THE KIDNEY

CIBA FOUNDATION SYMPOSIUM

ON

THE KIDNEY

Arranged jointly with the Renal Association

Editor for the Renal Association

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PREFACE

THE Ciba Foundation is an international centre, established as an educational and scientific charity under the laws of England. It owes its inception and support to its founder, Ciba Limited of Switzerland, but is administered exclusively by its distinguished British Trustees.

As one part of the Foundation's activities, informal symposia or colloquia, strictly limited in membership, are arranged, to which leading research workers from different countries and different disciplines are invited. As the smallness of the group necessarily excludes many people active and interested in the subjects discussed, the proceedings are being published and made available throughout the world.

"The Kidney" was arranged at the request and with the assistance of the Executive Committee of the Renal Association. It was initiated mainly by Mr. G. J. Sophian, formerly Hon. Secretary of the Association, and considerable help was given to the Director of the Foundation in planning the programme and selecting the membership by Dr. C. E. Dent, Prof. K. J. Franklin, Dr. A. A. G. Lewis (Hon. Secretary) and Dr. W. W. Payne.

The ground covered at this symposium included structural and functional relationships in the kidney, the regulation of acid-base balance, tubular functions other than the regulation of acid-base balance, general problems of electrolyte excretion, and the renal share in the volume control of body fluid.

As membership was severely limited and, apart from many workers in the field in other countries, the great majority of the members of the Renal Association had to be excluded, a public session was held at the Royal Society of Medicine on the day after the conclusion of the symposium at which Dr. Jean Oliver, Dr. J. V. Taggart, Dr. R. F. Pitts, Dr. N. Alwall,

and Prof. J. G. G. Borst reviewed in turn the proceedings of the five sessions of the symposium. A report of this public meeting was published in *The Lancet* (1953, Vol. II, page 120).

It is hoped that the full proceedings of the symposium recorded in this book will prove not only informative and stimulating, but will also give to readers a sense of participation in this informal and friendly occasion.

The Editors are greatly indebted to Mr. J. and Mr. John A. Rivers of Messrs. J. & A. Churchill, Ltd., for their courteous attention and ready advice at all stages of the preparation of this volume.

FOREWORD

A. A. OSMAN, *D.S.C.*, M.D., F.R.C.P.

THIS book embodies a verbatim account of the proceedings at an international symposium on The Kidney arranged jointly by the Ciba Foundation and the Renal Association, and held in London from the 7th to the 10th July, 1953.

To facilitate a free, informal and intimate exchange of views membership was limited to thirty-five participants, but the last day of the conference was devoted to a review of the previous sessions by five of the participants. This meeting, at the Royal Society of Medicine, was made open to the Profession and was very well attended.

The participating members were chosen for their eminence in those aspects of renal anatomy, physiology, pathology and medicine that it was desired to integrate for study, and included experts not only from this country but also from Belgium, Denmark, France, Holland, Sweden, Switzerland and the U.S.A.

Twenty-two papers were read and each was followed by a full, and often vigorous, discussion under a number of distinguished Chairmen.

No one who was privileged to attend this Symposium would, I am sure, regard it as having been other than a most stimulating and rewarding, if somewhat strenuous, experience, and it was generally felt that the mass of valuable information presented, together with the illuminating comments made in the subsequent discussions, should be permanently recorded and made more widely available.

That this volume will prove valuable to all interested in the kidney and its disorders can hardly be doubted, and is, I know, the earnest hope of the Editor for the Foundation, to whom, incidentally, credit for the success of the whole venture is chiefly due.

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Chairman: PROFESSOR R. A. MCCANCE, *CBE*, MA, PhD, MD, FRCP, FRS
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Part I—Structural and Functional Relationships in the Kidney

THE STRUCTURAL AND FUNCTIONAL ASPECTS OF RECOVERY FROM ACUTE RENAL FAILURE

JEAN OLIVER

For all the dynamic surge of the biological sciences in the present day, structural change—morbid anatomy to put it bluntly—still remains the chief business of the pathologist. In this somewhat old-fashioned, or may we say classical tradition, the pathologist need feel no sense of dragging feet, for now that morphological investigation is approaching the molecular level by a microscopy that uses not only the invisible energies of the spectrum but electronic forces as well, the old antithetical bugbear of structure as opposed to function has disappeared. Bertalanffy's (1952) postulate of a dynamic morphology is no longer a philosophical concept for the library but a matter of daily practice in the laboratory, for what the morphologist sees at any particular moment may now be regarded as a sort of arrested cinematographic image and these images can be fused by him into the dynamic continuum that is function.

These principles are illustrated by the newer morphological concepts that concern the nephron, a field long restricted by the technical method of the histological section. From this two-dimensional view we have progressed to add not only a third but, in the conceptual sense, a fourth dimension in the form of the continuum which results from viewing the nephron, as obviously it must be viewed if correlation with shifting gradients of functional activity is desired, in the integrity of its continuity. It is cheering, I think, that this forward step can be taken in a time of fantastically complicated technology by a return to a procedure well known to primitive man, namely the poking at mysterious things with a stick, or by some slight refinement on our part, with a needle.

The particular example that I wish to describe as an illustration of these newer concepts is the manner in which nephrons recover their functional capacities after an episode of acute failure. As will be evident, we cannot ask how the "kidney" recovers, for such a question allows of no intelligible answer since what is concerned is the integrity of some two million independent, individual organs (Oliver, 1950*a*). When I speak of the manner of recovery I am including both the functional and structural aspects of the process. For the former I shall follow the description of Dr. Graham Bull (Bull, Joekes and Lowe, 1950), and to his relation of what was happening during the considerable period that elapsed before renal activity was restored, I shall add what appears to a morphologist to be correlative structural mechanisms (Oliver, MacDowell and Tracy, 1951).

You will recall that in the typical case of acute renal failure, after a relatively brief period of anuria or oliguria, urine flow rather suddenly returns and increases to the extent of a considerable diuresis. For some time the urine has the general character of a simple glomerular filtrate and appropriate tests indicate that the tubules are functioning poorly, if at all. Yet, paradoxically, it is commonly observed that they do reabsorb the sugar of the glomerular filtrate, though the maximal rate of tubular transport of glucose (Tm_G) is reduced, an interesting anomaly to which we shall return. Over a considerable period, for weeks and even months, tubular function gradually returns, the urine volume is reduced, nitrogenous wastes are concentrated in the tubule lumen and eliminated with the urine, electrolytes are increasingly conserved and for all clinical purposes renal activity is restored. In many cases, however, more critical functional tests show a decrease in renal reserve.

What the morphologist sees in the earlier stages of the renal disturbance would seem to explain the mechanisms of altered function. During the period of oliguria, the histological section shows collapsed and bloodless glomeruli; it is notable that not all are equally affected, for in some the capillaries

contain red blood cells. Of considerable importance for what is to happen later, is the absence of any tissue damage in the tufts, save perhaps for collections of protein precipitated by the action of the fixing solution in Bowman's space, an indication that the permeability of the glomerular membrane has increased.

The condition of the tubules is less clearly determined in sections, though it is evident enough that they have suffered considerable structural change. This frank tissue damage is noticeable a few days after the beginning of the oliguric period and increases with the passage of time until it becomes the predominant feature of the histological picture. Associated as it is with evidence of loss of tubular function, Dr. Bull's designation of the renal status as Acute Tubular Necrosis summarizes the situation as accurately as a few words can.

It is meaningless, however, to talk of "tubular" damage in the light of our present knowledge of the structure and function of the nephrons (Oliver, 1950*b*). One must discriminate, and how accurately and specifically this can be done is illustrated by examining the anomaly I have just mentioned, wherein, with all other tubular functions eliminated, the reabsorption of sugar still persists.

One of the very few renal physiological processes of which we have what may be called reasonably certain knowledge, since it is based not on deduction but direct observation, is the location of the site in the tubule where sugar is reabsorbed; an extension of Richards' method of tubular puncture to the mammalian kidney (rat and guinea pig) has shown that practically all the sugar is removed from the tubule fluid by the time it has passed through the first half of the proximal convolution (Walker and Oliver, 1941). If one examines the proximal convolutions in dissected nephrons from human kidneys in acute renal failure, it is unusual to find much, if any, structural change in this first portion of the segment, though the second half and particularly its medullary part may be grossly damaged to the point of complete necrosis. From the structural standpoint it would appear that such a

proximal convolution, though its total ability to absorb sugar might be lessened, and this Dr. Bull observed in the form of a lowered Tm_G, could still deliver a glucose-free fluid to the lower reaches of the nephron.

A closer consideration of the tubular damage in dissected nephrons shows that there are not one but two sorts of tubular lesion. One, which may be called nephrotoxic, consists of epithelial necrosis but with a preservation of the integrity of the basement membrane. Such lesions are confined to the proximal convolutions and all the nephrons are involved to a remarkably similar degree. In the other, which can be called the tubulorhexic lesion, there is a disruption of the entire tubule wall as both epithelium and basement membrane are destroyed. This is the lesion first seen in histological section by Dunn, Gillespie and Niven (1941) in the distal portion of the nephron. Its recognition in other parts of the nephron is quite difficult, if not impossible, as the damaged nephrons can no longer be identified, but in dissected nephrons these lesions can be seen scattered at random from glomerulus to collecting tubule.

The causes of these two strikingly different tubular lesions are, as Dr. Bull deduced from his functional findings, quite distinct, for it can be shown experimentally that the nephrotoxic lesion is due to the action of poisons on the renal epithelium; since the proximal convolution is the portion of the nephron which we know absorbs and concentrates within its cells foreign substances, such as trypan blue, it alone is affected and all are involved. It can also be shown experimentally that the randomly scattered tubulorhexic lesions are due to patchy ischæmia. Furthermore, it can be experimentally demonstrated that a complication arises in that in instances of severe renal poisoning, after sublimate injection for example, a patchy focal cortical ischæmia occurs and the final result is a mixture of the two lesions (Oliver, MacDowell and Tracy, 1951). In the human example of acute tubular necrosis, the relative predominance of the two sorts of lesions varies with the nature of its clinical origin; in the case of

sublimate poisoning both lesions are found, the nephrotoxic being more impressive in appearance in the histological section. Conversely, in the "shock kidney" nephrotoxic lesions may be few and the tubulorhexic common.

With this description of the renal status at what might be called the acme of the structural and functional disturbance, we may now proceed to examine how restitution of renal activity comes about. A few general statements concerning the problems that must be solved in this recovery will be helpful.

First, though there are grave functional disturbances in the activity of both glomerulus and tubule of the affected nephrons, frank tissue damage occurs in the latter alone. Secondly, since there are two distinct sorts of tubular damage, two different problems of repair present themselves. And thirdly, that though a restitution of a functioning epithelium is an obvious requirement, even more essential to the output of urine is the restitution of the continuity of the individual nephrons.

Let us consider the last question first, for it is a matter in which considerable confusion has arisen in past studies of renal repair. In the histological section it is easy to see that at a certain period in the development of a renal lesion proliferation of tubular epithelium becomes a prominent feature. Such proliferations are not infrequently described as evidence of a "regeneration of tubules" and the implication is given that a rapid progress towards functional recovery is under way. But from the histological section nothing can be learned of the distribution of these proliferating cells along the considerable length of the damaged tubule nor can it be determined whether at some point a failure in the reconstitution of the tubule wall or an occlusion of its lumen has not defeated the entire functional effect of the regenerative processes. That such eventualities are common enough and in fact are prevalent when regenerative proliferation is at its maximum, is evident in dissected specimens from both experimental and human examples of acute tubular necrosis. It is therefore important

to appreciate that in the last analysis it is nephron reconstitution that counts and that this the pathologist can never see in his histological sections.

To return to our first point, the difference in the nature of the lesion in glomerulus and tubule, we find an explanation of the first and often dramatic clinical evidence of returning functional activity, that is, the appearance of a diuresis. This phenomenon seems clearly due to differences in the rate of recovery from the initial disturbances in glomerulus and tubule. As we have seen, there is little or no tissue damage in the former: the blood flow has merely to return and the glomerular activity of filtration begins, whereas months may elapse before the reformation of a mature epithelium affords a re-establishment of water absorption. From what is seen in dissected specimens, the sweeping out of débris and plugs from the tubule lumen that at times has been considered the important factor in the return of urine flow doubtless plays a subsidiary part but may in fact be more an effect than a cause of the diuresis.

And finally the two sorts of tubular lesion present entirely different problems of repair. In the nephrotoxic lesion the intact basement membrane affords the supporting surface of a tube which needs only to be relined with a new epithelium. In the tubulorhexic lesion of ischæmia, the entire tubule wall must be rebuilt and there is a great gap to be spanned by proliferating tissues that must be very exactly orientated if the continuity of the tubule is to be re-established. Moreover, the reaction in the interstitial tissue around the tubular defect is very apt, as Dunn, Gillespie and Niven observed, to intrude in the form of a capillary-rich granulation tissue into the tubule lumen and obliterate it. As a result of all these difficulties, complete repair of the severe lesions of tubulorhexis seems to be an exceptional occurrence. In a case of paroxysmal hæmoglobinuria in which the individual had suffered repeated attacks over several years, many remnants of disrupted nephrons were found along with other evidence of nephron distortion and disruption. The result is a lessened

number of nephrons, and this accounts, I believe, for Lowe's finding of a decreased functional reserve in patients who had clinically recovered (Lowe, 1952).

But many individuals do show an adequate physiological restitution of renal activity after acute renal failure. In the kidney of the fatal human case it can be seen that the tubular epithelium has begun its regenerative proliferation in the first days of the lesion and that it progresses not only extensively but exuberantly, so that in many examples the nephrotoxic damage has been repaired in so far as the tubules are again covered with new epithelium. The new-lined tubules characteristically show a wide lumen with a flat epithelium and this appearance, associated as it is at times with the large output of the concomitant diuresis, has led some to the conclusion that the atypical appearance of the renal cells, in particular the flatness, is the result of tubular dilatation that presumably results from the large volume of urine that is passing through the tubules. No doubt such a simple mechanical effect plays some part in the production of the flatness of the regenerated epithelium, but other and much more important cellular structural abnormalities can be demonstrated. The new cells are cytologically immature in many ways. If, therefore, the functional adequacy of the tubular epithelium depends on the original structural characteristics of the cells, then one would expect that return of tubular activity would be delayed over the considerable period that maturation of the new-formed cells might require. Concerning this protracted maturation we now have considerable information.

In 1916 (Oliver, 1916) I noticed in experimental animals that the regenerated epithelium after renal damage from heavy metals was not only atypical in its general structural characteristics, a fact that had long been known, but that the cells also lacked certain cellular organelles, the mitochondrial rodlets of Heidenhain, which are such a striking feature of their normal cytological configuration.

At the time and on the background of what was then known of functional cytology, this observation was more curious

than illuminating, and a considerable time has elapsed before it has fitted into a pattern of comprehensibility. Somewhat later my co-workers and I found that regenerated cells without rodlets were unable to absorb certain dyes, such as trypan blue, from the tubule fluid (Oliver *et al.*, 1941). No rodlets—no reabsorption still remained a logically unsatisfying correlation of disparate and apparently unrelated phenomena until Kabat and Furth's (1941) demonstration by histochemical methods that atypical regenerated epithelium cells are lacking in certain enzymes, and though their observations missed the exact point which was needed to complete our chain of evidence, since they made no examination of the mitochondrial content of the cells in their study, it gave the clue to the final correlation. For it is now known, from many sources, that it is the mitochondria of the renal cells that contain many of the enzymes which activate their functional energies. Our hypothetical causal chain would thus run: atypical epithelium—no mitochondrial rodlets—enzyme deficiency—functional inadequacy, or in our particular case, depressed tubular reabsorption.

The definitive experiments which suggest that the reasoning I have outlined may be valid can be briefly described (Oliver, 1953). Rats were poisoned with a moderate dose of sublimate in such amount that although a few died on the sixth to eighth day, the others recovered. No elaborate functional examination was made, but all showed a severe albuminuria and cylindruria with a period of oliguria that lasted a few days, which was followed by a diuresis in varying degree with ultimate return to a normal output. Those that were allowed to survive for fifty-seven days appeared clinically well and their urine was normal by microscopic examination and contained no abnormal amount of protein.

Histological examination of the kidneys on the third to fourth day showed extensive damage to the cortical tubules to such an extent that no normal cross-sections were apparent, the lesion varying from severe parenchymatous degeneration to frank necrosis. The glomeruli showed no tissue damage.

Dissected nephrons showed the typical nephrotoxic lesion in all proximal convolutions.

In order to follow the course of renal repair, animals were killed at periods from four to fifty-seven days. Mitotic figures were seen on the second or third day and by seven to ten days the tubules were re-lined with an atypical epithelium. This proliferation was in many instances so excessive that cords of cells rather than tubules had been formed and, as could be seen in dissected specimens, dilatation of the proximal convolutions had occurred above the point of occlusion. The dilatation and flattening of the new epithelium was therefore clearly not due to increased urine output, as has been suggested. By twelve to fifteen days the dilatation of tubules had disappeared and the new epithelium in large part assumed its original appearance. That the structural maturation of the regenerated cells was, however, a slow and greatly prolonged process was seen in sections stained for the mitochondrial rodlets. At twenty-four days large irregular areas of tubule cross-sections were still lined by an epithelium that contained no rodlets. Even at fifty-seven days, a considerable period in the life of a rat, clear islands of rodlet-free tubules could be found on the dark background of a cortical tissue composed of heavily stained, entirely normal appearing tubules which had re-acquired the mitochondrial organs of the original renal epithelium.

When these sections were stained by methods which demonstrate the seat of activity of alkaline phosphatase and lipase, the same pattern was found in the cortex: on the dark background of the mature restored tubules appeared the unstained scattered islands of tubules lined with enzyme-free cells which in the mitochondrial preparations were free of rodlets.

These experiments suggest an explanation, I believe, for the protracted course that is observed in tubular repair after acute renal failure: not only must the continuity of ruptured nephrons be re-established, but the functional activity of their epithelium must wait on a cytological maturation occurring

on the intracellular level of cell organelles and their enzymatic products.

