting additional groups, e.g., Rh factors. Simple grouping has a practical value in that certain clinical conditions appear to be linked to them and consequently suggest their genetic transmission. An example is gastric cancer which is more commonly found in blood group A than in the other groups.

Spontaneous Mutation.—Spontaneous mutation probably occurs continuously at a low rate and probably affects all genes. Generally the changes produced in individuals from this type of mutation are neither marked nor dramatic. The occasional alleles that do effect marked change are usually detrimental and tend to be eliminated because the individual affected 'dies out' or does not produce descendants. Gradations of consequences are the rule, and the slight differences in successive generations and between different races are the results of the interaction of many minor genetic variants.

Induced Mutation.—It may be argued that there is no such process as spontaneous mutation, but that all mutations are induced by environmental influence; most are minimal changes in which the inducing agent is too small to be recognized or measured. A proven mutagen (an agent causing mutation) is ionizing radiation, and it is suggested that many so-called spontaneous variations are in fact the results of unrecognized small doses of naturally occurring emanations.

Apart from physical agents like ionizing radiation, there are chemical mutagens, e.g., methylcholanthrene and mustard gas.

Genetic Effects of Radiation.—

1. Germ cells may be destroyed, in which event the lineage will cease. Chromosomal architecture may be altered. This usually prevents reproduction or causes early death of progeny. But, if chromosomal repair occurs, the descendants may live and be malformed.
2. Germ-cell gene mutation may be increased. It appears that the increase is proportional to the radiation dose, and is greater when this is given in a single intensive dose than when there are repeated small doses.
3. Somatic cell genes may be affected and undergo alteration.

There is evidence to suggest that the effects of ionizing radiations are related to alterations in the structure of DNA molecules which influence the function of somatic as well as germ cells.

Germ and Somatic Cell Mutations.—Gene mutants in germ cells are inherited by descendant generations of individuals. Mutations in somatic cells are passed on to descendant cells, affecting a limited segment of the body. Such variants are not transmitted to future generations of individuals. Mutations in somatic cells may affect either nuclear or cytoplasmic constituents of a cell.

Some of the concepts of the mechanism of change from a normal to a cancerous cell postulate somatic nuclear gene mutations in addition to cytoplasmic mutations.

BIOCHEMISTRY OF GENES

The functions of genes are mediated by two kinds of nucleic acid which act by coding or decoding so as to exercise control over the synthesis and sequential arrangement of protein. The two nucleic acids are deoxyribonucleic (DNA) and ribonucleic acid (RNA).

DNA is an integral part of genes which occupy specific loci in the nuclear chromosome. Each gene has a specific role controlling the synthesis of a particular protein which may be either structural or enzymatic in function. DNA is the basic carrier of this genetic process; it acts by directing the synthesis of RNA which itself acts as a chemical messenger in the cytoplasm, where it conditions the elaboration of the particular protein.

These ideas are included in *Fig.* 38, a diagram modified from that given by Mercer,* of current theories on cellular biochemical mechanism and behaviour.

* Mercer, E. H. (1962), 'The Cancer Cell', *Br. med. Bull.*, **18**, 187.

DNA, *continued.*

The molecule of DNA is physically constructed of two coiled strands, twisted around each other, like the strands of a rope, and linked with one another by chemical bonds. Reproduction of DNA is probably by separation of the strands, each of which is identical and builds a complementary partner from the metabolic environment. Each DNA strand thus forms a template from which further strands are replicated. DNA molecules differ from one another by the length of the coiled chain and by the sequence of the nucleotides which form them.

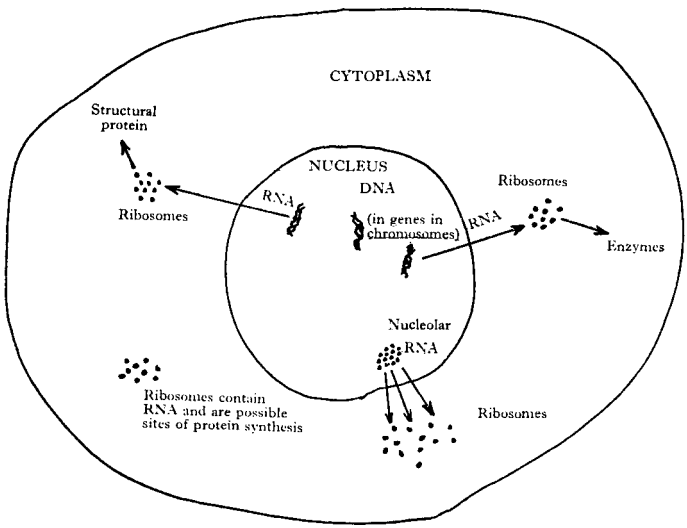

Fig. 38.—Diagram of theory of action of DNA and RNA.

RNA.—Ribonucleic acid is formed from DNA. Several systems or types are postulated.

MESSENGER RNA diffuses out of the nucleus into cytoplasm where it decodes the information in DNA and controls synthesis and arrangement of protein. Each m-RNA represents the transmitted code from one gene.

RIBOSOME RNA.—Ribosomes are small dense particles attached to reticulum in cell cytoplasm. Ribosomes are also found in clumps or agglomerations within cell nuclei and are there called nucleoli; they appear to be 'ready' to pass out into cytoplasm. Ribosomes contain RNA and are the sites of formation of proteins. Such synthesis in ribosomes requires the presence of messenger RNA.

TRANSFER RNA is the carrier of amino-acids from the general metabolic pool to the ribosome where the amino-acids are compounded and linked into various combinations and sequences to give the particular protein its specific character.

Modification of Protein Synthesis.—The system of biochemical reactions leading to the production of protein is liable to modification at different levels. The DNA code may undergo mutation: if it affects a gamete (ovum or sperm) the mutant is heritable: at a somatic level, a localized zone of replicated mutants is produced. The abnormalities of structure and arrangement, or the deficiencies of protein, are translated into clinical entities, called, in genetic terms, *phenotypes*.

This is to be differentiated from *genotype*, or *genome*, which connotes the genetic constitution of an individual; whereas the *phenotype* may be defined as the state resulting from changes in the genotype wrought by extraneous influences.

CHAPTER XXIII

MUTATION THEORY OF CANCER

Cowdry* and Koller,† among many other observers, have noted frequent nuclear abnormalities in cancer cells. A high mitotic index, 'stickiness' of chromosomes interfering with and distorting anaphase and telophase division of chromatin, and disturbances of spindle development, are common though not invariable features. They point to the possibility of mutations playing a part as a cause of cancer.

The frequency of abnormal karyotypes and nuclear patterns as an essential part of cancer, and their interpretation as indicating gene mutations, is not accepted by all authorities. Ludford‡ argues that chromosomal modifications can occur in the absence of malignancy, and that they may be absent in the presence of malignancy. It has also been noted that in many tumours there is a higher rate of abnormal mitoses near the centrum, i.e., the zone of advancing necrosis, than at the periphery where the tumour is growing and spreading. A further point quoted as negating the mutation theory of cancer is the difference in mitotic activity of differently situated tumours, suggesting that environment is a main conditioning factor of such differences.

The pendulum of this argument is tending to swing back again in favour of gene mutation because of the accumulation of evidence from a number of sources. Spriggs and others§ undertook cytogenic studies of teased-out solutions of lymph-nodes and brain tumours, and of cells in malignant effusions. In this latter state cancer cells have a unique form of independent existence, removed from most of the usual environmental influences which are stated to affect nuclear processes. It appears from their study that:—

1. Abnormalities of chromosome number and/or morphology are present, with different patterns in each case.
2. Chromosome breakage and reunion is evident in some cases; the similarity of chromosomal abnormality in different cells of the same case, even in the presence of different counts, supports the idea of descent from a common malignant parent cell.
3. Chromosomal abnormalities explain the disorderly function and formation of tissues in neoplasia.
4. There is evidence to suggest that the karyotype is altered before the development of invasive cancer, e.g., in carcinoma-in-situ of the cervix.

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* Cowdry, E. V. (1955), *Cancer Cells*. Philadelphia: Saunders.

† Koller, P. C. (1952), 'The Genetic Component of Cancer', in *Cancer*, Vol. 1 (Ed. Raven, R. W.). London: Butterworths.

‡ Ludford, R. J. (1954), 'Nuclear Structure and its Modifications in Tumours', *Br. J. Cancer*, **8**, 112.

§ Spriggs, A. I., Boddington, M. M., and Clarke, C. M. (1962), 'Chromosomes of Human Cancer Cells', *Br. med. J.*, **2**, 1431.