In looking back on the progress that has been made in correlating the structural and functional aspects of renal activity, the appropriately modest satisfaction that morphologists may feel in the part they have played in its accomplishment should perhaps be tempered by the recollection of that cynical definition of the specialist as he who learns more and more of less and less, for in a certain sense such would seem to be the direction of their intellectual progress. In the present generation their field in renal pathology has narrowed from correlations of the broad generalities of "Kidney" and "Renal Failure," through tentative speculations concerning ill-defined glomerular and tubular activities and what could be seen in the histological section, to the more restricted but definitive aspect of individual "Nephrons" and "Specific Tubular Dysfunction." At this level a certain discrimination has in fact become possible in the last ten years, for a linking of "Proximal Convolution" and "Reabsorption" has been firmly established and in the case of certain urinary constituents, glucose for example, in greater refinement to a restricted segment of that convolution. Morphologists now find themselves at the limits of the visible concerned with data on the intracellular level of "Mitochondrium" and "Enzyme." They have the means to go further and doubtless will; but is it not an auspicious moment for them to pause and consider the implication inherent in our definition that, knowing more and more of less and less, they may find themselves knowing almost everything about practically nothing? The totality of renal activity and its relation to other essential systems and indeed to that pattern which is the Organism itself, still remain in the background as the *desiderata ultima* of our progress.

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DISCUSSION

BULL: I think the evidence for proximal damage is fairly well established, but there is some doubt as to whether we can show definite evidence of distal tubule damage. Distal tubule dysfunction can be shown by the presence of low U/P ratios for creatinine or urea and low P/U ratios for Na and Cl under circumstances where we would expect high ratios. This dysfunction could be due to cell damage, or as Black has suggested it may result from an osmotic diuresis induced by the height of the blood urea. While an osmotic effect is likely, I think tubule damage is also present because one sometimes finds rising ratios despite a continued rise in blood urea.

MERRILL: If I may give Dr. Black's views in his presence and can do so correctly—I should like to defend the point that this may be an osmotic diuresis. Let us consider one of the points that Dr. Oliver made when he talked about the fact that they just do not have as many functional nephrons. Dr. Bull has pointed out, and we have found, that they don't have as many nephrons, or at least their kidney function remains poor for some time after this. I think that if you postulate that during this early stage of diuresis there are a number of nephrons totally knocked out, you get the same sort of effect that has been described when one hemisects a kidney or takes three-quarters of it out. You have a patient with a lot of solute to unload and perhaps only 25 per cent of the nephrons to do it. Therefore the residual nephrons are handling a high solute load. When one considers, in some of these patients, the ratio of the urine volume to the amount of functioning renal tissue, the picture certainly appears to be more that of an osmotic diuresis than it does of failure of a damaged distal tubule to resorb part of the small fraction of filtered water which reaches it.

BULL: Yes, I'm sure an osmotic diuresis plays some part.

BRADLEY: Can one produce an osmotic diuresis with osmotic loads at any time prior to the development of the diuretic phase?

BULL: I don't know for certain, but plenty of people have given sodium sulphate to these patients with a view to promoting diuresis without effect.

BLACK: I wonder if it would act in the phase of diuresis. I think they

are usually given sodium sulphate very early on and it never gets into the tubules.

MERRILL: One might expect no further increase, when you add an additional solute load if the patient, presuming this first theory is correct, is already excreting about 60 per cent of his filtered water.

REUBI: I think there is one more piece of evidence that tubular damage occurs in the late oliguric stage, not earlier. If you measure PAH, thiosulphate, urea and creatinine clearances simultaneously, you will find at the beginning normal ratios between them, as well as normal U/P ratios for each substance. I found for instance in a case of anuria-oliguria on the second day a PAH U/P ratio of 23, a urea U/P ratio of five, a creatinine/urea clearance ration of three and a PAH/urea clearance ratio of 4·8. The absolute clearance values at this point are sharply reduced, but the ratios are nearly normal. On the eleventh day there was a marked tendency for the clearances to become equal to each other and for the ratios to drop to unity (PAH U/P ratio three, creatinine/urea clearance ratio one, and so forth). I think this is indirect evidence that in the early stage there is a true ischæmia, because tubular function is still well preserved; but in the late stage, despite the fact that diuresis is starting, we observe severe tubular damage. At this point, urine is in many respects like a glomerular filtrate, as tubular excretory and reabsorptive mechanisms are no longer working. We may therefore assume that renal ischæmia is the initiating factor in anuria-oliguria and perhaps, at least in part, of the tubular lesion as well. During the late oliguric stage, back-diffusion of water and solutes may contribute to renal insufficiency. During convalescence tubular improvement may be inferred from the fact that the above-mentioned ratios again become progressively normal.

PLATT: We have seen one patient in my department, a terminal case in which there still was some excretion of urine, in which the serum creatinine concentration was more than twice the urinary creatinine concentration. So that if we are discussing U/P ratios in creatinine we have to take that possibility into account. Of course, there are a number of objections to regarding creatinine clearance as a measure of glomerular filtration. But if we do accept this as evidence we have to come to the conclusion that the urine volume in such a case is greater than the glomerular filtration rate. This is one of the reasons why Dr. Roscoe and I think that tubular secretion of water may occur possibly in the normal kidney, and demonstrably in the abnormal kidney. I won't go into all the possible objections to that theory. It is just something we have to bear in mind when we have a ratio like that.

DE WARDENER: May I ask Dr. Reubi what sort of case his was? Because you can't often get functional evidence of tubular necrosis so early. Usually functional evidence starts when the tubular necrosis has occurred. You seemed to start functional tests while the patient was anuric.

REUBI: It was on the second day of anuria, or, if you prefer, oliguria in the first stage. We had the chance to observe the case just at the beginning, after an abortion. The patient was admitted to hospital

about twelve hours after the beginning of the oliguria, and there was still some urine being passed (about 30 ml. a day).

MILNE: Were the PAH clearances controlled by a catheter in the renal vein? I have always thought that the PAH clearances were rather meaningless at such a time owing to an abnormally low extraction ratio.

REUBI: I think the test is not meaningless if you compare the different clearances and the U/P ratios, but absolutely speaking I agree that the clearance values are not reliable. Unfortunately we are not able in such cases to determine the extraction ratio on the first day of the oliguria. Prof. Bull and I have found that there is later always a decrease, practically nothing being extracted. In the particular case I am reporting now, the extraction ratio was 0·07 on the sixteenth day and 0·66 on the thirty-fifth day.

HELLER: May I ask if it is known whether in the diuretic phase the kidney responds to posterior pituitary extract?

BULL: On one or two shots, no.

DARMADY: I have got some evidence on this, which I am hoping to produce in a paper later, so it is a bit difficult for me to produce it now, but I hope to show that there is a certain amount of distal control in this condition in the recovery phase.

BRADLEY: Is it entirely justifiable to equate tissue hypertrophy with improved or increased function? There is no evidence of improved function of residual active tissue in the nephritic or nephrosclerotic kidney. Indeed, renal blood flow appears to be less seriously depressed than maximal tubular excretion of PAH. What is the relation between PAH excretion and renal blood flow in patients with "tubular necrosis"?

BULL: I don't know.

OLIVER: Certainly in the case of the handling of trypan blue by the tubule of the nephron, as the slide I showed indicated, the hypertrophied proximal convolution was handling 15 to 20 times as much dye as its normal neighbour. Of course, one can say that this is beside the point of Dr. Bradley's question, if one believes that the handling of trypan blue bears no relation to any physiological process. I have often wondered why it is that the handling of some foreign substances, such as hippurate, is so readily accepted as being of physiological significance by those who object to others, like trypan blue. In any case, it is a demonstrated fact that the hypertrophied nephrons in dogs handle trypan blue in the same way that normal nephrons do, but in greatly increased amount.

PLATT: I don't know if Dr. Bradley is referring purely to tubular function. We have some evidence from partial nephrectomy in rats that the glomerular filtration rate per unit weight of tissue remaining is very greatly increased. In other words the hypertrophied nephrons appeared to work at a high glomerular filtration rate. But as to their tubular function I don't know.

RUSSELL: Is there any reason to consider that there would be a significant difference between the hypertrophy that takes place in the conditions that were discussed this morning, and the compensatory hypertrophy that results from unilateral nephrectomy?

OLIVER: I suppose there might be a difference between the functional significance of the two types of hypertrophy, but I think they are usually considered to be analogous.

BULL: May I put one more question? Why is the renal blood flow reduced, although there appear to be intact glomeruli? We think that it is due to a rise in intrarenal tension; what do you think, Dr. Oliver?

OLIVER: I think there are demonstrable structural reasons why one might suppose that intrarenal tension is increased by œdema and even inflammatory reaction in the interstitial tissue. Perhaps this is what causes the ischæmia to continue long after the original physiological mechanism of vascular spasm.

PRELIMINARY EXPERIENCES WITH ASPIRATION BIOPSY OF THE KIDNEY

FLEMMING RAASCHOU

Two years have passed since Iversen and Brun (1951) published their first results with the percutaneous kidney biopsy method. The preliminary experiences which have been gained with the procedure since that time are due to collaboration between Bjørneboe, Brun, Gormsen, Hilden, Iversen and Raaschou (1952*a*, *b*, and *c*; 1953; Brun, 1954).

As regards *technique*, it may be briefly mentioned that the biopsy is always performed through the right lumbar region under local anæsthesia as an aspiration biopsy, in the same manner as a liver biopsy, using an Iversen-Roholm cannula. The biopsy is performed with the patient in the sitting position.

It has proved to be rather more difficult to secure a kidney biopsy than one would have anticipated in advance. In only 40 per cent of the attempts did we obtain a biopsy suitable for histological examination, in spite of the fact that all punctures were done by the same person (Iversen), that preceding intravenous pyelography had been obtained, and that the same patient had often been punctured several times. When no renal tissue is obtained, the specimen is generally found to be fatty or muscular tissue, and on rarer occasions liver tissue.

As regards *the histological technique* the specimen is placed on a small piece of cardboard immediately after the puncture and at once fixed in 93 per cent alcohol, this being done in an attempt to demonstrate the alkaline phosphatases using Gomori's (1941) method.

At present *the series* comprises 215 attempts at kidney biopsy in 171 patients and in only 82 of these attempts (that is 38·2 per cent) did we secure a suitable biopsy.

When the biopsy is successful, varying amounts of renal cortex and medulla, and occasionally only cortex or only medulla are obtained. The lengths of the biopsies (Fig. 1) vary from 1 to 27 mm., the average length being 10·2 mm. The number of glomeruli in one section varies from 0 to 35, the average being 10·5 glomeruli per section. This is actually

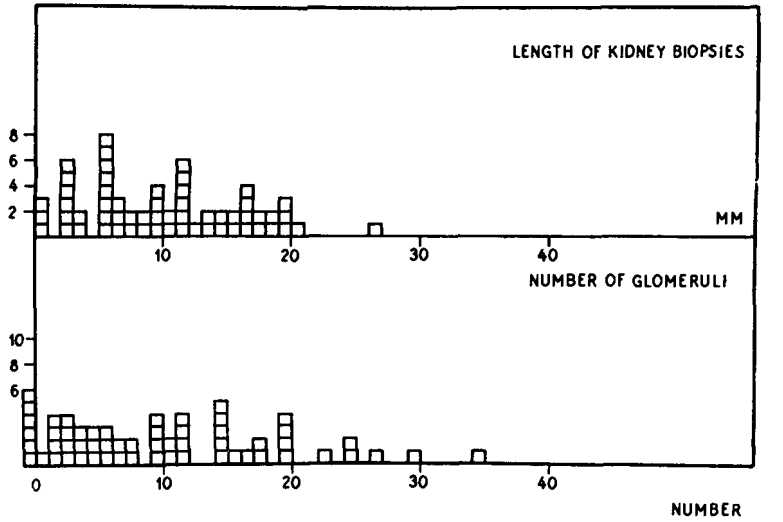

FIG. 1. The lengths of the kidney biopsies and the number of glomeruli in one section of the kidney biopsies.

a small number of glomeruli, considering that the number in a kidney is about one million.

There have been no fatal *complications* using this procedure. In the course of the puncture the patient feels a brief pain in the right renal region, but pain of longer duration is extremely rare. Generally a transitory microscopic hæmaturia is observed when the kidney has been punctured, but gross hæmaturia has been observed in only very few instances. Post-mortem examination of the site of the kidney puncture was made in a small number of cases and in a few of these a small hæmatoma was found in the renal capsule, but in most cases

the site of the puncture could not be demonstrated. We have thus had no deaths with the method, in contradistinction to Alwall, who, in 1952, reported that in 1944 he had one death after kidney biopsy in a series of thirteen biopsies. This occurred in a severely ill patient, and it seems difficult to decide if this patient's death was due to the procedure. However, Alwall abandoned the kidney biopsy method.

Because of the localization of the large abdominal vessels and the spleen, we do not consider that kidney biopsy should be done on the left side. The presence of a hæmorrhagic diathesis is a definite *contraindication*, and in cases of hydronephrosis, pyonephrosis and aplasia of the kidney the examination should not be done.

In order to evaluate the applicability of the kidney biopsy method, particularly the permissibility of a correlation between the results of renal function tests and histological findings, it is important to find out how representative the attainable amounts of renal tissue are in the various diseases of the kidney. This question should be dealt with statistically in greater detail, as has been done in the case of liver biopsy (Waldstein and Szanto, 1950; Billing *et al.*, 1953). So far, our experience seems to indicate that these biopsies are probably representative in the diffuse renal disorders, but hardly so in the focal ones, such as chronic pyelonephritis (Brun *et al.*, 1953).

We can only speak of the value of this procedure with some reservation at the present time, since our experience is as yet so comparatively limited. There can hardly be any doubt that its essential importance is in the investigative field, but already now it is possible to see certain other fields where it can be turned to practical clinical use.

With regard to the *investigative value*, this method permits a more accurate diagnosis of the various diseases of the kidney, and in this manner the knowledge of the clinical course of these diseases, their amenability to treatment, etc. will be considerably enhanced. Since the same clinical syndrome may be an expression of widely different pathological findings, it is

obvious that a purely clinical examination is accompanied by considerable error. By supplementing the clinical examinations with biopsies one gets a basis of comparison which actually ensures that the same disease processes are always discussed.

Secondly, the possibility of obtaining *fresh tissue specimens* affords an opportunity of studying the finer cytological structures in the tubular cells, thus circumventing the post-mortem autolytic processes which occur if autopsy specimens are not immediately obtained and fixed in formalin. In addition, the method renders possible histochemical studies, and at the present time our interest has been focused on a study of the presence of *the alkaline phosphatases*. A normal kidney biopsy, stained according to Gomori's method (Bjørneboe *et al.*, 1952*a*), shows a markedly positive reaction in the luminal part of the protoplasm of the proximal tubules and of the ascending part of Henle's loop. On the other hand, there is no phosphatase activity in the glomeruli and the distal tubules. In our series of patients we found weak or absent phosphatase activity in cases of acute anuria and nephrosis.

Thirdly, the method is a useful supplement in *the study of the normal renal histology*, a phase in which observations using this method are as yet very limited. Biopsies from two patients without renal disorders and with normal renal function showed features which differ from the histological picture generally described in autopsy preparations (Bjørneboe *et al.*, 1952*a*). For some unknown reason the height of the cells in the proximal convoluted tubules can apparently vary. The cells in the proximal tubules are not well-defined on the luminal side; the lumen apparently often contains protoplasmic débris. The capillary loops in the glomeruli as a rule contain no blood; this being in contrast to the usual findings at autopsy which are presumably due to stasis in the kidney. Finally, protein-like precipitates may be found in the capsular spaces, even in cases with no proteinuria.

Further studies in this field are very much needed, and at

present the most important observation seems to be that the cells in the proximal convoluted tubules normally have an appearance which corresponds to what has previously been described as cloudy swelling.

The importance of the kidney biopsy method in *the study of the pathological histology* of the kidney is twofold: (1) It enables observations to be made in the early stages of renal disease, and by serial examinations on the same patient to follow the development of the processes, and (2) it also permits a study of the mild renal disorders which are seldom autopsied. Our knowledge in both instances is deficient and some illustrative examples may be mentioned.

In *acute glomerulonephritis* there are amazingly few changes in the glomeruli, considering the clinical picture and the usual view of the histology of this disease. Our pathologist (Gormsen) could not establish a sure histological diagnosis of acute glomerulonephritis on the basis of these slight glomerular changes.

In addition, mention may be made of *the hepatorenal syndrome*, the definition and pathogenesis of which is somewhat obscure, although we consider the kidney biopsy to afford some further explanation. Our series of five patients—although small—showed that in four there was nothing abnormal apart from bile-stained casts in the distal tubules and collecting tubules. In the fifth, the clinical course and the histological changes were similar to those seen in cases of acute anuria.

The acute anurias (Brun, 1954) constitute an especially interesting group and at present 17 biopsies have been performed in 12 patients from the second to the sixty-second day after the onset of the anuria. The most important histological changes observed were as follows (Fig. 2): the glomeruli were normal (in spite of the very low insulin clearance values); the proximal tubules had a low flattened epithelium, and were dilated in some cases, the epithelium showing hydropic changes in some areas, and in the tubular epithelium as a whole both degenerative and regenerative

changes were present, often side by side. There were focal or diffuse interstitial changes consisting of œdema with or without cellular infiltration. Hæmoglobin-stained casts were seen in the distal tubules, Henle's loops and the collecting tubules. There were no vascular changes, and in most instances the phosphatase reaction was negative.

A comparison with previous descriptions of the histological findings in this disease shows the most important difference to be that the interstitial changes play a more predominant part, and that the degenerative changes in the tubular cells are also, and perhaps chiefly, localized to the proximal tubules. Thus, we too consider "lower nephron nephrosis" an inappropriate term for this disturbance of renal function (Brun, 1954). "Acute tubulo-interstitial nephritis" is a more correct term.

The fourth advantage of this procedure is that it enables a correlation between histology, histochemistry and function in renal diseases, and presumably in the future will afford information leading to an understanding of their physiopathology. So far, we have compared the histological findings with the inulin clearance, the maximal rate of transport of *p*-aminohippurate (Tm_{PAH}) and the ratio between these; a daily determination of the twenty-four-hour endogenous creatinine clearance has also been made in all patients. Briefly it may be said that there is often a good correlation between the structural and the functional findings, although it is far from being so in all cases. I shall give a few examples of poor correlation between morphology and function, as such examples are the most illuminating ones.

Normal histological findings are not infrequently combined with a varying degree of reduced renal function, *e.g.* by acute anuria (following the administration of sulphadiazine), by anuria as part of a hepatorenal syndrome or a diabetic coma, and by so-called genuine nephrosis.

Conversely, we have observed in diabetic nephropathy (Brun *et al.*, 1953), that the glomerular filtration rate may be relatively well-preserved in spite of pronounced hyalinization

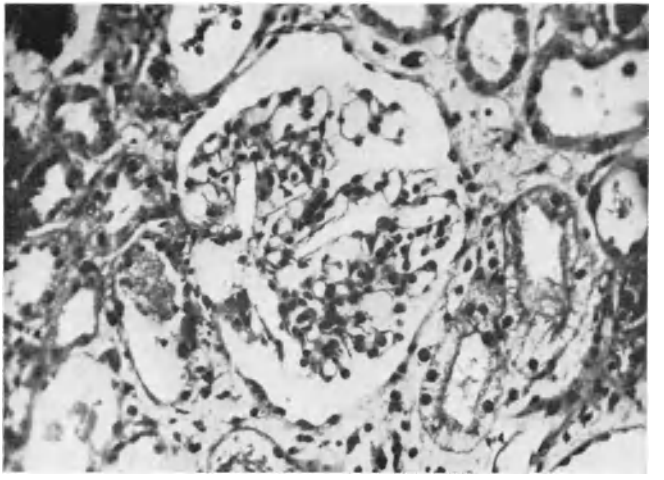

FIG. 2. Biopsy from a woman, aged 39 years, with anuria following an infected abortion and quinine intoxication. Performed on the eighth day of the disease, when the urinary output was 72 ml., the twenty-four-hour endogenous creatinine clearance 0·35 ml./min. This shows hydropic degeneration, rather low epithelium, interstitial œdema and casts.

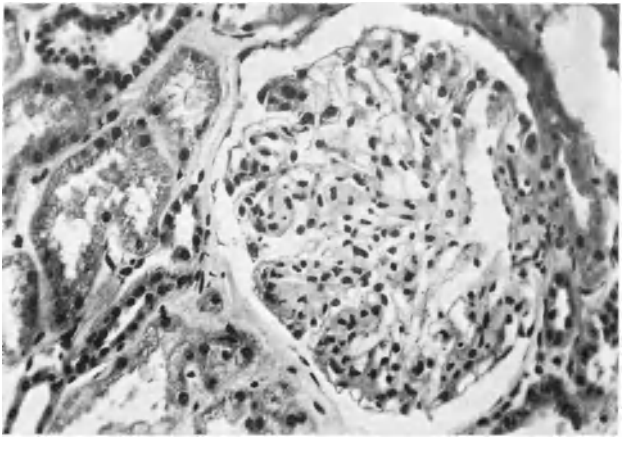

FIG. 3. Biopsy from a woman, aged 65 years, with diabetes mellitus for about 20 years. Nodular-diffuse, though chiefly diffuse, glomerular changes. The inulin clearance was 50 ml./min. and the endogenous creatinine clearance 52 ml./min.

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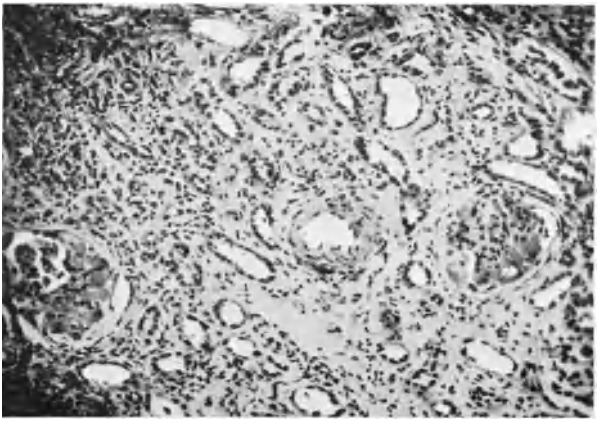

FIG. 4. Biopsy from a woman, aged 55 years, with a nephrotic syndrome of two years' duration. Chronic glomerulonephritis (Ellis type 2) with "prehyaline" precipitates in the glomeruli and tubular atrophy. Inulin clearance was 26 ml./min. and Tm_{PAH} 20·5 mg./min.

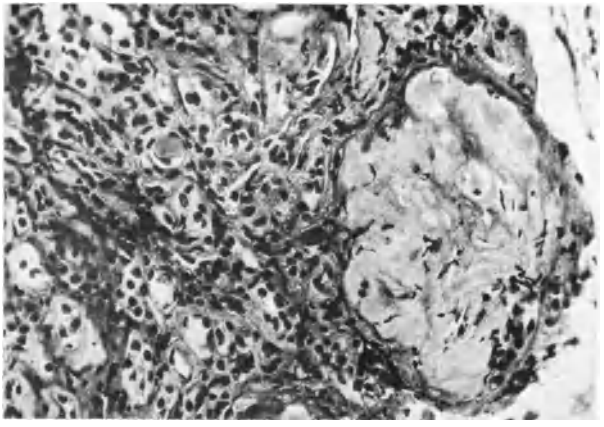

FIG. 5. Biopsy from a man, aged 38 years, who had been suffering from chronic polyarthritis since, in 1935, he had tonsillitis with rheumatic fever. Since 1945 proteinuria and since 1946 œdema. The biopsy was performed in 1951. Inulin clearance was 57 ml./min. and Tm_{PAH} 53·8 mg./min. Total amyloid degeneration of a glomerulus and pronounced tubular atrophy ("amyloid contracted kidney").

of the glomerular basement membranes (Fig. 3); when the renal affection is very pronounced, the inulin clearance/Tm_{PAH} ratio is increased.

In this connection it may be mentioned that the inulin clearance/Tm_{PAH} ratio is increased during the early stages of acute anuria (Brun, 1954).

We have observed another functionally interesting feature in diabetic nephropathy, namely that pronounced, diffuse, glomerular changes may be present, while at the same time it is impossible to demonstrate proteinuria.

In conclusion, I should like to mention that *the practical clinical importance* of this procedure is both of diagnostic and prognostic value. Moreover, there is every reason to believe that the method can be useful in the future in determining the choice of methods of treatment.

As to *the diagnostic value*, the method is apparently superior to the usual diagnostic procedures when attempting to establish an exact histological diagnosis in the *nephrotic syndrome* (Bjørneboe *et al.*, 1952*a*): in a group of ten patients with the nephrotic syndrome it appeared that six had amyloidosis, two chronic glomerulonephritis (Fig. 4), and two genuine nephrosis. Such a distinction between renal disorders with the same symptomatology, but with a different ætiology and course, is undoubtedly only possible by means of a kidney biopsy.

The great value of the procedure was demonstrated in the diagnosis of *renal amyloidosis* where only two of the six cases were suspected clinically. Especially in amyloidosis associated with rheumatoid arthritis has this method proved to be of diagnostic aid (Fig. 5).

In *nephrocalcinosis* (Bjørneboe *et al.*, 1952*c*), too, the method is of diagnostic value when rœntgenological examination fails to reveal the calcium deposits.

The diagnostic usefulness of the biopsy method in *diabetic nephropathy* (Brun *et al.*, 1953) has been demonstrated by examination of 14 patients with diabetes mellitus. These examinations confirm the view of previous investigators

(Rogers and Robbins, 1952) that the *Kimmelstiel-Wilson* syndrome is diagnosed clinically with great uncertainty. The method affords information about the nature and degree of the glomerular changes (whether diffuse or nodular-diffuse), and may reveal an incipient nephropathy when this has not been recognized clinically.

The demonstration of a varying number of *hyalinized glomeruli* in a kidney biopsy may be of importance in the *prognosis* of a renal disease; it is possible in especially good specimens to perform a "differential count" of the glomeruli in several sections through the biopsy. The usefulness of such an analysis of the glomeruli was demonstrated in two patients with *nephrocalcinosis* (Bjørneboe *et al.*, 1952*c*). Table I

Table I

FUNCTIONAL PATTERN OF TWO PATIENTS WITH NEPHROCALCINOSIS.

The kidney function was re-examined twelve and eighteen months after the first examination.

NO. INITIALS DATE	DIAGNOSIS	INULIN CLEARANCE (ML/MIN.)	ENDOGENOUS CREATININE CLEARANCE (ML/MIN.)	UREA CLEARANCE (ML/MIN.)	TM_{PAH} (MG/MIN.)	SERUM UREA (MG PER CENT)	SERUM CREATININE (MG PER CENT)	INULIN CLEARANCE/TM_{PAH}
E.R NOV. 27 1950	HYPERPARA-THYROIDISM	55,2	70,5	47,3	28,9	27	0,76	1,91
DEC. 5. 1951		42,8	58,7	25,2	—	52	1,32	—
G.Y.A. MAY 12. 1950	D_2-POISONING	42,9	53,3	26,4	30,3	42	1,50	1,42
NOV.11. 1951		75,6	68,6	39,3	—	43	1,37	—

demonstrates the functional pattern in these two patients before and after the treatment of the underlying cause (removal of a parathyroid adenoma and discontinuance of vitamin D_2); in the former patient the differential count showed 64 glomeruli: 30 of these were completely hyalinized, 17 were slightly to moderately hyalinized, and 17 were normal; one year later this patient's renal function had been further reduced. In the latter patient 50 glomeruli were counted: only one was completely hyalinized, all the others being normal, and eighteen months later this patient's renal function had increased.

Broadly speaking, an estimate of the importance of this method in determining *the choice of treatment* is a task for the future. All our patients with *nephrosis* were treated with ACTH (Bjørneboe *et al.*, 1952*b*); apparently the cases showing the slightest histological changes in the kidney biopsy were those that responded best to the therapy.

In *anuria*, kidney biopsy may afford some guidance, since we would much sooner attempt a dialysis in the acute cases than in the chronic.

In addition to what has been mentioned here it may be anticipated that in the future we can derive advantage from the kidney biopsy method in other fields. Serial examinations in the same patient may, for instance, contribute to an understanding of the course and prognosis of the various renal disorders; the method can be used in the examination of the influence of various drugs or operations on the histological processes; in enzyme studies; in measurements of tissue metabolism, and in examinations with the electron microscope.

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DISCUSSION

ALWALL: As long ago as 1944 I made 13 renal punctures with the same technique as that earlier published by Iversen and Roholm for biopsy of the liver, i.e. the technique now employed by Iversen and Raaschou for renal puncture. In ten cases I obtained renal tissue, but three yielded insufficient renal tissue, or none. Amyloid nephrosis was verified in two cases. One of the patients is still alive, the other died after an observation period of six years. The autopsy revealed amyloid contracted kidneys and generalized amyloidosis. The kidney tissue of the remaining cases was normal: there were cases of proteinuria and microscopic hæmaturia, hypertension, diabetes, acute hepatitis, hereditary non-hæmolytic bilirubinæmia and chronic ethylism. I discontinued the investigation after a short time on account of a serious shock complication in one case.

The details of the cases and the results have been published in the *Acta Medica Scandinavica*, 1952, **143**, 430.

RUSSELL: May I ask if the technique has been used at all in malignant conditions of the kidney, tumours and so forth?

RAASCHOU: We have not used this method in diagnosing tumours of the kidney, but Cazal in his book "La ponction-biopsie du foie" (1949) remarks that he has performed kidney biopsies in cases of tumours and cysts of the kidney. In addition it may be mentioned that Castleman and Smithwick (*J. Amer. med. Ass.* 1943, **121**, 1256) have made operation biopsies on the kidney in arterial hypertension during sympathectomy.

REUBI: How do you explain the flattening of the epithelium of the proximal tubules? I am interested in this question because there are, as far as I know, very few papers dealing with the histological findings in renal glycosuria. And in one case, that of Monasterio, the only finding was a flattening of the tubular epithelium. Do you consider it is normal or pathological?

RAASCHOU: I do not know exactly, as our normal material with the aspiration biopsy method is so small; but in one case with normal kidney function and without kidney disease we have found a low tubular epithelium.

OLIVER: I presume one artifact may be as good as another, but it does strike an old-fashioned morphologist that alcohol fixation is a perilous thing to use if one wants to examine the condition of the tubular epithelium.

RAASCHOU: Yes, we have been aware of this fact; the reason why we used alcohol fixation was that we wanted to investigate the distribution of alkaline phosphatases in kidney diseases with Gomori's method, which requires alcohol.*

OLIVER: One gets an entirely different picture by alcohol fixation. For instance, you emphasize the flattening of the tubular epithelium. I wonder if that flattening isn't largely the effect of dehydration from the violent action of alcohol, which most morphologists would consider about the worst way of getting a good fixation of renal tissue. I would hesitate to draw any conclusions concerning tubular epithelium after such a fixative.

REUBI: Could you make a comparison between your biopsy material and a small specimen taken during operation by a surgeon in living patients?

RAASCHOU: Yes, we have also performed operation biopsies, and they have shown the same findings, especially that the glomeruli only contain a few erythrocytes. It is a rather strange fact that we find so few erythrocytes in the glomeruli; perhaps they are lost during the preparation. We do not know either how many erythrocytes the normal glomeruli ought to contain in a single section.

DE WARDENER: Don't you think that as your needle approaches the area you are going to remove, the whole thing would go into spasm?

RAASCHOU: Well, it might be so. In collaboration with Dr. A. Tybjærg-Hansen I have done aspiration biopsies of dog's kidneys during operations; in most of these punctures we did not see any ischæmia, but in a single case we have seen a small circular ischæmic zone around the site of the puncture; how far in the kidney this ischæmia extends, we do not know. As our percentage of positive results is so relatively small (about 40 per cent) we have in collaboration with Dr. A. Tybjærg-Hansen tried to augment the positive results by localizing the kidney by its intrarenal pressure; the cannula has been modified so that we can measure this pressure through the stylet, which is connected through a heart catheter to a Tybjærg-Hansen manometer.

WINTON: Could you tell us anything about the pressures you found in kidneys using this technique?

RAASCHOU: We have first used this modified technique on dogs, and are now trying to use it in human beings. In dogs, the intrarenal pressure is about 20-25 cm. of water; it is accordingly higher than the pressure in the surrounding muscle and liver tissues; so, theoretically it is possible to locate the kidney by means of its pressure. So far, the experiments on human beings have not been successful, but we are continuing the experiments.

WINTON: As far as you are concerned this is a question of localizing the kidney rather than investigating the effect of disease on the pressure in the kidney?

*Addendum: Curiously enough, the biopsy which I showed, from the patient with acute anuria following enteritis, and in which we found a very flat epithelium in the proximal convoluted tubules, was fixed in formalin; in the beginning, indeed, we used formalin. By means of this fixative, flat tubular epithelium in acute renal failure can be seen.

RAASCHOU: Yes, our aim has been first and foremost to localize the kidney, because we get only 40 per cent positive results, but we hope later on to be able to investigate the intrarenal pressure in kidney diseases, especially in acute renal failure.

BORST: I think one of the problems is that we are still lacking in criteria of what is normal and what is abnormal. In animal experiments we have suddenly ligated the renal artery and renal vein simultaneously under anæsthesia and taken out the kidney while the animal was still living. Even then there were in different normal animals big variations in the filling of the glomerular tufts with blood; sometimes they were empty and other times they were filled with blood. And we have found animals with flat tubular epithelium and animals with high epithelium. In the classical book of Volhard and Fahr (1914) there are three pictures of acute glomerulo-nephritis and two of normal glomeruli; I must confess that I cannot distinguish between two of the pathological cases and the normal ones. Therefore I am not astonished that it is often impossible to make a diagnosis on biopsy material. We are accustomed to study old cases in which secondary changes have occurred, and then it is easy to make a diagnosis, but the early stages are difficult to distinguish. In an investigation into experimental glomerulo-nephritis we had to standardize the technique. Only in very thin sections could the dilated capillary loops containing swollen cells and plasma but no erythrocytes be clearly seen. Moreover the average number of nuclei per glomerulus was counted and the average size of the glomeruli measured. Only in this way could normal kidneys and kidneys in the early stages of glomerulo-nephritis be distinguished (Borst, 1929, Experimental glomerulo-nephritis, thesis, Amsterdam).

THE KIDNEY
G. E. W. WOLSTENHOLME

RENAL LESIONS IN RELATION TO AMINO-ACIDURIA AND WATER DIURESIS

E. M. DARMADY

I HAVE chosen the subject of renal lesions in amino-aciduria and water diuresis because I do not believe that these two subjects have been approached from the pathological anatomical point of view, and I thought the lesions we have found may help to crystallize our thoughts on the site of the functions within the nephron more clearly. I do not want you to think, however, that the functions of amino-aciduria and water diuresis have a common renal lesion, but it so happens that the phenomena of amino-aciduria and water diuresis are features of one of the stages of acute tubular necrosis, of which we are making a special study. The investigations we are about to describe were carried out to identify the site of each individual and separate lesion. Let me first consider the lesions associated with amino-aciduria, and secondly those found in water diuresis.

I have selected three conditions which are known to produce amino-aciduria, which have normal or subnormal levels of amino-acids in the blood, and which suggest the defect of renal reabsorption. These are:—

1. Fanconi Lignac Syndrome.
2. Hepatolenticular degeneration.
3. Acute tubular necrosis.

We decided first to examine the kidneys of fatal cases of Fanconi Lignac Syndrome, and have been fortunate in obtaining three cases; the first two from Dr. Dent and the third from Dr. Baar. The cases have been reported elsewhere in the literature* and I therefore do not propose to deal with

*Dent, C. E., and Harris, H. (1951). *Ann. Eugen.*, **16**, 60.
Baar, J. S., and Bickel, H. (1952). *Acta pædiat. Stockh.*, Suppl. 90.

these now, except to assure you that each conformed to the classical description of the disease, that is to say having massive amino-aciduria, phosphaturia and glycosuria associated in some cases with cystinuria (but without stone formation) and cystinosis.