Mutagens and Carcinogens.—A weighty stimulus to the formulation of a theory linking chromosomal variation and gene mutation to a causative role in cancer derives from the fact that many known carcinogens of different types are also mutagens. Strong* notes that all physical and chemical germinal mutagens also influence the origin of cancer, thus supporting the proposition that 'mutation is involved in the origin of cancer'.

A number of agents bring about gene mutations:—

1. PHYSICAL AGENTS.—Ionizing radiations, ultra-violet light, and actinic rays.
2. CHEMICAL SUBSTANCES.—Chemical carcinogens and alkylating agents, i.e., those inducing a chemical change by the addition of a methane group. Many alkylating agents are also carcinogens. Boyland† reports that one of the early morphological changes seen within a few hours of the administration of chemical carcinogens is the production of chromosome abnormalities similar to those caused by ionizing radiation. In fact, practically all chemical mutagens resemble radiation in their effect upon genes, so that their activity has come to be described as 'radiomimetic'.
3. VIRUSES.

Mutagenic and Carcinogenic Mechanisms.—The mode of action of physical and chemical mutagens appears to be a biochemical one in which cellular proteins, both structural and enzymatic, are altered. The alteration does not lead to the production of new macromolecules: DNA sequences, RNA messengers, and protein molecules have not been shown to be novel, but it is theorized that their proportions and absolute numbers may undergo change. Once such change has occurred, a modified genetic template, or mould, is formed from which are descended cells bearing the new allele.

Mercer,‡ from a consideration of the synthesis of macromolecules in epidermal basal layers, postulates the existence of a feed-back mechanism (i.e., a homeostatic process) between germinal cells of the basal layer and post-mitotic and differentiating cell zones. The germinal layer produces the latter cells which then act as checks upon, or stimulants to, further activity of basal cells. He poses the theory that a breakdown in this system could result in an overproduction of post-mitotic cells, and delay in differentiation by reduction of the synthesis of the proteins responsible, with ultimate tumour formation. Some morphological evidence for this proposition is to be found in cellular changes seen under an electron microscope. Moreover, structural proteins responsible for adherence between contiguous cell membranes may be quantitatively deficient, so providing alleys and pathways for actively growing

* Strong, L. C. (1947), 'The Induction of Germinal Mutation by Chemical Means', *Fourth Int. Cancer Res. Congr. St. Louis*; and (1949), 'The Induction of Mutation by a Carcinogen', *Br. J. Cancer*, **3**, 97.

† Boyland, E. (1962), in *Cancer and Hormones*, pp. 135–140. Chicago: Univ. Chicago Press.

‡ Mercer, E. H. (1962), 'The Cancer Cell', *Br. med. Bull.*, **18**, 187.

Mutagenic and Carcinogenic Mechanics, *continued.*

and migrating cells, and so offering a biochemical hypothesis for tumour spread (*see* Chapter II).

Physical and chemical mutagens appear to act upon chromosomal DNA, with consequent alteration of RNA, thereby upsetting cellular function and control and leading to abnormal and neoplastic growth.

Enzyme Aspects of Molecular Basis of Neoplasm.—Potter* propounded a *deletion hypothesis of carcinogenesis*: in this theory, the deletion of an enzyme resulting from gene mutation is the underlying reason for the change from a normal to a malignant cell. The lack of the particular enzyme induces alternative metabolic pathways necessary for the continuance of cell life and reproduction. The new form of growth is the neoplasm. Haddow† has a similar view, and expresses the theory that the cancer cell arises from a somatic mutation by loss: the deficiency being either genetic or enzymatic and the resulting cell-type developing its own new pattern of growth and organization.

Viruses and Mutation.—The mechanism of change of molecules composing cellular genetic material by radiation and radiomimetic chemical compounds is reflected in some of the theoretical explanations of the mode of action of viruses. (*See* Chapter XIX.) This is held by a number of authorities as vindicating the mutation hypothesis of cancer. However, it should not be forgotten that in the drive to find an intellectually satisfying all-encompassing theory for cancer there is necessarily a constraint to harmonize the action of all known tumour-causing agents. The explanation may thus be forced and stretched to fit such a preconceived necessity.

To give point to this reservation it is salutary to stress the differences in carcinogenesis between viruses and other agents.

1. SPECIFICITY OF ACTION.—Physical and chemical carcinogens have little recognized specificity of action: similar tumours are produced at the sites of application by different agents. Virus infection, on the other hand, shows quite marked specificity in that different viruses produce different diseases.
2. SPECIES SUSCEPTIBILITY.—Virus infection is influenced by such susceptibilities: certain animals get particular diseases from virus infection. Much less so is the reaction to physical and chemical agents.
3. FIELD OF ORIGIN OF CANCER.—In physically and chemically induced cancers, the origin is from a limited field. Viruses exercise their influence over a much wider area.
4. PERSISTENCE OF CARCINOGEN.—Physical and chemical agents induce changes in cells. Then, without further action and

* Potter, V. R. (1962), 'Enzyme Studies in the Deletion Hypothesis of Carcinogenesis', in *The Molecular Basis of Neoplasia.* Austin: Univ. Texas Press.

† Haddow, A. (1955), 'Recent Advances in the Study of Carcinogenesis', *S. Afr. med. J.*, **51**, 1185.

in the absence of the agent, cancer follows a variable latent period. Viruses usually remain with the cancer, in obvious or masked form, for a long period.

The theories of the mechanism of action of viruses are discussed in Chapter XIX. In the context of the present chapter, as part of the evidence favouring a mutation theory, some of the concepts of viral action require further mention.

Some viral bodies contain deoxyribonucleic acid, or DNA (e.g., vaccinia virus); some, mainly ribonucleic acid, or RNA (e.g., poliovirus); yet others possibly contain both. DNA and RNA are self-replicating bodies and they may, theoretically, affect normal host cells in a number of ways. Furth* classifies two types of action: cytogenetic and parasitic. In the latter class, parasitic viruses cause effects by independent self-replication, living off host cells in which they occur, forming their own systems of synthesis with possible contributions from host substrate. In this activity, host responses and normal feed-back homeostatic mechanisms are disturbed; such upset of normal control may arise by deprivation of an enzyme system and so fit the theory of deletion (*see above*), or excessive stimulation to hyperplasia may lead to uncontrolled growth. The virus of Shope papilloma may conceivably belong to this category: it is contagious; its tumour is virus-dependent for a long period, but the ultimate malignant tumour may not have demonstrable viral agents (a feature which rather weakens the theory, and has brought in several corollary hypotheses for its explanation).

The concept of a cytogenetic mechanism involves the alteration of host genes. This mutation may occur by transduction, transformation, or conjugation. The mutation is supposed to explain the conversion of a normal cell to one with neoplastic properties.

Several possible mechanisms of virus action have been tested by Vogt and Dulbecco† and by Dulbecco and Vogt.‡ On the basis of experiments using polyoma virus-induced neoplasms in cultured embryo cells from mice and hamsters, they rule out the hypothesis that the virus selects a pre-existing potentially malignant cell. Similarly, the concept that neoplasia is maintained by the presence of vegetative virus genomes which are able to produce mature viral bodies is also excluded. Whether or not viral nucleic acid becomes integrated with host genes, or whether the viral genome acts as a mutagen to cause a permanent change in host-cell mechanisms, are problems that still remain unsolved.

Genetic Initiation in the Two-stage Hypothesis of Cancer.—The two-stage theory of the evolution of cancer is discussed more fully in Chapter X. In the context of the present chapter it features as part of the framework of the mutation theory of cancer.

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* Furth, J. (1959), 'Mechanism of Carcinogenesis by Viruses', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

† Vogt, M., and Dulbecco, R. (1960), 'Virus-cell Interaction with a Tumor-producing Virus', *Proc. natn. Acad. Sci. U.S.A.*, **46**, 365.

‡ Dulbecco, R., and Vogt, M. (1962), 'Is there an Integrated Virus Genome in Virus-induced Neoplastic Cells?' in *The Molecular Basis of Neoplasia*. Austin: Univ. Texas Press.

Genetic Initiation in the Two-stage Hypothesis of Cancer, *continued.*

A comparison is drawn between mutation and initiation, a term used in the two-stage hypothesis which may be explained by reference to the following experiment.

Berenblum and Shubik* demonstrated that limited treatment of mouse skin by a standard carcinogen does not produce tumours; but that subsequent application of croton oil, by itself non-carcinogenic or only very weakly so, is productive of skin tumours in proportion to the concentration of the preliminary carcinogen. This led to the two-stage theory of cancer formation: an *initiator* conditions cells to a state of sensitivity; the second stage involves the action of a tumour-producing agent (e.g., croton oil), which is called a *promoter*. The agents in the two stages are cocarcinogens.