The first case was a child of fourteen months, whose immediate cause of death was hypopotassæmia; the second was an adult of forty-six, and the third a child of nine years.

In each case a portion of the kidney was dissected by the method described by Professor Oliver and also examined by normal histological technique. In spite of gross interstitial changes found in each of the kidneys, it was soon clear that three outstanding lesions could be made out.

First, although the glomerulus was normal in size, it was joined to the proximal tubule by an abnormally long and narrow neck. The following figures taken from the three cases demonstrate this point, and should be compared with the normal (Figs. 1–4). In fact, so common were these changes that they were seen 100 times out of 101 consecutive nephrons counted in Case 1. Histological preparations confirmed the presence of the narrow swanlike neck (Fig. 5). We were particularly impressed by the regular appearance of the epithelium, and because of this felt that this was a congenital lesion. Oliver (1939 and personal communication) has demonstrated a similar change in amyloid disease. Fig. 6 is taken from one such case, of two which we have seen. We were unable to find a neck that was as distinct as in the Fanconi; the length of the neck was variable. In places there was considerable ballooning and distortion of the proximal convoluted tubule and in others the whole of the tubule was atrophied. These changes were similar to those seen in chronic nephritis, and we have concluded that they are of a secondary nature.

The second change was that the proximal tubule (measurement of which was fraught with considerable technical difficulty) was soon found to be shorter than normal. For example, in Case II, two of the nephrons photographed and

measured were 0·34 and 0·6 cm. instead of the usual 1·4 cm. This was confirmed by the fact that a dissected kidney showed a cortex reduced in size.

The third change occurred in the distal tubule, but we believe that this is a non-specific change, and it will be discussed in the second part of this paper. We also observed doubly refractile crystals which may have been cystine. Because of unsuitable fixation we were unable to stain Cases I and II for the presence of phosphatase. However, in Case III alkaline phosphatase was not apparent, thus confirming Stowers and Dent (1947) and Cooke *et al.* (1947), who have found phosphatase absent in reported cases.

The hereditary nature of the syndrome has, I think, been well established by McCune *et al.* (1943), Fanconi (1931), Stowers and Dent (1947) and Dent and Harris (1951). We must therefore now ask the question: does the inborn error of metabolism cause the massive amino-aciduria, in turn causing secondary renal changes? Or alternatively is there a congenital defect of structure and property of the proximal tubule of the kidney? In support of the latter consideration we have: firstly, the hereditary nature of the disease; secondly, all reported cases have evidence of renal lesions, with the exception of one; thirdly, the frequency of the abnormal lesion and the regular appearance of the epithelium found; and fourthly, that no aglomerular nephrons were found, which we might expect if the lesion was of a secondary nature.

From a biochemical standpoint it is abundantly clear that the kidney is unable to retain amino-acids, phosphates, and glucose in the circulation. The absence of phosphatase may account for the loss of glucose and phosphates, but Stowers and Dent (1947) and Cooke and co-workers (1947) have already suggested that phosphatase may also play an important part in the reabsorption of amino-acids by interference with the normal phosphorylation. We feel that an added factor may be inadequacy of the proximal tubule, since we have already shown that this is shorter than normal. Homer Smith (1951), in reviewing the subject, has suggested

that there is a competitive nature about the transport system for amino-acid; it is possible, therefore, that relative reduction of the proximal tubules may cause this system to become saturated, with the loss of selective reabsorption and the subsequent loss of amino-acids in the urine.

We turned our attention to hepatolenticular degeneration or Kinnear Wilson Disease, and were fortunate in obtaining material from two cases, one from Dr. Milne, the other from Dr. Baar. From the biochemical point of view a renal defect for amino-acids has already been suggested by Cooper, Echartt, Falcon and Davidson (1950) who confirmed Uzman and Denny Brown's observations (1948) showing that plasma amino-acids were not necessarily raised, and that in three of their six patients there was also renal glycosuria. One case also showed osteomalacia with deranged calcium and phosphorylizing metabolism.

These kidneys were again studied by micro-dissection and histological technique. Anatomically no defect was found. Unfortunately we were unable to study the enzyme in the first case, but in the second case there was a complete absence of alkaline phosphatase. Baar (personal communication) also finds that a number of additional enzyme systems are absent.

In this disease we cannot explain the amino-aciduria on an anatomical basis, and therefore we must ask whether the increased tissue copper may not be responsible for the unselective paralysis of the enzyme systems with resulting loss of amino-acid reabsorption.

The question may, I think, be partially answered by studies of cases of acute tubular necrosis, in which we have found amino-aciduria without evidence of increased plasma levels. Our dissections have shown considerable disorganization of tubular epithelium, and we have confirmed the McManus (1950) observation that alkaline phosphatase was absent. We have also found that in the diuretic phase the urinary amino-acids are present at higher levels, but not in the abnormal amounts seen in the Fanconi Syndrome.

We feel, therefore, that phosphatase may be indicative of or may indeed be the operative factor in controlling reabsorption of the amino-acids, and that it is localized in the proximal convoluted tubules.

The Site of Action of Antidiuretic Hormone

Most of you would agree with the concept that the proximal tubule, by its ability or failure to reabsorb solutes, largely dictates the volume of urine formed. In spite of this, however, the volume of urine is also controlled by the presence or absence of circulating antidiuretic hormone, and its ability to affect the renal tubules. The exact site of water control is still open to question.

Diabetes insipidus resistant to pitressin has been familar to many. It would seem that there are two possibilities to account for this. First, the pitressin or antidiuretic hormone may be neutralized by a circulating antibody, and second, the renal epithelium may become insensitive to the hormone.

We have not sufficient evidence to discuss the former suggestion, but we would like to draw attention to a case kindly sent to us on the advice of Professor Platt by Dr. Roussak and Dr. Oleesky. This patient, who was suffering from myelomatosis with normal total protein but with a reversed albumin and globulin ratio, developed diabetes insipidus, that is to say persistently secreted urine of low specific gravity, and yet was resistant to pitressin. At death a portion of the kidney was kindly sent to us for dissection.

The dissection and histological preparation showed that the proximal tubule and the loop of Henle were normal. Surprisingly enough the changes found were localized to the collecting tubules and to a lesser extent to the distal tubule, in which massive cast formation was found, as shown in Fig. 7. (A normal collecting tubule is shown in Fig. 8.) Sometimes, however, there was marked atrophy of the epithelium with dilatation of lumen and tubule. This affected nearly all the collecting tubules, but some distal tubules had escaped (Fig. 9).

In the histological preparations the atrophy of the epithelium is clearly seen (Fig. 10); further, in places albuminous casts have evidently been deposited in the tubules, causing atrophy with subsequent re-epithelialization to one side of the cast.

Although we cannot at present exclude the neutralization of pitressin and antidiuretic hormone by unknown factors, it definitely suggests to us that the epithelial atrophy is responsible for the failure of antidiuresis, and the cause of the persistent secretion of urine of low specific gravity.

This atrophic change reminded us strongly of cases of acute tubular necrosis, and re-examination of our material brought out some interesting facts. In selected cases who died at the height of diuresis, and who were unable to dilute or concentrate their urine (osmotic diuresis), atrophic lesions of the distal and collecting tubules were found. Fig. 11 shows a dissection made from the kidney of a woman who died at the height of diuresis, following acute tubular necrosis due to ante-partum hæmorrhage, and who developed pneumonia and died. The kidney was sent to us by Dr. King and Dr. Shippam of Chichester.

A similar change was seen in the case sent to us by Dr. Dobson of Southampton, again from a woman who died at the height of diuresis following ante-partum hæmorrhage (Fig. 12).

The next case, sent to us by Dr. Shippam and Dr. King of Chichester, was recovering from acute tubular necrosis following self-induced abortion, and then died of over-administration of electrolytes in the post-diuretic phase. The patient was able to concentrate her urine to some extent. The epithelium had partially recovered, and patchy healthy areas are seen (Fig. 13).

Finally, I wish to mention a case of Mr. Foley's who had nineteen days anuria and oliguria following a concealed uterine hæmorrhage, and who died in the post-diuretic phase of cardiac failure following an embolism twenty-eight days after diuresis had begun. You will note in Fig. 14 an

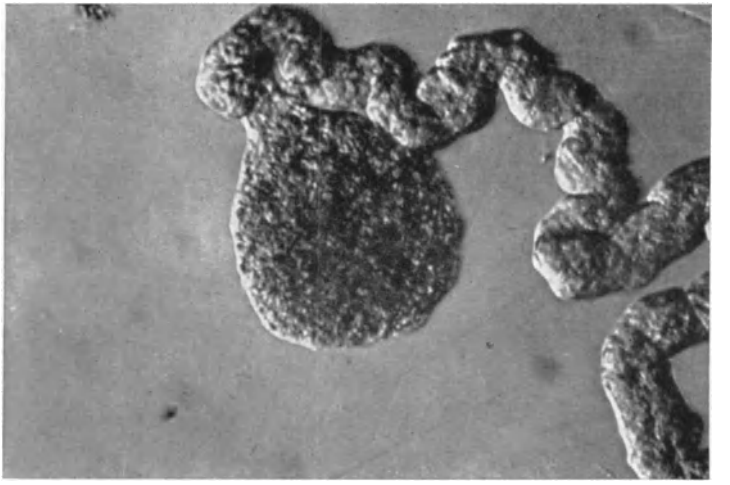

FIG. 1. Glomerulus from a normal kidney, showing how it communicates by a short neck with the proximal convoluted tubule. ×160. (*J. Path. Bact.*).

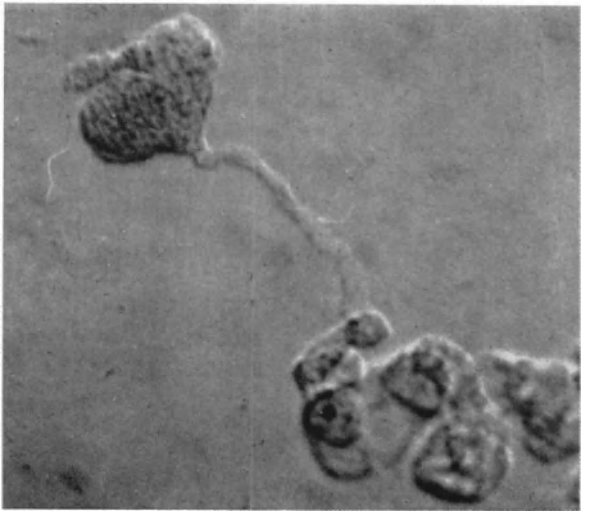

FIG. 2. Case 1. A dissected nephron in the Fanconi syndrome, showing the narrow elongated neck opening into the normal-sized proximal convoluted tubule. ×95 (*J. Path. Bact.*).

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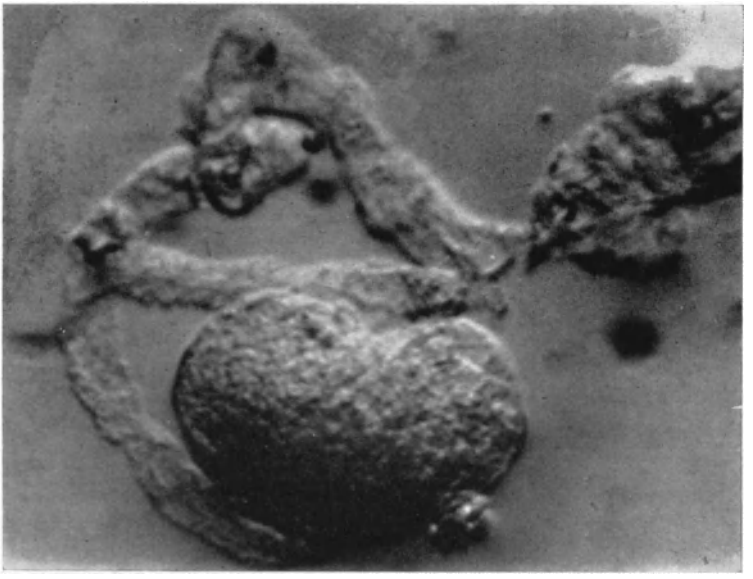

FIG. 3. Case 2. A dissected representative nephron in the Fanconi syndrome, showing the "swan-neck" appearance seen in histological preparations. A portion of the ascending tubule adherent to the glomerulus is also seen. ×270. (*J. Path. Bact.*).

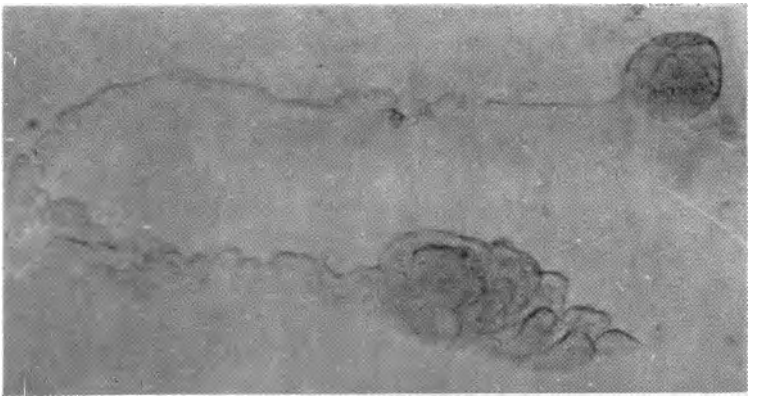

FIG. 4. Micro dissection of a nephron from a case of Fanconi syndrome showing the narrow elongated neck of the proximal tubule.

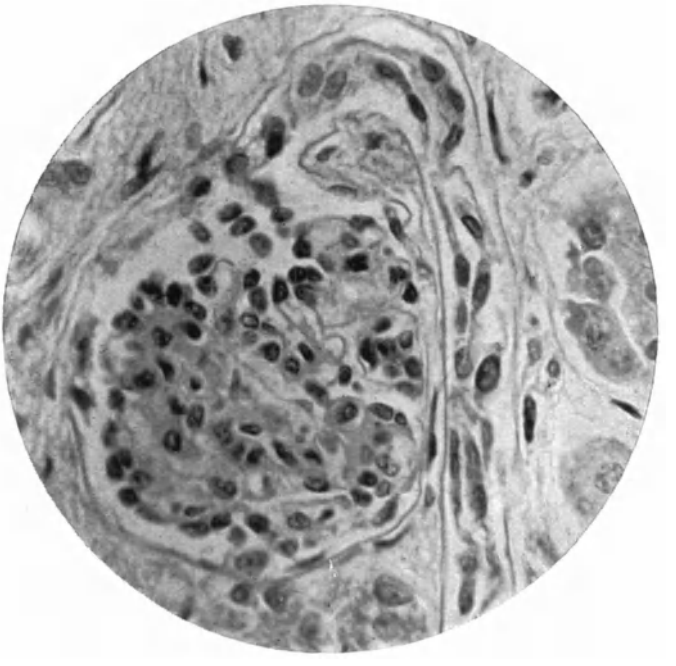

FIG. 5. Case 1. Sagittal section through a glomerulus in the Fanconi syndrome, showing the narrow elongated "swan-neck" appearance of the first portion of the proximal convoluted tubule. Hæmatoxylin and eosin. ×250. (*J. Path. Bact.*).

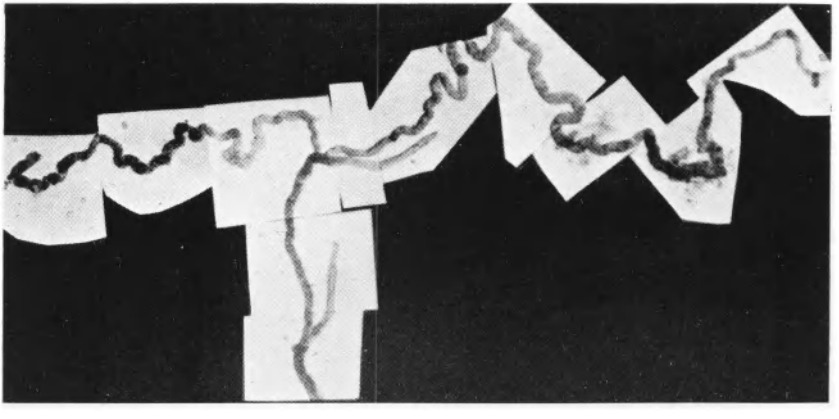

FIG. 6. Micro dissection of a nephron from a case of amyloidosis showing the distorted convoluted tubule and the narrow neck seen in this condition.

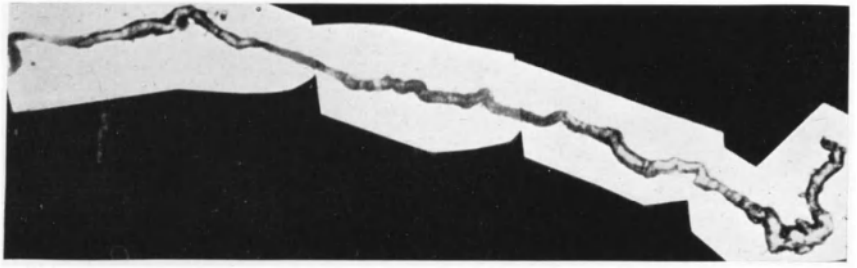

FIG. 7. A case of multiple myelomatosis showing massive cast formation and atrophy of the distal and collecting tubule.

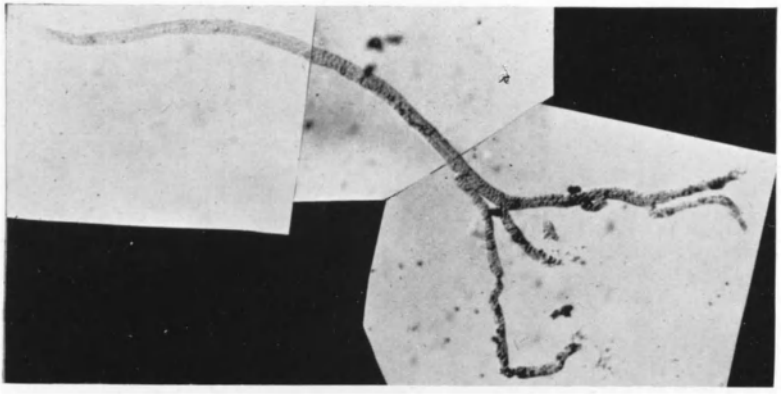

FIG. 8. A normal distal and collecting tubule from a normal kidney.

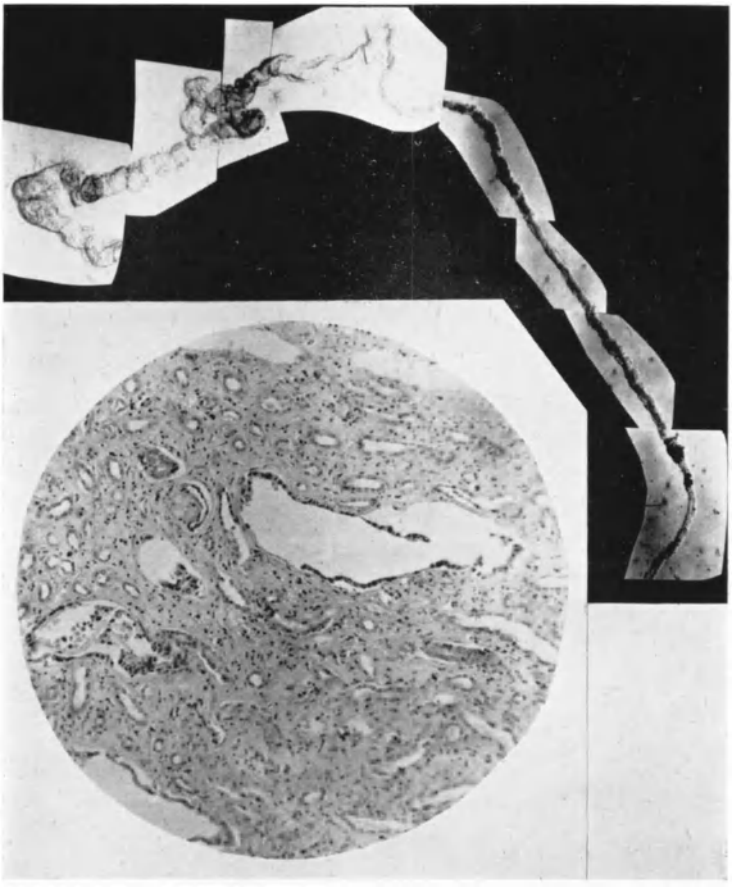

FIG. 9 (*above*). Distal and collecting tubule from a case of multiple myelomatosis with diabetes insipidus showing the localized atrophy and cast formation in the collecting tubule, but comparatively little damage in the distal tubule.

FIG. 10 (*below*). A case of multiple myelomatosis with localized cast formation and atrophy of the collecting tubules. In one area a cast is seen in which regeneration of the epithelium is occurring at one side.

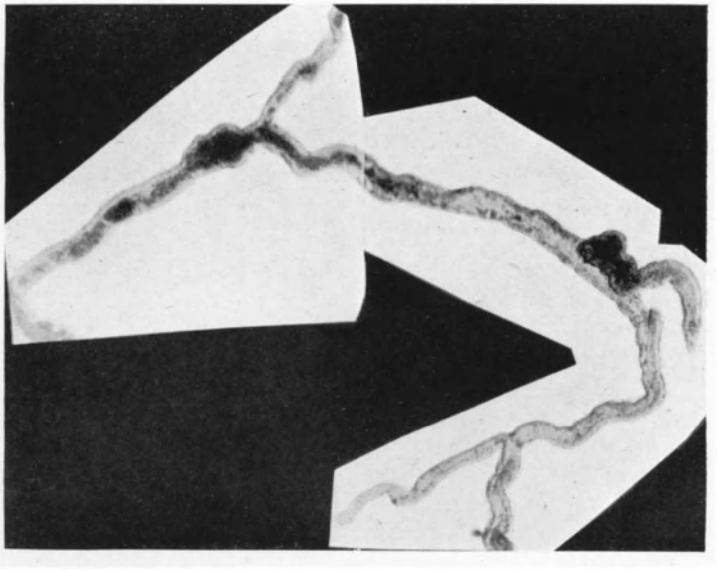

FIG. 11. Micro dissection of the distal and collecting tubules of a case dying at the height of diuresis showing the atrophied epithelium and cast formation.

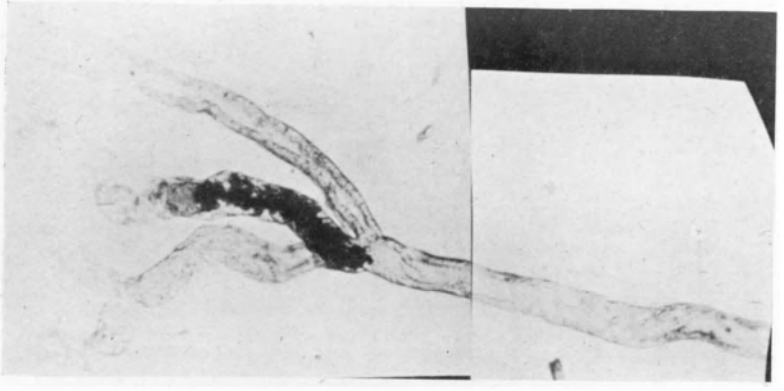

FIG. 12. Micro dissection of the collecting and distal tubules of a case dying at the height of diuresis showing atrophied epithelium and cast formation.

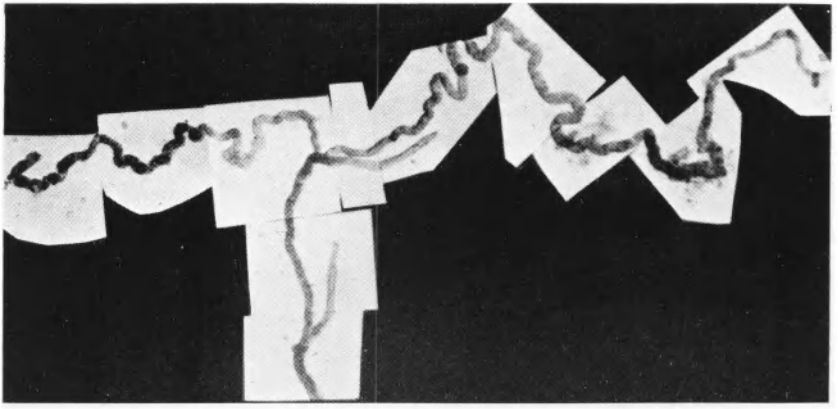

FIG. 13. Case of acute tubular necrosis showing the patchy regeneration of the epithelium in a case dying in the late diuretic phase.

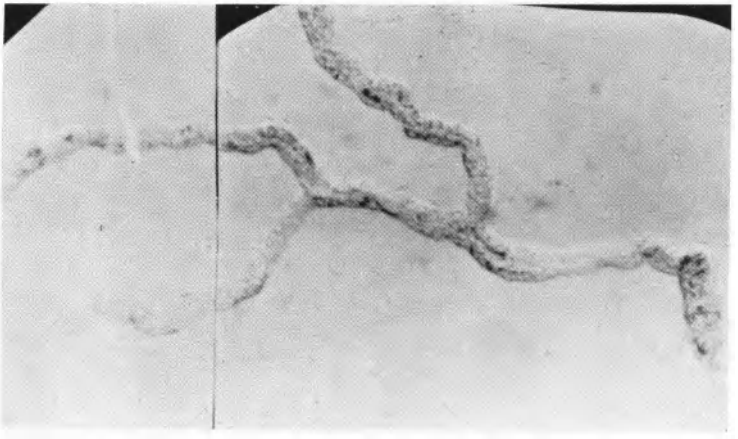

FIG. 14. Portion of the distal and collecting tubules from a case of acute tubular necrosis dying twenty-eight days after the commencement of the diuretic phase.

almost complete recovery of the epithelium of the collecting tubules, whilst lesions of the proximal tubules were still present in the healing stage and yet she was able to produce urine of specific gravity of 1018.

These changes are difficult to interpret in the presence of proximal tubular damage which is to be found in each case. However, it would seem that in the recovery phase the ability to concentrate urine accompanied the evidence of regeneration of epithelium. This suggested to us that the recovery of the epithelium of distal and collecting tubules enables antidiuretic hormone to come into action; and when we take this into consideration with Professor Platt's case, it is highly suggestive that the site of action of the antidiuretic hormone must be localized to the collecting tubules and to a lesser extent the distal tubules. As to whether this is a question of an active secretion or differential reabsorption of water cannot yet be determined by our present studies.

I should like to thank the Medical Research Council for providing an expenses grant for the work, and also to thank Dr. Clay, Miss Margaret Hawkins and Miss Fay Stranack, whose collaboration and dissection have made much of this work possible.

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DISCUSSION

PLATT: May I just say, for accuracy of publication before the discussion begins, that it was not my case of myelomatosis. Dr. Roussak

showed it at a clinical meeting and I suggested that the parts of the kidney should be sent to Dr. Darmady.

RUSSELL: The problem of the swan neck in the proximal convoluted tubule is very interesting. I hadn't heard about that before. In amyloid disease might it not possibly be due to hyaline thickening by amyloid deposit of the adjacent basement membrane? I only suggest that as a possibility. In Fanconi's disease, in which I have examined a number of specimens, I have been impressed by the evidence of regenerative activity in the epithelium of the proximal convoluted tubule, which has appeared to me significant. And in certain allied cases that I have been able to examine, there has been a profound sclerosis of the cortex which I have found very difficult to explain, and the laying down of peri-glomerular connective tissue, without any very obvious change in the glomerular tuft. The sclerosis induced a great deal of tubular atrophy. In the absence of evidence of transitional stages, I don't know how this comes about precisely.

STANBURY: I wonder, Dr. Darmady, if I could ask you for a few details concerning your cases of recovering tubular necrosis. Dr. Roussak's patient was producing what must be regarded as a water diuresis, with urine of a specific gravity of the order of 1·001; and hypotonicity of the urine was checked by measurement of freezing point depression. In the cases of recovering tubular necrosis, surely the polyuria was iso-osmotic. Is there any justification for considering these two groups of cases together?

DARMADY: It is certainly correct that the urines in those cases of tubular necrosis were iso-osmotic. The point that I was trying to make was that the last patient, whose epithelium had almost recovered, was able to concentrate her urine, a function which I thought might be associated with the ability of the antidiuretic hormone to come into play and therefore affect the renal epithelium. I don't think, except for the demonstration of site, that the two groups are really comparable.

DENT: I would like to take the subject back to the Fanconi syndrome, if I may. Firstly, Dr. Darmady has, I think, finally clinched the fact that there is a renal defect, which sounds like the primary hereditary mechanism of the condition. There are still some people in Birmingham (England) who don't believe in the renal theory as an important cause of the signs and symptoms of the disease and I think this is the end of that. What I am wondering, and what I'd like to discuss with the renal physiologists, is how far Dr. Darmady has pushed us completely out of the distal tubule into the proximal tubule. We used to think, of course, that as the Fanconi cases cannot pass an acid urine, and have this chronic acidosis, there was presumably some defect of the distal tubule. Dr. Darmady has now pointed out this very gross defect of the proximal tubule, which we also have thought was present originally—for other reasons, such as the associated glycosuria, amino-aciduria, and phosphaturia—but can we, however, possibly explain the whole thing now in the proximal tubules? One of the anomalies was that these patients could make ammonia fairly well, and that isn't usual when there is faulty acidification in the urine—here we have a curious

condition in which they cannot acidify the urine but can make reasonable quantities of ammonia. What the stimulus is to ammonia formation in such cases, I don't know. The other thing is—Dr. Darmady didn't mention it but it is now becoming more and more important—the loss of potassium from the body in this condition which all recent workers have noted; in fact a case of ours, I'm ashamed to say, died of low potassium syndrome, because we didn't get on to it quite quickly enough. We did give some extra potassium, but it was going out faster than we could put it in. Might it not be that if there is a proximal tubule defect leading to flooding of the distal tubules with potassium, that that also prevents the secretion of hydrogen ions? Might it not be that in losing that argument for defect of the distal tubules, we could now explain the whole renal disorder as being a defect of the proximal tubule? What this defect could be I cannot understand. It's a rather subtle one, presumably, because it isn't merely that the atrophy is such that some of the glomerular filtrate diffuses back unchanged into the kidney—if this were so it would amount to the same thing as a simple diminution in filtration rate.

DARMADY: I want to make it quite clear that the distal tubule is affected, but this is such a common lesion. I think Prof. Oliver and Prof. Sheehan would agree with me that it is very difficult to localize. So far as amino-acids are concerned, I really do think that that is associated only with the proximal tubule.

DENT: I am beginning to think that from the functional point of view there is no need to incriminate the distal tubule at all. I can imagine its being flooded with sodium bicarbonate, the sodium not being reabsorbed in the proximal tubule with the potassium, and everything else following.

BERLINER: We have come to the view, possibly as an oversimplification, that potassium excretion is largely attributable to potassium secretion, and that the filtered potassium rarely contributes to that excreted in the urine. It may be that the situation here is an exception. However, the general experience that, in renal acidosis, potassium excretion can be markedly reduced by giving sodium bicarbonate, can be interpreted in terms of potassium secretion as the mechanism for the potassium loss. The effect of giving sodium bicarbonate would be interpreted as repleting the body with sodium, thus reducing the stimulus for retention of sodium. (Potassium secretion in exchange for sodium is one mechanism for retaining sodium.) There is no other easy explanation for the phenomenon, because, ordinarily, the giving of large amounts of sodium actually increases potassium secretion, but in the case of renal acidosis it has the reverse effect.

PAYNE: The experience we have had clinically makes it rather difficult to accept a purely anatomical explanation. It is very difficult to understand, if it's a purely anatomical one of this character, why one should have such variations. Glycosuria, though it has been described as a common symptom, is frequently absent for months on end; occasionally it has to be searched for, and frequently it is present only in small degree. There are even amino-acidurias which are inconstant

in some cases and which return almost to normal. One gets patterns of this peculiar condition, for instance there is a potassium defect which is nearly always linked with the acidosis (as support for Dent's suggestion), but it is not invariably so. Occasionally one gets a potassium-losing case that appears to be able to produce an acid urine—not very acid, but in the neighbourhood of *p*H 6·2–6·3. There is such a variation in the clinical picture—and it varies even in the same case—that there must be a functional change, and this is supported by the fact that you have histological evidence both of degeneration and regeneration of the tubules.

DARMADY: Might that not be due to the differences in the hereditary nature of the enzyme systems handling such substances? It seems to me that phosphatase may only act as an indicator of other enzymes present. It seems quite possible that one or more enzyme systems may be absent as a hereditary factor.

PAYNE: That would give you a difference from case to case, but not in the same case from month to month.

DENT: I think the other factor to be considered here is temporary electrolyte imbalance, because you can make the patient so much better by correcting the electrolytes; they may have a phase of uræmia, with a blood urea of 150 or so, and then you correct their acidosis and it comes down to normal. I think that is the reason why we get the variation in glycosuria and the other things Dr. Payne mentioned. They are suffering from time to time from gross electrolyte imbalances which may vary according to diet and treatment.

MILNE: In relation to hepatolenticular degeneration I am not willing to accept that high plasma copper is inhibiting enzyme function. In this condition the plasma copper may be normal or low. I will accept however, that there is considerable evidence that high tissue copper may be inhibiting tubular enzyme systems for the reabsorption of amino-acids. And in this respect, I think it is significant that the amino-acid pattern of hepatolenticular degeneration is similar to that of galactosuria; I think that the effect of high blood levels of galactose is the reason for the amino-aciduria of that condition. I would like to ask if anybody has experience of the amino-acid pattern of uranium poisoning. Is it similar to these two conditions? If so, it would greatly strengthen the hypothesis.

DENT: We have studied lead poisoning and lysol poisoning, just because they turned up, but I don't know of anybody who has done chromatographic studies of the urine in experimental uranium poisoning.

ROBINSON: Is anything known about the carbonic anhydrase activity in this condition? I wondered if that came into it; and also whether the production of ammonia might have something to do with the presence of *a*-amino-acid oxidases, some of which were being presented with larger amounts of amino-acids than they were used to.

STANBURY: That last point is a hypothesis that we have already proposed (Milne, M. D., Stanbury, S. W. and Thomson, A. E., 1951, *Quart. J. Med.* **21**, 61), but, of course, experimental proof would be rather difficult.

DE WARDENER: Can one stain for enzymes other than phosphatase?

OLIVER: Lipase.

RUSSELL: There is a method for esterase now.

DE WARDENER: Not carbonic anhydrase?

RUSSELL: Not to my knowledge*.

ROBINSON: Is the zinc content of any value as a guide? I don't know whether that could be used.

STANBURY: Is it not important to realize that too much significance can be attached to the failure to demonstrate phosphatase? It is so very easy and convenient to demonstrate this entirely non-specific phosphatase; but its intracellular function is unknown and it is perhaps unwise to ascribe functional significance to its absence. If you failed to demonstrate succinoxidase in your tissue, would you consider that the tubular dysfunction resulted from deficiency of succinoxidase? I think not.

*Methods have in fact been described (*see* E. V. Cowdry "Laboratory Technique in Biology and Medicine," 2nd ed., Williams and Wilkins Co. 1948) but appear controversial (A. G. E. Pearse, "Histochemistry," Churchill, 1953).

THE KIDNEY
G. E. W. WOLSTENHOLME

THE PRODUCTION OF HYPERTONIC URINE BY THE MAMMALIAN KIDNEY

H. WIRZ

THE current theories of kidney function generally agree that throughout the length of the proximal convoluted tubule the urine either remains iso-osmotic with plasma or even becomes slightly hypotonic. We know from the micropuncture studies of Walker, Bott, Oliver and MacDowell (1941) that in this part of the nephron a large fraction of the filtered load is reabsorbed, that considerable concentration gradients for some of the individual constituents are brought about, but that the total concentration of the tubular content remains practically unchanged. If the mammalian kidney produces a hypertonic urine, the concentration process must take place somewhere distal to the proximal convolution. There is no generally accepted opinion on the accurate location of this concentrating region; but again all the current views either tacitly or explicitly assume that the tubular content is concentrated once and then stays concentrated until the urine leaves the kidney, and that the extracellular fluid is iso-osmotic with systemic plasma throughout the whole organ. This assumption implies large osmotic pressure differences across single epithelial layers not only in the concentrating region itself but also in all the subsequent parts of the nephron and the collecting ducts. The conjecture is therefore justified that the mammalian kidney might be equipped with a special device for the production of highly concentrated urines without steep osmotic gradients.