There are many indications that a two-stage mechanism is a common phenomenon in many forms of chemically induced cancer. As has been noted, it is probable that chemical carcinogens act as mutagens, and thus a theory has developed that mutation constitutes the basic stage of initiation, in which, although cancer is latent, the cellular change is irreversible.

Walpole† suggests that 'spontaneous' tumours may also arise in a similar manner. In many untreated laboratory animals, particularly as older ages are approached, the incidence of spontaneous tumours rises markedly and varies with species, sex, and selective inbreeding. Initiation may consist of some heritable factor, and promotion by endogenous or exogenous agent. Burnet‡ also postulates somatic mutation as an initiating phase; when the cells are later exposed to promoting agents, cancer becomes clinically evident. Court Brown§ gives further support to the thesis that genetic change is a stage of initiation.

Multiple Mutations, Ageing and Cancer.—There is some evidence that cancer arises by a multistage process. This does not negate the two-stage theory but is complementary to it, because the multiple stages in the formation of cancer are broadly divisible into initiation and promotion with degrees of enhancement or repression entering the field.

Earle‖ and Gey and others,¶ working on mice and rat fibroblasts, both report that cultures of normal fibroblasts on reintroduction into the respective live animals become sarcomatous. Although mutations were sparse, they were definite. The experiments indicate at least one mutational event during the culture and another on reimplantation.

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* Berenblum, I., and Shubik, P. (1949), 'A New Quantitative Approach to the Study of the Stages of Chemical Carcinogenesis in the Mouse Skin', *Br. J. Cancer*, **3**, 109.

† Walpole, A. L. (1959), 'Initiation and Promotion in Carcinogenesis', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

‡ Burnet, M. (1957), 'Cancer—A Biological Approach', *Br. med. J.*, **1**, 779.

§ Court Brown, W. M. (1962), 'Role of Genetic Change in Neoplasia', *Ibid.*, **1**, 961.

‖ Earle, W. R. (1943), 'Production of Malignancy in Vitro. IV. The Mouse Fibroblast Cultures and Changes in Living Cells', *J. natn. Cancer Inst.*, **4**, 165.

¶ Gey, G. O., Gey, M. K., Firor, W. M., and Self, W. O. (1949), 'Cultures and Cytologic Studies on Autologous Normal and Malignant Cells of Specific in Vitro Origin. Conversion of Normal into Malignant Cells', *Acta Un. int. Cancr.*, **6**, 706.

In the course of criticism directed against the theory of somatic mutation, Hieger* submits that the acceptance of the concept requires more than one mutation, in fact a series of mutations. In support he quotes the example of the carcinogenic property of uracil on the thyroid gland; the following being the minimum number of mutations in the process:—

1. Mutation for change from normal to hyperplastic thyroid.
2. Another mutation for the further change to benign tumour.
3. Another for conversion to malignant properties.
4. Another for rendering the tumour independent of uracil.

Jacobs and others† and Buckton and others‡ record that chromosomal variations in leucocyte counts bear a direct linear relationship to age, increasing in number with advancing years. There is a corresponding direct relationship between cancer and age. Nordling,§ because of the relation between cancer rate and age, dismisses the view that a single allele gives rise to cancer, and claims that multiple mutations occur. In summary, his theory is constructed on the assumption that a few mutations occur in a large number of cells; some of these mutants undergo further mutation, so producing two variant genes; further mutations are superimposed until a sufficient number of abnormal genes are formed to constitute cancer. On the basis of logarithmic graphs of cancer death-rate compared with age, Nordling calculated that 7 successive mutations were required to explain the relationship.

Armitage and Doll|| applied this concept to various cancers. The arithmetical basis seemed to hold for some tumours, but there were deviations of different degrees from the curves of theoretical expectation in others. The influence of environmental carcinogenic agencies and the different ages of exposure to the carcinogen may offer explanations for these divergencies.

At present, the work and thinking on the score of the correlation of age and cancer must be regarded as a beginning, and the current concept as being rather tenuous.

Inbreeding of Experimental Animals.—As a final segment of evidence supporting mutations as a fundamental part of the origin of cancer, inbreeding of animals and their varying susceptibility to tumours may be quoted. The purposive evolution of 'high-cancer strains' and 'low-cancer strains' is testimony suggestive of genetic selection of certain alleles in each case. Susceptibility and resistance may thus be determined by genetic factors in which mutations are closely associated with the aetiology of cancer.

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* Hieger, I. (1959), 'Theories of Carcinogenesis', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

† Jacobs, P. A., Court Brown, W. M., and Doll, R. (1961), 'Distribution of Human Chromosome Counts in Relation to Age', *Nature, Lond.*, **191**, 1178.

‡ Buckton, K. E., Harnden, D. G., Baikie, A. G., and Woods, G. E. (1961), 'Mongolism and Leukaemia in the same Sibship', *Lancet*, **1**, 171.

§ Nordling, C. O. (1953), 'New Theory on Cancer-inducing Mechanism', *Br. J. Cancer*, **7**, 68.

|| Armitage, P., and Doll, R. (1954), 'Age Distribution of Cancer and Multistage Theory of Carcinogenesis', *Ibid.*, **8**, 1.

Refutation of Mutation Theory.—Hieger* argues strongly against the concept of nuclear or cytoplasmic genic mutation as a basic factor in carcinogenesis. He points to the contradiction between theories based on somatic mutation and on viruses as the cause. Either viruses are mutagenic, in which event there is no need for their continued presence for the cancer to continue, or the continuing action of viruses is part of the neoplastic process, in which case there is no need for a mutation hypothesis.

This author, moreover, does not accept the parallelism between mutagenesis and carcinogenesis.

A substantial criticism developed against the mutation hypothesis derives from his support of the concept of the multicentric origin of cancer. Hieger argues that whereas mutation is a relatively rare event, neoplastic change affects a large number of cells in one anatomical region. The lack of adequate reconciliation of these two circumstances provides the refutation of the mutation theory.

Despite the strong argument marshalled against the mutation theory, and the doubts about the manner of development of neoplastic mutant cells, it is difficult to escape the compelling necessity to account for what seems to be an irrefutable mutation process.

The premises justifying the conclusion that mutation takes place begins with dicta on biological behaviour that have been accepted for a century or more. If 'all cells come from pre-existing cells', as proclaimed by Virchow in 1855;† and 'all cells come from genetically similar parent cells', as asserted by Thiersch 10 years later;‡ and cancer cells originate from normal body cells, then somewhere along the line of change from normalcy to malignancy there must be one or more mutations.

* Hieger, I. (1961), *Carcinogenesis*. London: Academic Press.
† Virchow, R. (1855), 'Cellularpathologie', *Virchows Arch. path. Anat. Physiol.*, **8**, 1.
‡ Thiersch, C. (1865), *Der epithelial Krebs*. Leipzig: Engelmann.

CHAPTER XXIV

IMMUNOLOGY AND CANCER

Immune Phenomena in Chemically-induced Cancer.—Experimental demonstrations of the development of antigenic properties in tumours induced by methylcholanthrene have been repeated on many occasions. Foley* is credited as the first to show this. Specificity of immunological activity was demonstrated by experiments conducted by Pollard and Russel.† Cancers were induced in a pure strain of mice by subcutaneous injections of three different polycyclic hydrocarbons, viz. methylcholanthrene (MC), dibenzanthracene (DBA), and benzpyrene (BP). Cultures of spleen on chicken plasma were made, the spleens being taken from untreated mice of the same strain and from injected mice at varying intervals after injection. To such cultures, tumour cells were explanted. Methylcholanthrene-induced tumour explants grew profusely when added to spleens taken from either normal animals or from those mice which had been given one of the other two hydrocarbon carcinogens; but growth was absent when the spleen came from a methylcholanthrene-treated mouse (*see Fig.* 39).

This proved the presence of a specific antibody in the form of a tumour growth-resistant factor in the spleen. Further experiment showed that the antibody persisted until the methylcholanthrene had induced a tumour; thereafter the spleen no longer prevented growth of cells on chicken plasma, i.e., the antibody remained effective until the carcinogen had produced a tumour in that animal.

Another phenomenon relating to alteration in the quality of immune reaction has often been shown: repeated passage of a tumour leads to reduction of its antigenicity, permitting 'takes' in a wider range of host strains than was possible at the commencement of the series of transplants.

Prehn and Main‡ found that when antitumour factors had been formed against methylcholanthrene-induced tumours, excision of the tumour did not remove the factors, and the host remained resistant to transplants of the same tumour.

There is much experimental work showing that acceptance or 'take' of tumour transplants is more successful in genetically closely related animals, and, conversely, there is less success as the

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* Foley, E. J. (1953), 'Antigenic Properties of Methylcholanthrene-induced Tumours in Mice of Strain of Origin', *Cancer Res.*, **13**, 835.

† Pollard, M., and Russel, P. H. (1953), 'Antineoplastic Factor in Spleens of Mice Previously inoculated with Methylcholanthrene', *Proc. Soc. exp. Biol. Med.*, **83**, 671; and (1954), 'Specificity of Antineoplastic Phenomenon induced in Mice by Carcinogenic Agents', *Ibid.*, **86**, 186.

‡ Prehn, R. T., and Main, J. M. (1957), 'Immunity to Methylcholanthrene-induced Sarcomas', *J. natn. Cancer Inst.*, **18**, 769.

Immune Phenomena in Chemically-induced Cancer, *continued.*

relationship becomes wider. The genetic influences in immunology have been studied in inbred mice, and, to an extent, the problems have been unravelled by Snell.*

Medawar† has shown that biological behaviour in tissue transplantation is similar to tumour transplantation in many respects and is fundamentally dependent upon the same processes. This applies also to second set reactions, which are considered by Hirsch‡ as a specific type of immunological response. It consists

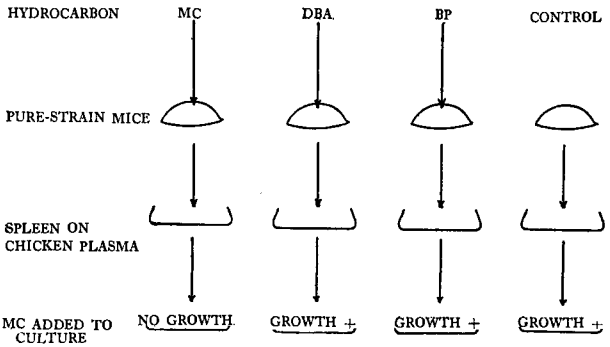

Fig. 39.—Diagram of experimental demonstration of tumour immunological specificity.

of an increased antitransplant reaction to a second graft as compared to that against a first graft of identical tissue. An illustrative example of second set reactions is to be seen in two successive skin homografts from one donor to the same recipient: the second skin homograft is rejected much more rapidly than the first graft. Such reactions occur in the same manner in transplants of cancer in animals.

In man, the patterns of behaviour are not dissimilar. Autografts of a wide range of tissues are highly successful in man and have been used in different branches of surgery for many years. Homologous grafts, in the absence of de-immunizing devices, are accepted in man in a very limited field: in identical twins; with cartilage and corneal grafts, i.e., structures without a blood-supply; and with vascular segments, bone and soft connective tissues, which endure sufficiently long to act as a scaffold upon which the host lays down new tissue. Apart from these examples, homologous grafts are rejected.

* Snell, G. D. (1957), 'Incompatibility Reactions to Tumor Homotransplants with Particular Reference to the Role of the Tumor', *Cancer Res.*, **17**, 2.

† Medawar, P. B. (1944), 'The Behaviour and Fate of Skin Autografts and Skin Homografts in Rabbits', *J. Anat.*, **78**, 176.

‡ Hirsch, H. M. (1959), 'Tumour Immunity and Tissue Transplantation', *Lancet*, **79**, 340.

Immune Phenomena in Viral Cancer.—A considerable body of evidence attests to the existence of positive immune phenomena in viral cancers in animals. Stewart and others* demonstrated the highly antigenic properties of polyoma virus. It is an haemagglutinating virus, and it stimulates the formation of antibody which is detectable by neutralization of the tumour-inducing effects of a cell-free filtrate, and by inhibiting haemagglutinin. The fact that passive immunization with rabbit anti-virus serum inhibits tumour production is further evidence of the antigenic activity of this virus. Rowe and others† developed a complement-fixation test for the detection of polyoma virus. Intraperitoneal injection of highly diluted virus and intranasal applications of more concentrated preparations gave rise to complement-fixation antibody, invariably in newborn and adult Swiss mice, and in different proportions of other mice. It has also been shown that in mice with polyoma-induced parotid tumours, complement-fixation antibody was always present; but it was generally absent in mice of high leukaemic strain, and in those with spontaneous leukaemia, thus indicating serological specificity.

Further confirmation of immune processes in cancer is embodied in the work of Klein and others,‡ who report the experimental production of host resistance against isotransplantation of Gross virus lymphomas. This relative immunity was achieved in inbred mice by two methods: by preliminary treatment of prospective hosts with homografts of other Gross lymphomas induced in genetically incompatible mice; and by sub-threshold doses taken from isologous lymphomas.

Habel§ adds another angle to the design of immune reactions in viral cancer. Polyoma virus inoculation of newborn mice gives rise to 3 major effects: virus multiplication in a number of organs; antiviral antibody formation; and the later development of different tumours. In adult mice, the first 2 effects occur, but the third, viz. the appearance of tumours, does not. However, the adult mice become resistant to subsequent challenge with isologous transplants of polyoma tumour.

Habel reasons that virus infection alters normal cells in both infants and adults; the altered cells contain a new antigen, which, in infants, does not evoke an antibody response as infants are immunologically immature; but in adults, the immune reaction is stimulated and is sufficient to inhibit tumour formation.

Evidence of specific antigenicity is extant for other tumour- and leukaemia-producing viruses. Graffi's agent (a cell-free filtrate

* Stewart, S. E., Eddy, B. E., and Borgese, N. G. (1958), 'Neoplasms in Mice inoculated with a Tumor Agent carried in Tissue Culture', *J. natn. Cancer Inst.*, **20**, 1223.

† Rowe, W. P., Hartley, J. W., Brodsky, I., and Huebner, R. (1958), 'Complement Fixation with a Mouse Tumor Virus (S.E. Polyoma)', *Science*, **128**, 1339.

‡ Klein, G., Sjorgen, H. Q., and Klein, E. (1962), 'Demonstration of Host Resistance against Isotransplantation of Lymphoma induced by the Gross Agent', *Cancer Res.*, **22**, 955.

§ Habel, K. (1961), 'Resistance of Polyoma Virus Immune Animals to Transplanted Polyoma Tumors', *Proc. Soc. exp. Biol. Med.*, **106**, 722; and (1962), 'The Relationship between Polyoma Virus Multiplication, Immunological Competence, and Resistance to Tumor Challenge in the Mouse', *Ann. N.Y. Acad. Sci.*, **101**, 173.

Immune Phenomena in Viral Cancer, *continued.*

from transplantable tumours causing a myeloid type of leukaemia in mice) is not particularly antigenic for mice, but rabbit antivirus serum is very strongly inhibitory. Friend's virus, also derived from a transplantable mouse tumour and leukaemogenic in adult mice of particular strains, gives rise to immune sera in rabbits and mice which neutralize its leukaemogenic effects; mouse serum from other viral leukaemias has no immunizing action on the Friend virus.

Rabbits which recover from the fibroma induced by the Shope virus exhibit active resistance to reinfection, and they develop antibodies with complement-fixation and neutralizing and agglutinating effects on the virus.

Immune Mechanisms.—The reaction of immunity is probably the end result of a chain of biological mechanisms. It has been described by Calne* as starting with an antigenic stimulus, followed by its recognition in host tissues with messages transferred to reaction centres, and ending with an effector mechanism that reacts with the provoking antigen (*see Fig.* 40).

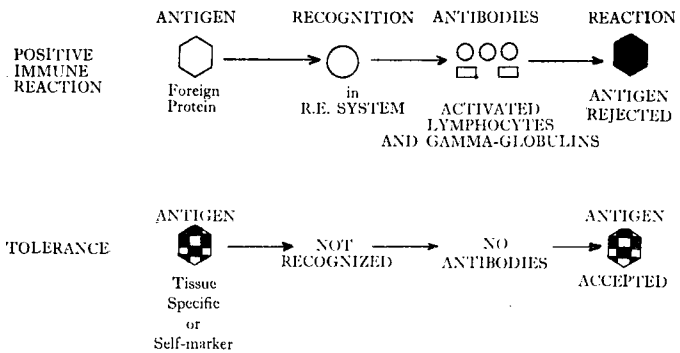

Fig. 40.—Immune mechanisms.

Antigens may come from a wide range of foreign proteins: animal, vegetable, bacterial, and viral; and also from many simple chemical compounds which become antigenic when attached or coupled to a carrier protein. The bodies' own tissues or metabolites may also become immunogenic as in auto-immune diseases.