It has long been known that the ability to produce hypertonic urines is restricted to kidneys bearing a well-developed thin segment. This may or may not mean that the concentration process actually occurs in the thin segment. According

to the theory first advanced by Hargitay and Kuhn (1951) it is the spatial arrangement of this part of the nephron rather than any cytological feature which enables the kidney to produce hypertonic urines. Whenever there exists a thin segment, it invariably forms a part of a loop diving more or less deeply into the medulla and entering into a close topical relationship to the collecting ducts.

An approach to this problem has been sought on different experimental lines. The first biological experiments were those of direct cryoscopy on rats' kidneys (Wirz, Hargitay and Kuhn, 1951). The kidneys were cut out of anæsthetized animals and immediately frozen in liquid air. Slices of 30μ were cut perpendicularly to the course of the medullary tubules. The slices were shielded with paraffin oil and cover glasses and placed in a cooled glycol bath arranged for microscopic examination. The whole procedure was carried out in a refrigerated room, and the tissue was not allowed to thaw before the microscopic examination was made. Ice crystals are birefringent and show up brilliantly against a dark background in a polarizing microscope at crossed Nicols. The temperature of the glycol bath was controlled by a Beckmann thermometer. As the temperature was slowly increased the ice crystals first diminished in size and then rather suddenly disappeared at a well-defined temperature, indicating the freezing point depression of the fluid within the environment of each crystal.

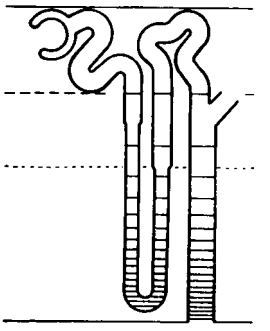

FIG. 1. Variation of osmotic pressure in a single nephron and collecting duct.

The results are shown schematically in Fig. 1. Within each section the osmotic pressure is approximately the same in all of the tubes. In the cortex the freezing points are close to $-0{\cdot}56$°C., i.e. the tubular content is iso-osmotic with normal systemic plasma. In the medulla, the osmotic pressure

of all the tubules in a section depends on the part from which it was taken; the deeper in the medulla, the higher the osmotic pressure. From this we conclude that the tubular content is isotonic in the proximal convolution, becomes increasingly hypertonic in the descending limb of the loop, but is rediluted in the ascending limb to isotonicity, which is kept throughout the distal convolution. The final concentration of the urine occurs in the collecting ducts. The effect is a high urinary osmotic pressure, without—at least so far as the tubular contents are concerned—any steep osmotic gradients. All immediately adjacent tubules show practically the same osmotic pressures in the cortex as well as at any level of the medulla.

Unfortunately these experiments by direct cryoscopy are not absolutely conclusive as they do not exclude *post-mortem* diffusion within small distances. Furthermore these experiments do not yield any information on the conditions between the tubules—in the interstices and in the blood vessels.

More conclusive evidence could be obtained by micropuncture experiments. However, it is well known that micropuncture is only possible on objects which can be visualized microscopically. Consequently it is restricted to the surface of the kidney, i.e. to proximal convolutions, occasional Malpighian corpuscles and, also exceptionally, some distal convolutions. All the rest is hidden in the depth of the kidney and not accessible from the surface.

The kidney of the golden hamster (*Mesocricetus auratus*) offers an opportunity to approach directly the innermost part of the medulla (Wirz, 1953). This kidney (Fig. 2) is unipapillar as in other small rodents; but the papilla, which is long and thin, extends beyond the hilus into the ureter. The end of this papilla, about two millimetres at best, can be exposed *in vivo* by opening the ureter and part of the renal pelvis. On histological examination this papilla contains collecting ducts and some thin segments of long Henle loops. *In vivo*, however, the papilla is optically homogeneous,

slightly opaque, and (in contrast to the kidney surface) it has not been possible so far to visualize the different tubules. On the other hand, the superficial papillary blood vessels (*vasa recta*) are clearly visible by their contents. As the osmolarity of the blood is of equal importance, some micropuncture experiments have been carried out on these blood vessels.

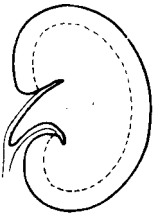

FIG. 2. Kidney of a golden hamster (×3½).

A quartz tube, diameter 10 to 15 μ, was inserted by micro-manipulation into one of the capillaries and a droplet of blood was collected. A fraction of a microlitre is enough to yield a clear reading of the freezing point. Immediately afterwards an equally small sample of the freshly formed urine was collected from the tip of the papilla with a similar quartz tube, and finally a droplet of systemic blood. The osmotic pressures were determined cryoscopically using the method of Hargitay, Kuhn and Wirz (1950) in the same quartz tubes which previously had served for collecting the samples.

The osmotic pressures of the urines varied between 300 m.osmol/l. and 620 m.osmol/l., i.e. the osmotic U/P ratios varied between 1 and 2. The osmotic pressures of the papillary blood samples were either the same or, at most, 30 m.osmol/l. higher than those of the corresponding urines. The systemic blood showed a normal osmotic pressure of 280 to 320 m.osmol/l. in all experiments.

As the walls of the blood vessels are no serious barrier to osmotic equilibration, the blood drawn from papillary blood vessels may be taken as representative of the papillary extracellular fluid. So it may be concluded that at the tip of the papilla there exists a hypertonic region including the contents of the tubules as well as of the extracellular space. Such a hypertonic region could be brought about by the Henle loops working as a "hair-pin countercurrent system" as suggested by Hargitay and Kuhn (1951). This system is best understood by examining their working model.

This model consists of a rectangular tube (Fig. 3), 4 m. long, 5 mm. wide, and 2 mm. high. It is divided by a cellophane membrane (*M*) into two compartments (*a* and *b*), each 1 mm. high. At one end (*A*) a small tube (*T*) of adjustable bore connects the two compartments. At first the whole system is filled with an aqueous acid solution of sodium polyacrylate. This compound was chosen for practical reasons: the polyacrylate molecule is large enough not to pass the cellophane membrane, yet it sets up an appreciable osmotic pressure due to the large number of dissociated sodium ions

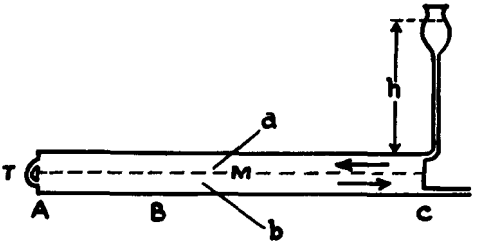

FIG. 3. The working model of Hargitay and Kuhn (1951). (For description, see text).

(in one particular experiment the osmotic pressure was measured as 58 cm. of water). One of these compartments (*a*) is now exposed to a hydrostatic pressure (*h*) (in this experiment it was 88 cm. of water), the tube *T* being closed. A certain amount of solvent is pressed through the membrane to the other compartment (*b*), whereas the polyacrylate and the sodium ions stay behind. The result of this first stage in the experiment is an increase in the osmotic pressure in compartment *a* and a corresponding decrease in compartment *b*. The system reaches equilibrium when the osmotic pressure difference between the two compartments equals the hydrostatic pressure *h*. For our experiment it can be calculated from the above figures that the highest theoretically possible osmotic pressure due to this "single effect" was 126 cm. of water in compartment *a*.

We now open the connecting tube *T* for a limited period of time. The more concentrated fluid of compartment *a* passes through *T* and replaces the fluid in compartment *b*, which passes out at *C*. Let us suppose that the more concentrated fluid reaches *B* in compartment *b* when *T* is closed. Within the region *A–B* both sides of the membrane are now in contact with fluid of the same elevated osmotic pressure. There is no osmotic pressure difference and the permeation of water from *a* to *b* is resumed until a new equilibrium is established. The osmotic pressure in this region of compartment *a* is now higher than could be due to the single effect alone.

This process may be repeated, or the connecting tube may be opened to allow a slow but continuous flow. When a steady state is established the incoming fluid is more and more concentrated in compartment *a* up to the return point, and rediluted on its way back in compartment *b*, and it leaves the system at *C* in the same state as before. The osmotic pressure in both compartments near *A* is several times higher than the hydrostatic single effect. In the quoted experiment an osmotic pressure of 252 cm. of water was measured.

We may now try to apply the principle of this working model to the mammalian kidney. The countercurrent system is represented in the kidney by the loops of Henle.

The single effect in the kidney is not likely to be produced by hydrostatic pressure. Urinary osmotic pressures of up to 70 atmospheres have been measured in some mammalian species, and the hydrostatic pressures available in the kidney—even if applied to a countercurrent system—seem too small to account for these. We must think of a metabolic function of some epithelial cells of the loop creating an osmotic pressure difference between the two limbs of the loop. This metabolic single effect could consist of an active water transport from the descending to the ascending limb as well as of an active transport of any solute, e.g. sodium, in the opposite direction. This osmotic pressure difference may be small as compared with the total osmotic effect of

the kidney. It is limited by osmotic gradients rather than by any absolute amount of water or solute handled by the process.

The limited but significant single effect is multiplied by the function of the countercurrent system. As fluid of slightly elevated osmotic pressure is carried around the bend from the descending to the ascending limb, the limiting osmotic pressure difference between the two limbs disappears and the single effect is enabled to go on. In a steady state the contents of both limbs near the return point may be several times as concentrated as the single effect alone could account for.

Since the water (or solute) is carried actively from one limb of the loop to the other across the extracellular space, the osmotic layering not only involves the contents of the tubes but also the interstitial fluid. And it is this *milieu* through which the collecting ducts and the medullary blood vessels pass.

The blood vessels of the medulla form another hair-pin countercurrent system. They originate in the isotonic region of the cortex, and they turn back to the arcuate vein at the cortico-medullary boundary—again an isotonic region. Between these two points the blood reaches down more or less deeply into the medulla and accordingly to a more or less hypertonic extracellular space. As all of the medullary blood vessels scarcely exceed the size of true capillaries, their membranes are most likely to be permeable not only to water but also to crystalloids. Thus the descending (arterial) blood adapts its osmotic pressure to the surroundings by taking up osmotically active solutes from the interstitial fluid, whereas the same amount of solutes is given up by the returning (venous) blood.

In the outer zone of the renal medulla arterial and venous capillaries are assembled in "vascular bundles." In these, most of the blood vessels are not in contact with tissue, but are in close contact with each other. This unique feature of the mammalian kidney, though well known to morphologists, has never aroused any physiological interest. Yet this is a most economical method of blood supply having regard to its

metabolic task. The incoming blood is from the beginning equilibrating with that which is going out, so that any exhaustive utilization of the blood becomes quite impossible. Yet just this facilitation of an arterio-venous interchange seems to favour a transient rise of the osmotic pressure of blood in the deeper regions of the medulla.

The epithelial walls of the collecting ducts, in contrast to the vascular walls, are impermeable to most of the urinary solutes. However, they may be permeable to water, and so the final urine concentration is achieved by a passive drain of water to the hypertonic surroundings. There is no need to attribute to the collecting ducts any active part in the concentration process.

The water which is drawn out of the collecting ducts by osmotic forces first reaches the interstitial fluid and is eventually taken up by the ascending limbs of the loops and by the venous blood vessels. Therefore it impairs the efficiency of the active countercurrent system to a certain extent. To what extent, depends largely on the velocities within the respective systems. It is reasonable to assume that in the distal convolution an iso-osmotic reabsorption process takes place similar to that of the proximal convolution. So the amount of fluid entering the collecting ducts is only a fraction of the fluid passing the system of the Henle loops. Hence the water passing the walls of the collecting ducts impairs the action of the countercurrent system only to a small and tolerable degree.

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DISCUSSION

BERLINER: I wonder how Dr. Wirz visualizes the transport of water or solutes across the interstitial space between the two limbs of the

loop. In the model there is contiguity of the two limbs of the countercurrent system, but in the loop of Henle there is a space between, across which the transfer of substances has to occur.

WIRZ: The thickness of a cellophane membrane is at least as much as the distance between two limbs of a loop.

OLIVER: I don't think the two limbs necessarily lie side by side.

WIRZ: It is not necessary to deal with two limbs of the same loop. The descending limb of the model is represented in the kidney by a large number of descending limbs, all having the same function, and the same applies to the ascending limbs. If—in the kidney—water is forced out of one descending limb, this may be taken up by any number of ascending limbs which happen to be in the immediate vicinity.

DENT: Have you done the reverse experiment of making them pass very dilute urine?

WIRZ: I have tried, but I have not been able to get the hypotonic urine *in anæsthetized and laparotomized animals. I hope I shall be* able to do that because I think this question is very important. One could think of the possibility that the dilution of urine is achieved by the same system working the other way round, but it is not sure.

DENT: On that model of yours, was there a constriction on that tap—was it partly open? I can't see how the hydrostatic pressure can vary as shown.

WIRZ: The connection must be small enough so that the hydrostatic pressure difference is kept up. At the end is a free opening. The flow is a very slow one, and in this model with distances of millimetres instead of microns it takes days until the steady state is established.

DENT: Is that very similar to the situation in the tubules?

WIRZ: No, and I must emphasize that, because in the kidney you have not a hydrostatic force pressing water from one limb to the other.

DENT: It's not really a valid model then.

WIRZ: But it is valid in this, that you can measure what you have. You can measure your single effect, which in the case of a single experiment was 88 cm. of water, and with this single effect you can compare the achievement of the countercurrent system, which is more than this. But you can have any mechanism of transfer from one of these compartments to the other.

DENT: You are replacing it all the time by the same isotonic fluid?

WIRZ: What enters is isotonic, and what leaves the system is the same. It must be, because nothing has been added and nothing has been taken away.

DENT: Then what you are doing is an ultrafiltration, aren't you? And the longer it is in the tube the more it ultrafilters, and therefore it gets more concentrated as it goes down. I don't see that you have shown any more than that that is an ultrafiltration apparatus. The constriction, which isn't present in the tubules, is the strongest criticism that it is in fact not a true model. What you are doing is ultrafiltering the top compartment into the bottom, and the longer it takes to flow along there, the more water you squeeze out, and therefore it concentrates at the bottom end; the fluid that is going through the hole to

the bottom obviously is not going to remain concentrated, but will suck a little water through, and will become more and more dilute during the time it takes to come out. I think that all you are showing is that you can do ultrafiltration under pressure.

ROBINSON: Yes, but supposing that you have no constriction, and no difference of hydrostatic pressure, and that the membrane is not a passive piece of cellophane, but something that can nonetheless feebly push water from one side to the other; what happens then? I think it does work.

PITTS: Fundamentally, couldn't you forget the presumed activity of the descending and ascending limbs, and put your whole transport system into the collecting duct? What have you gained by implicating the loop as an important link in the system? Could not the osmotic pressure of the tubular urine in the loop merely follow passively that of the surrounding interstitium? The osmotic pressure in the interstitium would then be determined by the active transport mechanism of the collecting duct.

WIRZ: What I have gained is that there is no large or possibly no gradient at all between the hypertonic urine at the end of the collecting duct and what is just around the collecting duct.

PITTS: Have you not in the whole system performed the same amount of work?

WIRZ: Yes, I have. I have not gained any energy. It is just a transformer mechanism, to be compared to an inclined plane or something like that. The same amount of work must be done.

BERLINER: The energy required in forming concentrated urine in this way is the same as if the urine were concentrated by the ducts themselves *only* if all the reactions are reversible. If the reactions are not reversible, the energy expended in concentrating the urine is dissipated when it becomes diluted again. That energy has been lost, and therefore in actual energy expenditure your theory requires a great deal more, since it calls for concentrating all the urine twice.

WIRZ: I don't think it is very important to stress the energetics. After all, we still don't know what a kidney does with all the energy which it spends.

BERLINER: I agree wholeheartedly. I just wanted to point out that although the total work performed is the same by either mechanism, the energy expended in performing the work is very different.

WINTON: But I take it the model is designed to explain observations made on the kidney, which include the fact that the blood taken from the medullary part is more concentrated osmotically than blood in the systemic artery. Isn't that why this loop mechanism has been introduced, in order to explain that? That implies diffusibility, so that the collecting duct would have to be surrounded by tissue fluid of high osmotic pressure.

DENT: But the actual concentration of urine, from the physiologist's point of view, takes place at the beginning, doesn't it, and after that it just goes backwards and forwards again and ends up as it was; and the actual concentration mechanism is on the left of your model, isn't it?

WIRZ: Yes, but it is not the total of 70 atmospheres which is brought across this membrane, but just say one atmosphere.

PITTS: In connection with Dr. Dent's question, I am again confused. Could you not produce exactly the same result if you put into your collecting ducts the active transport mechanism, assuming that changes in concentration in the loop of Henle follow passively? Why must you put your emphasis on the descending and ascending limbs?

WIRZ: Let me try to do that. You have an active membrane, water being turned out from the collecting duct to the surroundings. That would make the surroundings hypotonic, wouldn't it? Without that countercurrent in your kidney you could not get hypertonic urine *and* blood down there.

OLIVER: I can't follow this very closely, but it seems to me that from your description, considering the volume of water to be disposed of, would not the distal tubule be the place where the main removal of water is performed, much more than in the proximal? It seems to me that the whole burden of water removal is shifted from the proximal to the distal tubule.

WIRZ: This is true only for that part of the filtered water which must be removed to make the urine hypertonic. It has been shown that along the proximal convoluted tubules about 80 per cent of the filtrate is reabsorbed, and I do not doubt this. Whatever happens in the proximal convolution, this does not affect the urinary osmotic pressure. However some concentration gradients of urea, creatinine, glucose, etc. are built up.

MILNE: If this principle of osmotic equilibrium between blood and urine were extended to a hypotonic urine, surely the animal would die of intravascular hæmolysis? How do you consider that the mechanism is excluded when hypotonic urine is secreted?