Recognition is probably a function of lymph-node cells, and there is evidence that this property is shared by other cells of the reticulo-endothelial system. Lymph-nodes, acting as reaction centres, produce activated lymphocytes and antibody molecules. McMaster

* Calne, R. Y. (1963), 'Biological Factors in Tissue Transplantation', *Proc. R. Soc. Med.*, **56**, 935.

and Hudack* report evidence that lymphocytes produce gamma-globulins which are the substance of antibodies. These immune bodies have been shown by Biozzi and others† to be concentrated in reticulo-endothelial cells.

The effector mechanisms are thus humoral (i.e., the various antibodies such as antitoxins, agglutinins, precipitins, opsonins, etc.), and cellular elements, consisting mainly of lymphocytes and plasma cells.

Immunological Tolerance.—A theory to explain the mechanism for safeguarding body tissues from destruction by its own immune reactions has been evolved by Burnet and Fenner.‡ The reticulo-endothelial system is conditioned during embryonic life to distinguish 'self' from 'non-self', i.e., native from foreign elements. Native constituents have a 'self-marker' or 'identity factor' which ensures their protection; these 'self' antigens, also known as 'tissue-specific antigens', then contribute to the maintenance of tissue organization and are not subject to recognition and reaction by the reticulo-endothelial system (*see Fig.* 40).

If the embryo is challenged by foreign material, it accepts it, learns to tolerate it, and does not react against it.

Mitchison§ describes the advent of tolerance (or paralysis) in the adult. At one extreme, some antigens have strong immunizing effects and are named 'obligatory immunogens'; at the opposite extreme there are antigens which are ineffectual without the addition of chemical substances, e.g., alum. These are known as 'zero immunogens'. Between the two extremes there are all grades.

The weaker antigens are capable of inducing tolerance; e.g., in adult mice, large doses of pneumococcal polysaccharide will result in immunological acceptance of pneumococci without the usual host defensive reactions.

Obligatory immunogens may be rendered weaker, and thus more tolerated by the recipient, by several devices. Irradiation, which has been used in various forms, viz. whole body, spleen, thymus, site of application of the antigen, or of the antigen itself, reduces the recipient's immune reactions; so do radiomimetic agents like alkylating substances; antimetabolites, e.g., 6-mercaptopurine and actinomycin C; and steroids.

In terms of the biological behaviour of grafts, transplants, and cancer: the purposive induction of tolerance may make practicable the acceptance of grafts and tumour transplants by recipients, and thus aid cancer research in a number of fields. It is already proving of some value in aiding the 'take' of transplants of whole organs to

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* McMaster, P. D., and Hudack, S. S. (1935), 'The Formation of Agglutinins within Lymph Nodes', *J. exp. Med.*, **61**, 783.

† Biozzi, G., Halpern, R. N., Benacerraf, B., and Stiffel, C. (1957), *Physiopathology of the Reticulo-endothelial System.* Oxford: Blackwell.

‡ Burnet, F. M., and Fenner, F. (1949), *The Production of Antibodies.* Melbourne: Macmillan.

§ Mitchison, N. A. (1963), 'Immunological Paralysis in the Adult', *Proc. R. Soc. Med.*, **56**, 937.

Immunological Tolerance, *continued.*

replace defunct tissues in humans. Secondly, the presence of immunological tolerance may explain the occurrence and maintenance of spontaneous cancer. This has a corollary: the possibility of discovering methods of diminishing or eliminating tolerance, by active or passive immunization against cancer, by an antiserum or by transplantation of immunologically competent cells.

Graft-versus-Host Reactions.—Cells of a graft may be competent immunologically and react in terms of the host's antigenicity. In other words, the usual antigen-antibody reaction is reversed and the graft rejects the host. This concept has been used to explain runt disease in mice and various other conditions. It is a subject looming larger in the field of immunology, and it may come to play an increasing part in explaining aspects that are still not clear.

Immunity in Human Cancer.—The processes of immunity probably operate in spontaneous tumours as well as in transplants. They are not as obvious or as marked; in fact, in the vast majority of human spontaneous tumours, immune defences are inadequate to prevent advance of the cancer. Burnet* postulates 'that it may be that the most important effect of immunological processes' on human cancer is to be seen in the age distribution of malignant disease. The incidence is high in early childhood, due to the immunological immaturity of the neonatal period; then, after an interval of 4 or 5 decades, the curve again rises to higher levels, associated in part with progressing inability to elaborate new immunological responses.

Occasional examples of spontaneous regression are dramatic evidence of defence mechanisms, which may in some cases be immunological. Gordon-Taylor† suggested the possibility of such host defences, and Smithers,‡ in a review of the subject, calls attention to 112 cases of histologically confirmed spontaneous tumour regression. The tumours include neuroblastoma, renal carcinoma, chorionepithelioma, melanoma, sarcoma, and carcinomas of the bladder and breast. Reactions of immunity are not necessarily the explanations for all these cases. Most of the breast-tumour regressions occurred at the time of the menopause, suggesting their hormone-dependency, and bringing the effect upon the tumour into line with forms of therapy altering hormonal status, e.g., the known beneficial outcome of oophorectomy. In neuroblastoma, Willis§ suggests that regression may constitute delayed maturation of embryonal cells to adult types, as occurs in the course of normal growth at an earlier age. In some cases of bladder tumours, regression appears to follow withdrawal of

* Burnet, F. M. (1964), 'Immunological Factors in the Process of Carcinogenesis', *Br. med. Bull.*, **20**, 154.

† Gordon-Taylor, G. (1959), 'The Incomputable Factor in Cancer Prognosis', *Br. med. J.*, **1**, 455.

‡ Smithers, D. W. (1962), 'Spontaneous Regression of Tumours', *Clin. Radiol.*, **13**, 132.

§ Willis, R. A. (1958), *The Borderland of Embryology and Pathology.* London: Butterworths.

continued carcinogenic application and action, e.g., in urinary diversion by implanting the ureters into the colon. Chorion-epitheliomas are comparable to homografts, and their regression may be occasioned by a combination of hormone influence and immune reaction. Because of the entry of factors other than those pertaining to immunity, spontaneous regression cannot be regarded as proof of immunological response.

Similar evidence, with similar qualification, arises from examples of prolonged survivals of patients after incomplete removal of their cancers, and of cases of sudden and often overwhelming appearance of extensive metastases after a long period of apparent cure following extirpation of the original tumour.

Watne and others,* Moore and Grace,† Southam,‡ and others, have drawn attention to the frequently observed fact that in patients with cancer, although cells are present in blood- and lymph-vessels and in peritoneal and pleural fluids, they do not all take seed to give rise to metastases. Many authors argue that this is evidence of host resistance in the form of immune activity. The argument may be valid, but it is still subject to doubt as many other considerations, e.g., the adequate nutrition of the migrant cells, must be taken into account.

An indication of immune cellular reaction to human spontaneous tumours may be present in the form of infiltration by lymphocytes, plasma, and mast cells. The fact that prognosis in such cases is more favourable lends some weight to the idea that it is basically an immunological phenomenon.

Nairn and others§ report a case of advanced renal cancer which was given two courses of a specially prepared emulsion containing kidney tumour which had been removed from another patient one year earlier. The donor patient had the same blood group (ABO and Rh) and the same type of tumour as the recipient. The clinical course and advance to death were not influenced. However, studies on serum demonstrated the presence of a precipitin which appeared to be specific against renal cancer; and immunohistological examinations showed that globulin, possibly antibody, had localized on the surface of cancer cells. Whilst this work carries promising potential, it has barely begun and requires wider confirmation before deductions are in order.

There are many phenomena suggesting a host-antibody response in cancer, but the demonstration of specific antigens in man has not yet been unequivocal. An attempt has been made by Hughes and Lytton‖ to discover whether spontaneous cancer in man is antigenic to an extent to produce delayed cutaneous hypersensitivity (as

* Watne, A. L., Moore, G. E., Oliver, R. J., and Kondo, T. (1961), 'Modification of Host Resistance to Malignant Cell Growth', *Archs Surg.*, **82**, 478.

† Moore, G. E., and Grace, J. F. (1960), 'Cancer Immunity', *Surgery Gynec. Obstet.*, **110**, 234.

‡ Southam, C. M. (1960), 'Relationship of Immunology to Cancer. A Review', *Cancer Res.*, **20**, 271.

§ Nairn, R. C., Philip, J., Ghose, T., Porteous, I. B., and Fothergill, J. E. (1963), 'Production of a Precipitin against Renal Cancer', *Br. med. J.*, **1**, 1702.

‖ Hughes, L. E., and Lytton, B. (1964), 'Antigenic Properties of Human Tumours: Delayed Cutaneous Hypersensitivity Reactions', *Ibid.*, **1**, 209.