DE WARDENER: How long does it take for a red cell to break up under such conditions?

WIRZ: I don't know that and I can't answer these criticisms. If you have a hypotonic urine and if you think of a mechanism just the same but the other way round then you must have a metabolic force in the epithelium of the loop which can work either way. On the other hand, if you have a mechanism which can facultatively work just one way, then you wouldn't have the condition implied in the production of a hypotonic urine. The other thing is that most vertebrates are able to produce dilute urine, whereas hypertonic urine only occurs in animals with Henle's loops.

OLIVER: You are quite sure of that? Because there are many species that Sperber has shown to have no long medullary loops, and of course man has only one out of eight nephrons.

WIRZ: I have not stressed the point of the thin limb, and I have not stressed the point of the so-called long loops. I don't know anything about the cytological aspects. It could be the short loops as well. There seems to be a correlation between the length of the loops and the hypertonicity of the urine; I gather that from the paper you mention by Dr. Sperber.

OLIVER: He did no determinations of the tonicity of the urine to correlate with the number of medullary loops; he simply relied on the literature.

WINTON: From what type of blood vessel did you take your blood?

WIRZ: Mostly venous capillaries, returning blood. Sometimes you are not quite sure which is an arterial capillary and which is a venous one, because there are many interconnections between these vessels.

HELLER: In connection with the question of intravascular hæmolysis, have you by any chance seen or measured the blood corpuscles in your concentrated blood? Are they in any way deformed or shrunk? Is there sufficient time for them to show the effect of a rise of osmotic pressure?

WIRZ: I haven't done that, but even if I had I couldn't answer your question of time, because the time which I require to take blood out of the vessel and measure the size of the cells would be much more than the time the cell takes to pass that spot.

WINTON: Well, it is in the literature, there was somebody at Berlin working on it who showed that the time curve of the change of form of blood corpuscles varies with various dilutions. And it's not as quick as one had imagined; I should think it would pass through the kidney quite comfortably without irreversible changes.

HELLER: Is it a question of seconds, do you think?

WINTON: Yes.

HELLER: Well, that would answer this objection.

Part II—Tubular Functions other than the Regulation of Acid-base Balance

DISTRIBUTION OF FUNCTIONAL ACTIVITY AMONG THE NEPHRON POPULATION

S. E. BRADLEY, E. LEIFER, and J. F. NICKEL

THE several million tiny units or nephrons comprising the kidney in man and other mammals, are similarly constructed and apparently function in a similar manner. Each possesses a glomerulus and a segmented tubule, each is normally supplied with blood which has traversed the arteriolo-capillary nexus of a glomerulus, presumably under more or less the same pressure head. Hence it is appropriate to discuss renal function in general terms as if one were dealing with but a single nephron and to discuss the behaviour of the different parts of the tubule on the basis of urine-plasma concentration relationships, as if the urine was the product of a single unit—and not the pooled product of all. On the whole this simplification has proved exceedingly useful and fruitful, as other contributions in this conference will testify. Nonetheless, such a device may tend to obscure the fact that one is actually dealing with a population of nephrons which may vary widely as individuals. Indeed, the anatomical evidence strongly suggests that this may be so. The diameters of the glomeruli have been found (Peter, 1927) to vary over a two-fold range, and a similar variation appears to affect the lengths of the proximal segments. The distal tubular segment displayed a much greater range of difference in length, varying from a short, scarcely detectable structure to one that pursues a lengthy tortuous course through cortex and medulla before it terminates in a collecting duct.

Certain studies of renal function indicate that the anatomical variety in the nephron population is associated with functional variation: (a) A study (Smith *et al.*, 1943) of the relationship between glucose or diodrast loading (so-called "titration") and the rate of tubular transfer has

indicated relatively uniform distribution of filtrate and perfusate in tubular tissue. A two- or three-fold range in activity ratio of filtrate formation relative to glucose reabsorption (proximal tubular) observed in this work conforms with the evidence of a similar range in the size of glomeruli and proximal segments. (b) The changes in renal function observed during abdominal compression (Bradley and Bradley, 1947) are difficult to explain without assuming considerable variation in the end-tubular urinary pressures. (c) Kennedy, Orloff and Berliner (1952) have suggested that the relationship between carbon dioxide tension in bladder urine and the total urinary concentration of buffer other than bicarbonate might be explained by the final assembly of nephron urines of differing buffer concentrations and *p*H at some point in the kidney where adjustment by back-diffusion of carbon dioxide cannot occur. And finally, (d), a consideration of the times required for appearance and equilibration leads to the same conclusion: only two or three minutes are required for the appearance of dye or other substances in the urine following intravenous injection (Morales *et al.*, 1950), but at least twenty minutes are required for equilibration of the clearance values once a constant blood level has been established (Mitchie and Mitchie, 1951), suggesting a comparable spread in the delays imposed upon the passage of filtrate from the glomerulus in different groups of nephrons.

Further evidence for such a concept of a distribution or spread in delay times among the nephron population has been adduced in a study of the disparity observed between plasma and urinary specific activities for long periods following injection of radioisotopes. Handler and Cohn (1951) noted the phenomenon following administration of radiophosphorus. A similar disparity was found to persist for as long as one hour following injection of ^{15}N labelled urea (Fig. 1) in the course of a study made in collaboration with Drs. D. Rittenberg and San Pietro. In ten experiments with radio-potassium the urinary specific activity exceeded that of the plasma by

as much as 700 per cent during equilibration (Fig. 2), and in nine experiments with radioactive sodium by as much as 400 per cent. The values for specific activity of radioactive

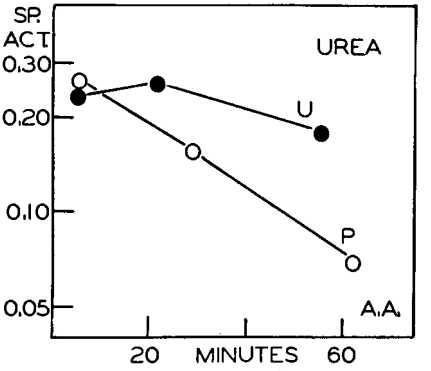

FIG. 1. The specific activity of isotopically (^{15}N) labelled urea in urine (U) and plasma (P) following intravenous administration (Patient A.A.). (From Bradley, Nickel and Leifer, 1952).

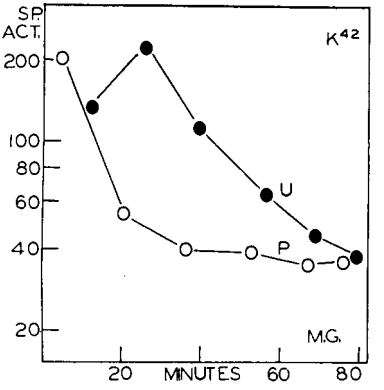

FIG. 2. The specific activity of radioactive potassium (^{42}K) in the urine (U) and plasma (P) following intravenous administration (Patient M.G.). (From Bradley, Nickel and Leifer, 1952.)

sodium presented in Fig. 3 show the smallest disparity encountered. The rather large turn-over of sodium and urea by the kidney seems to rule out the possibility of isotope

storage, with subsequent feed-back from tubular cells. No correlation between the degree of disparity and urine flow or electrolyte excretion could be detected over a ten-fold range of variation. The single factor of delay appears to provide the simplest explanation, differences in extent of the disparity being attributable to differences in absolute, average, and peak levels. The specific activity of labelled

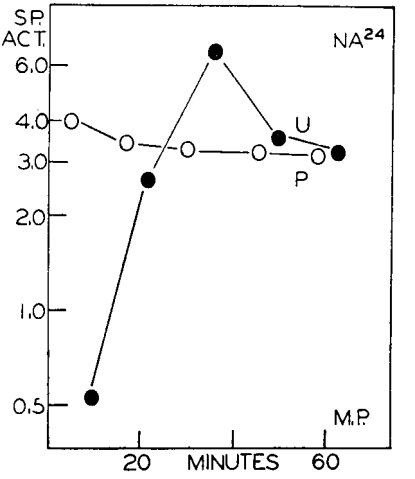

FIG. 3. The specific activity of radioactive sodium (^{24}Na) in the urine (U) and plasma (P) following intravenous administration (Patient M.P.). (From Bradley, Nickel and Leifer, 1952.)

urea, radio potassium, and radio sodium in the glomerular filtrate should not be affected by subsequent reabsorption of water or solute from tubular urine since the isotope distribution should remain unchanged.* The changes in urinary specific activity should therefore be referable solely to changes in plasma specific activity and to the times required for filtrate to pass from plasma to bladder. Similarly the inulin excretion

*To some extent dilution respectively in urea, potassium, or sodium already in the urine in the tubules and urinary tract would operate to reduce the initial specific activity but should have little effect thereafter.

would be expected to change in relation only to the load (filtration rate multiplied by plasma inulin concentration) and to the delay times, since output is not affected by reabsorption of water. When the output and load of inulin are plotted against time, a disparity resembling that between specific activities is found (Fig. 4). If delay and plasma activity or concentration are the only variables affecting the

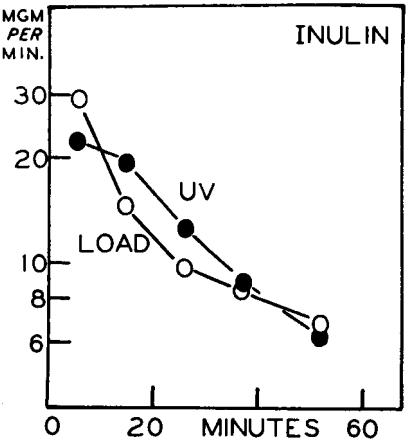

FIG. 4. Filtered load (open circles) and urinary excretion (UV-closed circles) of inulin following intravenous administration. (From Bradley, Nickel and Leifer, 1952.)

urine then the frequency distribution of delay among the renal tubules may be calculated.

As a first approximation we may examine the situation in which a spread in the distribution of delay alone is responsible for a disparity between urine and plasma. A simple analogy will aid in making this calculation. A reservoir drained by a manifold of pipes which empty into a single terminal mixing pipe may be used as a schema representative of the kidney. In such a system the outflow from the mixing pipe (or bladder) is equal to the sum of flows from all the contributory pipes (tubules—assuming for the moment no reabsorption). If

we take an arbitrary number of pipes—say ten—each putting out 1 ml. per min. (total outflow=10 ml. per min.) we may set up an arbitrary distribution of delay as follows: the time required for fluid to move from the reservoir is one minute in one pipe, two minutes in two pipes, three minutes in four pipes, four minutes in two, and five in one (Fig. 5). If a concentration of inulin of 100 mg. per cent (or a specific activity of 100 for some isotope) is now instantaneously established in the reservoir, we may calculate minute-to-minute changes in inulin concentration (or specific activity) to be expected in the fluid issuing from the mixing pipe. Thus, between zero time and one minute the concentration (or specific activity) is zero, between one and two minutes the pipe with a one-minute delay begins to contribute one ml. to the ten flowing out, and the resultant concentration in the outflow must be 10 mg. per cent (or the specific activity, ten). And so on from minute to minute the concentration (or specific activity) mounts to one hundred as in the table in Fig. 5. It can be seen that the changing outflow concentration (or specific activity) describes a cumulative frequency distribution curve from which the percentage of tubes contributing at any minute can be determined. Increasing the number of units yields a curve that approaches closer and closer to the typical sigmoid curve of a normal population. Now when the concentration (or specific activity) is allowed to change in the reservoir, as the plasma values are observed to change in practice, the concentrations (or specific activities) in the outflow may be calculated as follows: assuming that each pipe or sequence of pipes throughout each time period is receiving fluid having an *average* concentration equal to the *average* concentrations in the reservoir during that period:—

$$U_1 = S_1\%P_1$$
$$U_2 = S_1\%P_2 + S_2\%P_1$$
$$U_n = S_1\%P_n + S_2\%P_{n-1} + S_3\%P_{n-2} \quad . \quad . \quad .$$

where U_1, U_2, U_3 etc., are successive average concentrations in the outflow throughout each period of collection; P_1, P_2, P_3

etc., are successive average concentrations in the reservoir; and $S_1\%$, $S_2\%$, $S_3\%$ etc., are the successive percentile contributions of each pipe or sequence of pipes to the total

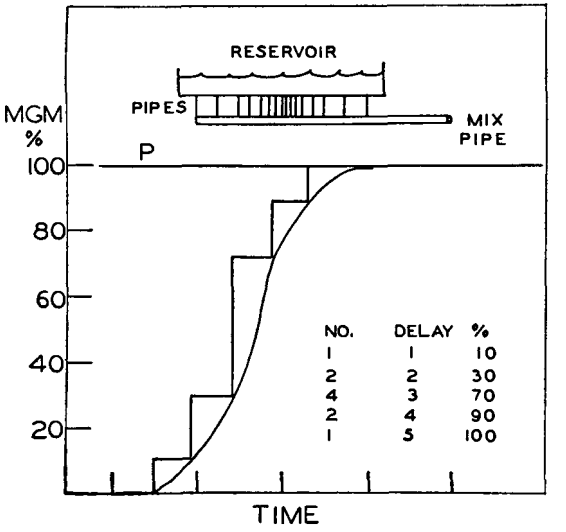

FIG. 5. Schema showing the change in outflow concentration as a function of delay alone. A reservoir filled with fluid drains through a manifold of pipes into a mixing pipe (mix pipe). Taking an arbitrary number of pipes (say 10) each with 1 ml. outflow per minute (total outflow of 10 ml. per minute), a distribution of delay may be set up as shown in the Table. Here the number of pipes for each delay time is given; i.e., in one pipe fluid takes one minute to travel from the reservoir to the end of the mixing pipe, in two it takes two minutes, in four three minutes, and so forth. The cumulative frequency distribution of delay is presented in the last column of the Table. If the concentration (P) of some substance (such as inulin) in the reservoir is instantaneously raised from zero to 100 mg. per cent at the first upright mark on the time scale, the concentration in the outflow will change as indicated. During the first minute the outflow contains none of the tracer material, between the first and second minute the concentration rises to 10 mg. per cent as the outflow from the pipe with a delay of one minute contributes to the total outflow, to 30 mg. per cent in the next minute as two additional pipes contribute, and so on. It can be seen that these values agree with those of the cumulative frequency distribution. On increasing the number of pipes and maintaining a normal distribution, the concentration change in the outflow approaches closer to the typical sigmoid cumulative distribution curve. (From Bradley, Nickel and Leifer, 1952.)

outflow. Thus in the first pipe or sequence of pipes (S_1) transfer is completed in the first period of outflow collection and is in equilibrium with P_1 during that period. When mingled with the outflow from the other units, the outflow from S_1 gives rise to U_1. At each step, each sequence contributes additively to the outflow concentration. The curves so obtained closely resemble those observed experimentally (Figs. 1–4).

Values for the frequency distribution of delay may be computed from the experimental data by substituting successive values of plasma and urinary specific activities or of the filtered load and output of inulin for P and U respectively, solving at each step for the sequence percentage in question (S%). In using inulin, the inulin clearance may be measured at a constant plasma level following the period of equilibration, or computed from the collected urine and plasma values throughout the period of equilibration as the average clearance. For both radioactive isotopes and inulin the percentile values should indicate the percentage of the isotope or inulin which reaches the bladder during the period of measurement. If glomerular filtration is uniform throughout the kidney and each glomerulus forms approximately the same volume of filtrate, the figures may be interpreted as indicating the percentage of the nephron population contributing to urine formation during each collection period. Sodium *p*-aminohippurate (PAH) may be used in the same fashion as inulin, providing relatively small doses are employed, under the assumption that all of the substance entering the kidney is removed in the urine. The successive loads of PAH are determined from the renal blood flow (determined at a constant blood level subsequently or estimated as an average value during equilibration) and the average successive plasma concentrations. Here the values indicate the times required for PAH to move from blood to bladder urine. In practice urine is obtained by indwelling catheter and the bladder washed out with 20 ml. of sterile distilled water at each collection period. Blood is obtained

continuously from a peripheral artery at a constant rate throughout each ten minute collection period following injection of inulin (10 per cent—20 ml.), PAH (25 per cent—5 ml.) and radioactive sodium or potassium (100 μc.).

Table I presents figures for sequence percentages obtained following simultaneous injection of inulin and PAH in 14 normal human subjects. The values for both are in good

Table I

DISTRIBUTION OF DELAY FOR INULIN AND SODIUM P-AMINOHIPPURATE IN NORMAL HUMAN SUBJECTS

TIME AFTER INJECTION

Subject	10 *min.*		20 *min.*		30 *min.*	
	*I** *per cent*	*P** *per cent*	*I* *per cent*	*P* *per cent*	*I* *per cent*	*P* *per cent*
McL. . . .	70	76	36	23		
Fl.	69	42	35	25		
Ro. . . .	74	73	27	25	11	5
Ve. . . .	66	52	34	44		4
Yo. . . .	40	51	60	42		6
Ma. . . .	61	47	39	44		4
Al. . . .	65	64	25	21	11	11
Kc. . . .	60	65	33	25	8	9
El. . . .	78	73	9	18	11	6
Go. . . .	61	65	39	27		6
Gi. . . .	64	65	30	31	4	4
Le. . . .	52	52	33	38	12	10
McF. . . .	48	53	30	32	22	15
McK. . . .	60	65	40	31		4

*Percentage of filtered inulin (I) or PAH load (P) first appearing in the bladder urine.

agreement in indicating that on the average, 60 per cent of the load of inulin and PAH reaches the bladder in ten minutes, 30 per cent requires twenty minutes, and 6 per cent, thirty minutes. The agreement between these values strongly suggests that the region in the tubular system in which glomerular urine is delayed lies beyond that part of the tubule into which PAH is secreted. Otherwise, PAH would reach the bladder before inulin. Furthermore, no correlation between these values and urine flows ranging from 0·5 to 10 ml. per min. has been observed, presumably indicating

that the final volume of the urine must be determined at some point distal to the region of delay, possibly in the lowermost portions of the collecting tubules. In these individuals (Table II), sequence percentages were determined with inulin and PAH before and after induction of cyclopropane anæsthesia. No change was evident despite marked reduction in urine flow. In these experiments little alteration occurred in filtration and further work is necessary to determine whether filtration changes can affect the distribution of delay.