Immunity in Human Cancer, *continued.*

is found in homografts). In 50 patients with a variety of cancers involving breast, colon, lung, stomach, and other organs, about one-quarter had such delayed reactions to injections of cell-free extracts of their own tumours, suggesting that there were specific antigens and that the response was indeed immunological.

Theoretical Potential.—A number of theoretical possibilities arising from immunological phenomena may come to aid clinical management of human cancer. These aspects have been included in a review by Southam,* who stresses that, at present, they are largely speculative.

CANCER PREVENTION.—A method may be found by which immunological defence mechanisms may be enhanced. Both passive and active immune reactions are being intensively sought; some examples of the type of work have been referred to in the preceding section of this chapter.

CANCER DETECTION.—Many investigations into the possibilities of detecting specific antigens and antibodies, in serum, urine, and various body tissues have been, and are currently being, undertaken.

TESTS OF PROGNOSIS.—The spread of cancer causes increasing destruction or radical alteration of normal tissues. Such interference with normal function might be amenable to discovery by immunological techniques.

THERAPY.—Immunological mechanisms have been used extensively and successfully in many non-cancerous conditions. The possibilities of making or finding anti-cancer antisera, of eliminating tolerance, of reinforcing active and adoptive immunity (i.e., continuing antibody production by lymphoid cells transferred from an actively immunized donor), and of stimulating cellular defences, have inspired considerable research. So far, the results are puzzling and disappointing.

Immunological Theory of Carcinogenesis.—This theory involves reference to three types of antigenic activity:—

1. SPECIES SPECIFIC ANTIGEN.—The cells of one species are antigenic in another, inducing a specific antibody effective against the antigen.
2. ISO-ANTIGEN.—The cells of one individual are antigenic in a recipient of the same species, unless, during embryonic life, the potential antigen has been introduced to the recipient so that it becomes recognized and tolerated; in fact, it evolves into the third type of antigen.
3. TISSUE SPECIFIC ANTIGEN.—This is the 'identity factor' or 'self-marker' (*vide supra*), brought into the hypothesis explaining

* Southam, C. M. (1960), 'Relationship of Immunology to Cancer: A Review', *Cancer Res.*, **20**, 271.

the absence of antigen-antibody reaction within one body and a supposed factor for maintaining normal tissue organization and relative disposition of different bodily constituents.

Green* has been a chief proponent of the immunological concept of carcinogenesis, the origin of which may be said to spring from the observation that certain carcinogenic and non-carcinogenic hydrocarbons exhibit tumour-inhibiting (T I) properties upon homologous transplanted tumours, but do not have this effect on spontaneous or chemically induced tumours. In the latter varieties, the tumours arise from the animal's own tissues, and they do not, therefore, provoke antibody formation. When such tumours are transplanted, they exercise an iso-antigenic action upon the new host; this may be strong initially, but repeated transplantation leads to the elimination or weakening of the iso-antigen. The supposition then arises that T I activity is due to the induction or re-invigoration of iso-antigens.

There are differences in the immunological activity of carcinogenic and non-carcinogenic T I agents. Carcinogens stimulate mitosis, even in the presence of cortisone (which ordinarily suppresses cellular activity). The other T I agents do not stimulate mitosis; and cortisone, which is known to suppress reticulo-endothelial activity and thus reactions of immunity, effectively negates non-carcinogenic hydrocarbon tumour inhibition. It is postulated that the introduction of a carcinogen into an animal results in a combination of the carcinogen with the tissue-specific or 'identity' proteins, forming a complex which is antigenic; continued exhibition of the carcinogen, or the addition of a promoting agent, leads to an adaptation with loss or neutralization of the new antigen by the development of host cells in which tissue-specific antigen is deleted. The outcome is the absence of recognition of these new cells, the loss of normal control over them and their consequent proliferation without regard for organization or tissue boundaries, i.e., neoplastic behaviour.

Green considers the behaviour of cancer induced by radiation, hormones, and viruses in the light of his theory, and speculates on the possibility of their origin from loss of immunological identity.

Many facts and ideas leading to the immunological concept of cancer are of unquestionable importance, but the final conclusions are supported only by a number of hypothetical assumptions, and judgement must therefore be reserved on the subject. As Hieger† points out, there is cogent argument in the work by Soroff and Cohen,‡ who find a deficiency of a basic cytoplasmic protein in tumour cells and suggest that this is the fundamental change to

* Green, H. N. (1958), 'Immunological Basis of Carcinogenesis', *Br. med. Bull.*, **14**, 101; (1958), 'Immunological Aspects of Cancer', in *Cancer*, Vol. 3 (Ed. Raven, R. W.). London: Butterworths; and (1959), 'Immunological Aspects of Cancer', in Ciba Symposium on *Carcinogenesis*. London: Churchill.

† Hieger, I. (1961), *Carcinogenesis*. London: Academic Press.

‡ Soroff, S., and Cohen, P. P. (1951), 'Electrophoretic and Ultra-centrifugal Studies on the Soluble Protein of Various Tumours and of Livers from Rats fed 4-dimethylaminoazobenzene', *Cancer Res.*, **11**, 376.

Immunological Theory of Carcinogenesis, *continued.*

neoplasm. There is also the work of Miller and Miller* on amino-azo-dye hepatoma in rats. They hold that the carcinogen is metabolized to a derivative which combines with liver protein, the role of which in determining cell response to intrinsic and extrinsic controls is thereby upset. Protein synthesis is interfered with; and it is supposed that the deletion of a specific enzyme is responsible for the loss of control of cellular activity, so leading to cancer. The three theories of deletion, of tissue-specific antigen, basic cytoplasmic protein, and specific enzyme have certain similarities. Which, if any, theory is true is a question not yet resolved.

THE THYMUS IN IMMUNOLOGY

An investigation, reported by Kay and others,† into the development of the human thymus, produced evidence compatible with the hypothesis that lymphoid tissues were colonized by cells originating from the thymus.

Miller‡ showed that thymectomy in mice in the neonatal period led to severe reduction in lymphocytes and serious lowering of immune responses; later grafts of thymus into the mice restored lymphoid tissues and immunological competence. Similar experiments in rabbits and chicks are referred to in support of the findings. In a review, Miller and others§ quote an impressive number of experiments and observations which support the hypothesis of the thymus having the highest lymphopoietic activity among all lymphocyte producers.

It would appear that lymphocytes produced in the thymus leave it to enter the general circulation. In addition, the thymus may possibly function by secreting a lymphocyte-stimulating hormone which effects maturation and/or production of lymphocytes elsewhere in the body. The fact that thymectomy in experimental animals at increasing intervals after the neonatal stage have progressively less effect on the lymphocyte population indicates that thymic lymphopoietic function is greatest absolutely and relatively at very early ages; and that at later ages, production by other organs may compensate for loss of the thymus.

The relation between lymphocytes and immunity is very close, and the significance of the thymus in immunology is similarly marked by an intimate association; so much so, that observations on lymphocytic responses to experiments on the thymus show parallel effects in immunity reactions. Gowans and others‖ have shown that plasma cells,

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* Miller, J. A., and Miller, E. C. (1953), 'The Carcinogenic Amino-azo-dyes', *Adv. Cancer Res.*, **1**, 339.

† Kay, H. E. M., Playfair, J. H. L., Wolfendale, M., and Hopper, P. K. (1962), 'Development of the Thymus in the Human Foetus and its Relation to Immunological Potential', *Nature, Lond.*, **196**, 238.

‡ Miller, J. F. A. P. (1962), 'Effect of Neonatal Thymectomy on the Immunological Responsiveness of the Mouse', *Proc. roy. Soc.*, B, **156**, 415.

§ Miller, J. F. A. P., Marshall, A. H. E., and White, R. G. (1962), 'The Immunological Significance of the Thymus', *Adv. Immun.*, **2**, 111.

‖ Gowans, J. L., Gessner, B. M., and McGregor, D. D. (1961), 'The Immunological Activity of Lymphocytes', in *Biological Activity of the Leucocyte.* Ciba Study Group 10. London: Churchill.

which are known to play a major role as antibody producers, are most probably derived from small lymphocytes. Fichtelius* traced thymus cells to splenic perifollicular red pulp, the region of plasma-cell proliferation during antibody production. The accumulating data and observations suggest the possibility of the site of origin of precursors of immunologically competent cells being in the thymus. It seems reasonable to anticipate that further discovery in this young field of biological research may illuminate many problems of immunology and cancer.

* Fichtelius, K. E. (1960), 'On the Destination of Thymus Lymphocytes'. In Ciba Symposium on *Haemopoiesis: Cell Production and its Regulation*. London: Churchill.

CHAPTER XXV

PREVENTION AND TREATMENT OF CANCER

The main objective of research into the origin and behaviour of cancer is to provide preventive measures, and, if the disease occurs, to find methods of curative treatment. The tactical planning for the achievement of such a strategic objective may be based upon several main waves of attack: prevention; recognition and elimination of precancerous lesions; treatment of early cancer; and treatment of developed cancer. The tactical approach is listed in order of priority; each succeeding move is employed for the failures resulting from evasion of the more important and earlier manœuvres. Only a bare outline, directing attention to the main lines of action, is appropriate in this book.

PREVENTION

Epidemiological Studies.—The greatest contribution to the prevention of many cancerous diseases has emerged from different forms of epidemiological investigation involving geographical pathology, demographic and ethnic factors, industrial and occupational conditions, correlations of habits, customs, and social observances with the diseases, and the study of incidence, prevalence, and mortality rates in relation to the foregoing information.

Statistical description and analysis is a fundamental part of such investigations. Despite the inaccuracies, variables, and the inevitable human errors of judgement, statistical methods have proved of considerable value. Two discoveries, substantially aided by statistical analysis of environmental circumstances and their relation to neoplasms, exemplify the practical importance of the method. Cigarette smoking as a cause of lung cancer and aromatic amines as a cause of bladder tumours, whilst not completely proven, represent major breaks in the sombre clouds shrouding the knowledge of cancer, and the discoveries have led to an improved capacity for combating these diseases.

The prize to be won makes the battle well worth while. Increasing recognition of environmental carcinogens and internal influences, with their early effects, have made prevention potentially possible in more than three-quarters of human cancer (W.H.O.*). The dimensions of this objective are immeasurably enlarged by the ever-widening horizons of biological science that cancer research brings within the grasp of human knowledge. Conquest in the war against cancer has an attraction and fascination, and promises far

* World Health Organization (1964), *Prevention of Cancer*. Technical Report Series, No. 276.

greater reward and booty than any other war in human history; it has, moreover, prospects for success at a much lower cost than the destructive world wars of this century.

Industrial Cancer.—It was in the sphere of malignant tumours arising from industrial occupations that the first significant success raised hopes for an extension of control and prevention of many cancers. Success did not come suddenly with a flash of new disclosures; it was the outcome of long years of clinical observation and experimental research by many investigators in different medical and biological disciplines. Some of the outstanding discoveries have been noted in different chapters on geographical pathology, occupational cancer, and the carcinogenic agents.

The range of substances in industry, so far known or suspected as causes of cancer, is wide and extensive. They may be present in raw material, as in the ores mined at Schneeberg; as an ancillary part of a manufacturing process, as in the lubricating oil in cotton spinning; in intermediate products, as in the chemicals of certain dyestuff production; in by-products, as in chromium processing; or in the finished product, as in artificially produced ionizing radiations from X-ray tubes. Some carcinogens are active from their crude source through to the consumer form, as is the case with arsenic and asbestos.

The agents are of different types: physical, such as actinic rays; chemical, which probably accounts for the bulk of industrial cancerigenic agents; and others, like parasites, which are not so strongly supported by the evidence.

Examples illustrative of the many successful planned campaigns against the occupational hazards of cancer are the virtual elimination of shale-oil cancer; the considerable reduction of incidence of mule-spinners cancer, mainly by changing the type of lubricating oil; the lawfully enforced protection of personnel exposed to ionizing radiations; and the reduction of urinary bladder cancer in dye factories.

Cancer due to Habits and Customs.—The elimination or control of tumorigenic agencies in industry is eminently practicable and enforceable by legislation and regulation. The picture is very different in regard to habits and customs, for which the cancerigenic potential is supported by reasonably good and sufficient evidence. Certain habits, and particularly social customs, have acquired an aura, time-ingrained rather than time-honoured, which grips their practitioners in an obstinate renunciation of any evidence which might point to a beneficial change of habit. Sometimes it is vested interest that opposes the eradication of the noxious substance; at other times, resistance comes from those addicted to the habit and are unable to liberate themselves from it. It is remarkable and significant that among the physicians who do not accept the case for incriminating cigarette smoking as a cause of lung cancer, disproportionate numbers are inveterate smokers.

Educational and publicity programmes for the elimination of cigarette smoking are of recent origin. So far, they have had but slight effect, and they require reinforcement and wider application.

Cancer due to Habits and Customs, *continued.*

Further research to determine doubtful aspects is essential, but there are already adequate grounds to justify full-scale, confident approaches to the public.

In the light of the difficulties besetting the campaign to reduce lung cancer, it is not surprising that efforts to reduce oral cancer by dissuading people to abandon betel and tobacco chewing have met with little success. The mass of habitués of this practice is uneducated, backward, and unaccustomed to hygienic principles; they are thus less amenable to proposals for reform than the presumably better educated and more advanced 'Western' cigarette smoker.

Where custom becomes incorporated into traditional and religious belief, anti-cancer programmes are even more difficult to apply. The Egyptian fellah's habits in defaecation hygiene and his ritual oral washing incite bilharzial infection of the individual and spread the disease in ever-widening circles. Another example of this aspect of the problem is the marriage of very young Indian girls. Their early intercourse is one of the factors responsible for the excessive prevalence of uterine cervical cancer; but it is unlikely that this fact will have much influence over a traditionally hallowed practice.

The causative effects of persistent smegma for both penile and cervical cancer could be eliminated by a relatively simple régime of personal cleanliness. The very simplicity of the necessary preventive measures throws into prominence the tremendous difficulty impeding their introduction: the lack of trained personnel to teach the people, and the pathetically low standards of the people affected, are perhaps the greatest obstacles.

Prevention by Treatment of Predisposing Conditions.—Cancer comprises many different diseases; some afflict large groups of people, such as those referred to above; others affect much smaller numbers, but prevention is still desirable. A number of congenital conditions have a recognized proclivity to malignant transformation; prevention of the defects that are heritable may be possible by eugenic measures, e.g., for the condition xeroderma pigmentosum. In others, e.g., multiple polyposis of the colon, early treatment may prevent malignant evolution. The treatment of moles, especially in the more dangerous situations, as on the soles of the feet, adjacent to or on the external genitalia, and on the face, is probably of value as a prophylactic measure.

The high incidence of oral cancer following long-continued dental irregularities and sepsis emphasizes the importance of proper dental care as an effective preventive procedure.

Dietary and nutritional factors are of known influence in malignancy. The Plummer-Vinson syndrome is an outstanding example: anaemia, malnutrition, and vitamin-B deficiency are precursors of hypopharyngeal carcinoma in comparatively young females. Prevention is possible by treatment of the initial non-malignant state.

These examples of several fields of cancer prevention bring to light major problems that may be likened to logistical considerations in

destructive warfare. A prime requirement in the cancer battle is to bring trained and accoutred personnel to the proper places in order that they may effectively use their knowledge and equipment at the right time.

Prevention by Treatment of Precancerous Conditions.—Precancerous lesions are those in which an appreciable proportion evolves into malignant tumours; the proportion that does undergo change is sufficiently significant to justify radical treatment of the early suspect condition. This form of prevention offers an extending battle-field with great promise and inspiring opportunity for subjugation of many potential enemies.

The prime needs are recognition and diagnosis of the premalignant conditions, and the discovery of an ever wider variety of such lesions. The experimental laboratory, with its facilities enabling close study of progressive pathological states caused by different carcinogens and combinations of carcinogens, has been of tremendous help to clinical assessment and management of abnormalities that bear malignant potentialities.

Examples of premalignant conditions amenable to therapy or control will indicate the importance of this form of cancer prevention. Hyperkeratotic lesions of the skin are premalignant. They may follow many different stimuli and circumstances: the advance of years, exposure to ionizing radiation or sunlight, repeated injury or persistent unhealed wounds, old burn scars, arsenical medication, and contact with tar and other chemicals of the polycyclic hydrocarbon group. Chronic ulcers, apart from hyperkeratotic change, may properly be considered precancerous; on mucosal or mucocutaneous situations, their danger is enhanced, and chronic fissures and ulcers on the lips and within the mouth are highly suspect. Alterations in the clinical qualities of moles and naevi call for the most careful appraisal including biopsy excision.

Leucoplakia, affecting mucosal surfaces, is comparable to hyperkeratosis of the skin in origin and potential danger. Warts, nodules, and papillomata on mucous membranes are precancerous in an appreciable proportion and they call for biopsy excision. On the skin, they are not as serious; but the development of certain characters, such as ulceration, rolling or irregularity of edge, or in fact any clinical change, are warning signs of greater danger.

In certain organs, papilloma has a marked tendency to cancerous change, and consequently requires more intensive management. Papilloma of the urinary bladder is particularly susceptible. At a somewhat lower grade of incidence of malignancy following a benign condition, but still highly dangerous, are papillomata of the rectum and the remainder of the gastro-intestinal tract.

There are several areas of possible premalignant pathology in which there are differences of attitude. One such area is that of thyroid nodules, whether single or multiple, toxic or simple. Some hold that carcinoma follows in 10–15 per cent and that this is a sufficient incidence to justify early surgery; others deny this high rate of tumour development, and advocate surgery only in suspicious cases. Circumcision is another instance: a few authorities recommend it as a general measure to prevent penile cancer and to reduce

Prevention by Treatment of Precancerous Conditions, *continued.*

uterine cervical cancer. This seems an unnecessarily extreme measure, as proper hygiene has proved an adequate prophylactic in large national groups. However, it is reasonable to recommend circumcision as cancer preventive treatment for cases of recurrent phimosis and balanitis.

The question of the relationship of adenofibrocystic disease of the breast and cancer is not definitely settled. But this should not interfere with the necessity for histopathological examination of breast lumps, as benign mastopathy may mask a malignant condition. Here the imperative need is for accurate diagnosis.

TREATMENT OF EARLY CANCER

The disciplines and organization for treating early cancer are extensions of the preventive measures applied to precancerous conditions. It has been fully realized for 4 or 5 decades that eradication of early cancer is curative and that delay brings death; the consequential corollary that it is of vital import to find and publicize methods of diagnosing early malignancy has also been appreciated for as many years. It is only in the last decade or so that planned activity and specially designed projects have begun to meet the problems of finding, and then bringing, premalignant and early cancer to adequately equipped units.

The education of the public is a major contribution to the grand scheme. Free discussion and unrestrained reference in all the media of public communication is a necessary background and helps to spread the circulation of knowledge of the manifestations of early cancer and its curability. Methods of self-examination, e.g., for breast lumps, at one stage criticized as a stimulus to an upsetting cancerphobia, has proved physically valuable and psychologically harmless.

The controversial subject of whether all medical practitioners should be instructed in the recognition of early cancer, or whether the public should be directed to special diagnostic or 'detection' centres, is being solved in a practical manner. They are not mutually exclusive arrangements and both may be fostered as complementary to one another and to the ultimate advantage in waging war on cancer.

Certain specialized techniques of early diagnosis call for particular establishments. Radiography is an obvious case in point; hospital and clinic departments, however, have not satisfied the whole demand, and mobile units, using conventional and miniature films, have improved the position. Histopathological services have been extended to cope with the response to educational emphasis on the need for early pathological examination. New cytological techniques to find evidence of early cancer lesions by examining cells from suspect sites have called for greater decentralization of pathological units. Papanicolaou and similar methods are now applied not only to suspect cells but also to routine clinical surveys.

Schemes for regular periodic examinations in certain centres have demonstrated the practicability of eliminating cancer of the uterine cervix as a fatal disease. Routine annual clinical and cytological reviews of women over the age of 45 may also reduce the problem of corpus carcinoma to minor proportions.

Cells in sputum, cells from the rectum and colon, and from urinary sediment, in suspect cases, are examples of relatively simple forms of examination with an increasing degree of accuracy as personnel acquire training and experience.

All this requires organization on a national or paranational level; pilot schemes have proved themselves and have set the stage for grand designs involving large populations.

TREATMENT OF ESTABLISHED CANCER

Two forms of treatment, surgery and irradiation, are available for the eradication of cancer. The treatments are used separately as well as in combination in various forms. Palliation of incurable cancer is also possible by both therapeutic agents, and a third class, chemotherapy, forms part of the armamentarium for this type of treatment.

Surgery.—Surgical extirpation has long been the main prop of curative therapy. Improvements in technique, refinements of knowledge of cancer behaviour, and advances in anaesthetic and metabolic services, have extended the applicability of surgery to every organ of the body, and have enhanced its value in a wide range of tumours. The principles of surgery for malignancy include complete excision of tumour tissues, removing *en bloc* the initial focus, the neighbouring tissues involved in direct spread, and the lymphatic field of drainage.

'Cancer' surgery, in every branch of practice and applied to every part of the body, constitutes a substantial part of surgical therapy as a whole. The subject is of such an extent that there are no convenient short references that will do justice to its scope. A bibliography, constructed in moderate measure, would necessarily embrace many standard monographs as well as more comprehensive works, and would therefore not be suitable in this present context.

Radiotherapy.—A similar comment is applicable to radiotherapy, and the following bare indication of its place cannot here be buttressed by an adequate bibliography.

Radiotherapy is used for curative and palliative purposes. The objective of complete destruction of malignant cells has its greatest degree of success in early, superficial, and accessible tumours. This is the same field as that in which surgery is most efficacious, so that two modes of healing are available for certain cancers, whereas others are not so beneficially influenced by either instrument of therapy. The two forms of treatment are often used in combination; frequently, irradiation is administered post-operatively, to cater for tumour cells missed by the scalpel or for those beyond its safe reach. Radiotherapy has, perhaps, its greatest value in palliating the distress of the inoperable and generalized malignancy.

Of the several methods of administration, roentgen rays and the emanations from radium are the most commonly used; but rays from radioactive isotopes and other forms of electromagnetic and particulate energy have attained special usefulness in specific

Radiotherapy, *continued.*

situations. A classic example of such specificity arises from the fact that the thyroid gland takes up iodine and so makes the use of radioactive iodine particularly suited to irradiation of thyroid tumours.

Advances in construction of apparatus for the production of X-rays have provided new heights of power and accuracy of application and concentration in deep tissues. The new techniques are still under trial and their full therapeutic capacity has not been evaluated.

Chemotherapy.—The efforts to discover a drug with selective effects on tumour cells have been disappointing, and chemotherapy is still mainly indicated for generalized malignant spread, for leukaemia and lymphosarcoma, and as an adjunct to surgery and radiotherapy in earlier cases. The agents, in addition to effects upon malignant cells, have similar effects upon normal cells, and may therefore cause severe and dangerous complications.

Considerable numbers of drugs have been tested; those with practical therapeutic possibilities may be classified in the following main groups.

ALKYLATING AGENTS.—During World War II, studies of the physiological effects of nitrogen and sulphur 'mustard gas' revealed their cytotoxic action. This led to their trial in Hodgkin's disease, where partial success stimulated extensive investigations of many aspects of such drug activity. The more commonly used cytotoxic agents in this group are nitrogen mustard, melphalan, busulphan ('myleran'), chlorambucil, thiotepa, and endoxan.

The cytotoxic effect of alkylating agents is proportional to the proliferative activity of the cells, and thus normal, actively dividing cells, such as haemopoietic, gastro-intestinal epithelial, germinal epithelial, and cutaneous epithelial cells, may be seriously affected.

ANTIMETABOLITES interfere with a stage in the biosynthesis of purines or pyrimidines, resulting in disturbances of cell growth and division. Some of the more successful of this group are methotrexate (extensively used in leukaemia in children), 6-mercaptopurine, 5-fluorouracil, and 6-azauridine.

ANTIBIOTICS.—An appreciable degree of anticancer activity has been shown in very few of those tested: actinomycin-D and mitomycin are among the more promising; they seem more effective when combined with other chemical antitumour agents.

HORMONES.—It would seem appropriate to include hormones in this list. Their place as therapeutic agents is noted in several chapters on hormones as carcinogenic factors.

TECHNIQUES OF ADMINISTRATION.—The disadvantages arising from harmful effects of anticancer drugs on normal cells have already been referred to; to overcome this, different techniques of concentration of the drug have been devised.

Local regional perfusion, by adaptation of extracorporeal pump oxygenation apparatus, is particularly applicable to a limb. Continuous infusion, by cannulating an artery as closely as possible to the tumour, is another practical method of applying concentrated antitumour agents in a localized zone.

A somewhat different class of the 'local' use of a cytotoxic agent is the 'washing' of a surgical wound made during extirpation of a tumour. At intervals in the course of operation and prior to final wound repair, the wound may be flushed with an agent to devitalize tumour cells on the wound surfaces and floating freely in blood and exudate. Instruments and gloved hands are subjected to rinsing with the same chemical solution. The benefits are difficult to assess statistically, but appear to be highly probable.

The whole subject of chemotherapy in cancer is in a state of flux and uncertainty. A short and balanced review is presented in a W.H.O.* Technical Report.

* World Health Organization (1962), *Chemotherapy of Cancer*. Technical Report Series, No. 232.

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