Table II

DISTRIBUTION OF DELAY FOR INULIN AND SODIUM p-AMINOHIPPURATE BEFORE AND AFTER CYCLOPROPANE ANÆSTHESIA

TIME AFTER INJECTION

Subject	10 *min.*		20 *min.*		30 *min.*	
	*I**	*P**	*I*	*P*	*I*	*P*
El. before	78	73	33	19	4	2
after	63	79	39	27		6
Gi. before	64	65	30	31	4	4
after	61	60	29	36	9	4
McK. before	60	65	40	31		4
after	51	56	39	34	3	7

*Percentage of filtered inulin (I) or PAH load (P) first appearing in the bladder urine.

Four patients with severe renal disease and functional impairment (uræmia in patients Tu, La and Ca) (Table III)

Table III

DISTRIBUTION OF DELAY FOR INULIN AND SODIUM p-AMINOHIPPURATE IN RENAL DISEASE*

TIME AFTER INJECTION

Subject	10 *min.*		20 *min.*		30 *min.*	
	I	*P*	*I*	*P*	*I*	*P*
Re.	29	27	27	36	41	34
Tu.	21	32	73	54	6	6
La.	32	45	55	39	12	14
Ca.	3	10	81	78	16	12

*The subjects are arranged in order of severity of the renal disease; in subjects Re, Tu, and La, malignant hypertension, and in Ca, chronic diffuse glomerulonephritis. Tu, La, and Ca were in uræmia. For abbreviations and procedure see footnote to Table I.

showed a definite reduction in the amount of inulin and PAH reaching the bladder in the first ten minutes following injection, but total delay seemed not much greater than normal. Although additional data are obviously needed, these preliminary findings may indicate that nephrons through which filtrate passes most rapidly are particularly susceptible to damage in the course of renal disease.

In theory, the distribution of delay for the radioactive isotopes and inulin should be identical. In the first five studies, comparison of inulin and electrolyte curves (Table IV) supported this conclusion. Thereafter, however, in 14

Table IV

DISTRIBUTION OF DELAY FOR INULIN AND RADIOACTIVE ELECTROLYTES*

TIME AFTER INJECTION

Subject	*Electrolyte*	10 *min.*		20 *min.*		30 *min.*	
		I	*E*	*I*	*E*	*I*	*E*
Fo. . . .	Sodium	30	29	53	63	15	1
Gl. . . .	Sodium	78	73	18	38	18	
Ha. . . .	Sodium	76	86	29	14	3	6
Gu. . . .	Potassium	34	29	36	41	35	30
Sh. . . .	Potassium	68	60	19	68	18	9

*Radioisotopes of sodium or potassium (100 μc.) were administered simultaneously with inulin. None of these subjects presented definite evidence of renal disease. Subject Fo was extremely ill as the result of carcinoma of the breast and Gu was under treatment for severe gout. Renal involvement in these individuals may account for the low initial values for inulin delay.

normal subjects and three patients with renal disease, more than 90 per cent of the radio sodium or radio potassium appeared in the first ten minutes, much more than could be accounted for by filtration in terms of the distribution of delay for inulin. It is noteworthy that simultaneous determination of PAH delay was made in all of these except the first five subjects and it is possible that rapid sodium and potassium trans-tubular exchange may be related to PAH secretion. Additional work is required to define and clarify the phenomenon. Possibly technical difficulties of sampling blood at a constant rate, the changes in total electrolyte

reabsorption with changes in intra-renal admixture during PAH secretion, errors resulting from the arbitrary identification of peripheral blood activities with those in glomerular capillary blood, streamline effects, or other as yet unknown factors may account for this finding.

It must be emphasized that the term "delay," as used in this presentation, relates only to the time required for the passage of molecules from the glomerulus to the bladder in a single tubular unit or category of units. It is not the "dead-space time," the time between concentration peaks in the blood and urine, or the time for equilibration. In the sense used here, filtrate delay time has been found to vary from ten minutes for approximately 60 per cent of the filtered molecules that reach the bladder to thirty minutes for approximately 10 per cent of them. Such a broad distribution of delay times implies an equivalent range in structural and functional differences between nephrons, and stresses the need for evaluation of renal function in terms of the nephron population.

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DISCUSSION

WINTON: I was particularly interested in your interpretation of the change as being independent of urine flow. Those of us who have studied the dead space of the kidney have usually been appalled by this problem; the dead space seems to vary with the urine flow, which is another way of saying the same thing that you were saying, but your interpretation sounds a much more probable one.

BRADLEY: We assume that delay is primarily a result of the length of the tubular system between the bladder and the glomerulus. This is undoubtedly a simplification of the true situation, and it is probable that factors other than the total volume of fluid within the kidney and the length of the tubule are involved.

WINTON: But there is in fact some difference in dead space in the kidney with the change of urine flow, isn't there?

BRADLEY: Yes, there certainly seems to be, and there is a change in the delay between the time of injection of a dye and its appearance in the urine. However, we have failed to find any significant change in the distribution of delays between the glomerulus and the bladder.

You are quite right in calling attention to the importance of the volume of the dead space and the nature of its content in dealing with electrolytes. Specific activity should not be altered by reabsorption of electrolytes or of water. But it will be altered if there is dilution of the electrolyte already present in the dead space. This fact has worried us a great deal. The fact that the initial figures agree so closely makes it very difficult for us to believe that the error so introduced is very important—unless a change occurs. It may be that in the case of PAH we are dealing with the active movement of sodium across the tubular epithelium with the PAH. That I find extremely difficult to accept, but it is one explanation for a very rapid accretion of radioactive material within the urine, in excess of what can be accounted for by filtration alone. On the other hand, the phenomenon may be attributable to a rapid change in the electrolyte content of tubular urine.

BERLINER: Is that with large amounts of PAH?

BRADLEY: Five millilitres of 20 per cent solution. We assume that there is 100 per cent extraction by the tubules under these circumstances, and that we are justified therefore in relating the total load of PAH coming to the kidney with the amount appearing in the bladder. The distribution curve expresses the percentage of excreted load which arrives in the bladder at various times, just as the inulin curve expresses the percentage of inulin filtered which arrived in the bladder at different times.

DENT: Why can't it just be due to the residual urine in the pelvis? Wouldn't that give you the dilution effect?

BRADLEY: Not to this extent. For example, with a urine flow of 10 ml. per minute such a dilution effect would be minimal, since the maximal volume of urine in the pelvis of the human kidney scarcely amounts to 15 ml.

DENT: Yes, but it may be in eddies with little bits left behind.

BRADLEY: We had three ten-minute periods, and it seems scarcely possible that this is not washed out after the first ten minutes.

BERLINER: Michie's experiments were done with a catheter in the pelvis.

WINTON: Have any observations been made on dogs, for example, in which if you suddenly inject phenol red or a tracer substance and maintain its concentration by perfusion, you can then follow the transient changes in concentration in the urine? Haven't I heard of something like that?

BRADLEY: This is the kind of experiment the Michies conducted. They injected thiosulphate, mannitol, PSP and PAH and followed the changes in the urine concentrations relative to the blood levels. They found that the point at which the clearance value became stabilized occurred approximately twenty to thirty minutes after the blood concentration levelled off.

LEWIS: Do you remember a paper of Burch's, when he was first studying ^{24}Na? There was a very surprising delay sometimes before ^{24}Na appeared in the urine after it had been injected intravenously. He commented on that, but he never explained it.

BRADLEY: Burch's figures and Handler's figures for phosphate all fit in with this study. Of course we are really interested in whether a change in the population of nephrons will bring about changes in urine formation. For example if 50 per cent of the nephron population were highly active in forming a dilute urine, and 50 per cent were active in forming concentrated urine, then removal from function of a fraction of the nephrons forming dilute urine would result in the excretion of a more highly concentrated urine without any real change in the activity of the active tubules. Such a "population effect" may provide a possible explanation for the observations we have made on abdominal compression. It is extremely difficult otherwise to understand how residual nephrons, apparently continuing to function without any change in filtration, should produce urine containing less water and less sodium, without calling upon improbable humoral or neural responses.

RAASCHOU: In connection with Dr. Bradley's paper, I should like to tell you briefly about some experiments which Brun, Hilden and I have made on the delay time of the kidney and the urinary tract (to be published in *J. clin. Invest.*).

We inject intravenously very quickly small amounts of sodium thiosulphate (0·3 ml. of a 10 per cent solution) or sodium *p*-aminohippurate (1 ml. of a 2 per cent solution); thus we get a transitory peak in the arterial blood. We collect the urine every minute and follow the concentration of the substance in the urine. The delay time is estimated as the time from the injection to the peak on the concentration curve in the urine. The delay time was examined in patients with medical kidney diseases.

We found no differences in the delay time between the normal material and the kidney diseases, even if the kidney function was reduced to very low levels. Furthermore we have examined the delay time in cases of bilateral hydronephrosis. Normal values of the delay time with this technique are four to ten minutes, varying with the urine volume; in bilateral hydronephrosis we have found values between eighteen and forty minutes.

The fact that the delay time is of the same order of magnitude in the normal kidney as well as in the diseased and even in the contracted kidney, while it is prolonged very much in hydronephrosis, implies that most of the delay takes place in the pelvis and ureter and not in the nephron.

DENT: When you get a very long delay, the curve is presumably very

much smoother too—it is a flatter curve, isn't it? Which suggests dilution in the pelvis.

RAASCHOU: Yes, we get a longer and flatter curve.

DENT: But it is further along. That's a bit odd, isn't it? The injection material is being diluted into a larger volume in the pelvis.

BRADLEY: I think we are using the term "delay" differently.

RAASCHOU: Our "delay time" means the time from the peak of the high and narrow concentration curve in the arterial blood to the peak of the corresponding concentration curve in the urine. We are of the opinion that we must use this delay time for the correction of our clearance values, when the arterial blood concentration varies.

BRADLEY: In our work "delay" refers to the time between filtration and appearance in the bladder. There is no single "delay time" in this sense, but rather in the proportion of filtered inulin that is delayed ten minutes, another that is delayed twenty minutes. As your "delay time" refers to the time between concentration peaks, I cannot correlate it with any discrete segment or summation of nephron delays.

THE KIDNEY
G. E. W. WOLSTENHOLME

SOME BIOCHEMICAL FEATURES OF TUBULAR TRANSPORT MECHANISMS

JOHN V. TAGGART

MUCH that I shall say today has already been reported elsewhere over the course of the last three years. However, each of the earlier reports has tended to emphasize certain limited aspects of the problem at the expense of a more integrated approach. Consequently, it is hoped that this opportunity may be used to bring together the scattered facts and speculations and to provide a better perspective of the whole. The use of the word "whole" is perhaps unwise, for the investigator who dares to venture into the biochemical features of cellular transport should bear in mind the limitations of our present knowledge of intermediary metabolism. The volume of current biochemical literature, together with the excitement that attends each new development, tends to exaggerate the real extent of our information. I trust that Dr. Baldwin will agree that we now possess a fairly detailed knowledge of the major energy-yielding reactions of anaerobic and aerobic glycolysis, some insight into certain coupled reactions whereby energy is conserved, and as yet only a limited number of model biosynthetic reactions in which the energy source is clearly defined. When one attempts to translate this information into as complex a physiological process as cellular transport, it should not be surprising that the picture obtained is a fragmentary one.

During the past five years, several of my associates and I have examined certain of the biochemical events which may participate in the tubular excretion of such compounds as phenol red, *p*-aminohippurate (PAH), diodrast and the penicillins. More recently, Dr. Gilbert Mudge and his associates in our laboratory have explored the mechanisms underlying potassium transport. In the time available, I shall

have to confine most of my comments to the anion excretory mechanism; reference to potassium transport will be made only when it serves to point out important analogies or dissimilarities between the two systems.

In both systems we are dealing with "active transport," that is a process in which the cell must perform work in order to achieve a concentration gradient. In the one case (PAH), the concentration gradient is across the tubular epithelium, while in the other (K) it is across the cell membrane. Work implies the mobilization and expenditure of free energy. In keeping with current views in biochemistry, that is almost the same as saying that we believe that energy-rich phosphates must be generated and utilized at some stage in the overall process.

It has now been fairly well established that the tubular excretory mechanism does operate at the expense of phosphate bond energy. Some of the earlier observations were made with the isolated renal tubules of the flounder (Forster, 1948; Forster and Taggart, 1950). The fish kidney was originally selected because a sparing intertubular cement substance permits the teasing out of long segments of the individual tubules. When such tubular segments are suspended in an oxygenated balanced-saline solution containing phenol red, they rapidly accumulate the dye within the tubular lumen; a concentration gradient of 100-fold or more is achieved in thirty to sixty minutes. Various agents which are known to interfere with aerobic phosphorylation were tested in this system (Taggart and Forster, 1950). The studies with 2,4-dinitrophenol (DNP) and a series of related compounds were perhaps the most revealing, for these agents are among the most specific of the known enzyme inhibitors. DNP, in a concentration of molar/10,000, blocks the generation of energy-rich phosphates (ATP) which normally accompanies the aerobic oxidative reactions of the citric acid cycle; this had been observed in earlier studies with rabbit-kidney mitochondria (Cross, Taggart, Covo and Green, 1949). Unlike many inhibitors of aerobic phosphorylation, DNP does not

inhibit, and may actually stimulate, cellular respiration. DNP was found to inhibit completely, but reversibly, the transport of phenol red by the isolated flounder tubules. The graduation of inhibitory effects obtained at various concentrations of DNP paralleled almost exactly that which had been observed in the phosphorylation system of rabbit-kidney mitochondria. Many closely related nitrophenols are available, some of which are more active than DNP as uncoupling agents, and others of which are completely lacking in this property. When tested with the fish tubules, their effects on phenol red transport were found to conform to their activities as uncoupling agents.

The observations on transport inhibition by DNP were confirmed and extended in the intact dog by means of clearance techniques (Mudge and Taggart, 1950*a*). In the usual experiment, the maximal rate of transport (Tm) of PAH, diodrast or phenol red was measured during three control periods and for approximately one hour following a single intravenous injection of DNP (10 mg./kg.). The administration of DNP resulted in a prompt and prolonged suppression of Tm. In general, the rate of transport was diminished by about 60 per cent, although the plasma levels of DNP were as high as M/4,000. However, dialysis equilibrium studies showed that 90 per cent of the DNP in plasma is protein-bound. Consequently, the concentration in plasma water is actually M/40,000, a level which yields a comparable degree of inhibition in the other systems in which DNP has been examined.

At this juncture, one may properly ask the following question: if phosphate-bond energy is presumed to be the common currency of biological systems, should we not expect DNP to interfere with almost every energy-dependent physiological process? Fortunately for the investigator, such does not appear to be the case. Further studies on the dog demonstrated that DNP, in the usual dose, had no effect on glucose Tm, nor in casual observations were there detectable effects on the tubular reabsorption of sodium, potassium or orthophosphate. The lack of effect on glucose transport was

particularly surprising for two reasons. First, the free DNP in plasma is filtered and almost completely reabsorbed, a circumstance which should lead to a relatively high concentration of DNP at the luminal margin of the tubule, that is at the site at which reabsorption is initiated. Second, it is quite generally accepted—although by no means established—that glucose transport involves the formation of glucose-6-phosphate, presumably through ATP and the hexokinase reaction. If such were the case, one would certainly expect DNP to be a potent inhibitor. I hope that this point may be dealt with later in the discussion. In any event, it is quite clear that the inhibitory effects of DNP on tubular excretion are not completely non-specific.

The chemical nature of the compounds actively excreted is such that their direct phosphorylation during transport appears unlikely. Therefore, it is assumed that phosphate bond energy is used in priming or activating some cellular element of the transport system. Our attention was next directed toward the identification of certain metabolic factors which might participate in tubular excretion. In these studies, thin slices of rabbit-kidney cortex were prepared as for respiration studies. Approximately 300 mg. of slices were introduced into a Warburg vessel with 2·7 ml. of a saline medium containing dilute PAH. The vessels were gassed with oxygen and shaken in a 25°C. bath for the desired time, usually one hour. Respiration was measured and expressed as a Q_{O_2} (cu.mm. of O_2 consumed per mg. initial wet wt. of tissue per hr.). Later on, the slices and an aliquot of medium were recovered for PAH estimations. Under suitable conditions, the slices accumulate PAH, so that in one to two hours the concentration in the slices (S) is 10 to 20 times that in the medium (M). Results are expressed in terms of the S/M ratio. However, it should be noted that the bulk of the accumulated PAH is probably contained in the tubular lumina, and therefore the true gradient across the tubule epithelium is at least 10 to 20 times that indicated by the S/M ratio (Cross and Taggart, 1950).

The accumulation of PAH by tissue slices is confined to those of the kidney cortex; slices of the renal medulla are completely inactive. Satisfactory results have now been obtained with kidney slices from the dog, rat, guinea pig, pigeon, and dogfish, as well as the rabbit. Various compounds other than PAH, which are excreted by the same mechanism, have also been examined by the slice method. The relative rates at which they accumulate parallel the rates at which they are excreted by the intact kidney.

The accumulation of PAH by kidney slices is coupled with respiration and is dependent upon the maintenance of a relatively high oxygen tension. It has been of obvious interest to examine various metabolic intermediates for their effects on respiration and particularly on PAH transport. The endogenous substrate contained in the slices supports a limited accumulation of PAH. The addition to the medium of glucose or the phosphorylated intermediates of anaerobic glycolysis has in general little effect on either respiration or transport. On the other hand, lactate, pyruvate or acetate consistently increase both respiration and transport. From this it might be concluded that any oxidizable substrate will support a more active transport. That such is not the case was demonstrated with members of the citric acid cycle. The tricarboxylic acids stimulate respiration, but have no effect on transport. The dicarboxylic acids, α-ketoglutarate, succinate, fumarate and malate, stimulate respiration as much or more than does acetate, yet all have proved to be potent inhibitors of PAH transport. Similar inhibition is observed with those amino acids that feed directly into the cycle and with fatty acids of C_6 to C_{12} chain length. The concentrations at which the various substrates exert their maximal stimulatory or depressant actions are fairly striking; acetate may double the rate of transport at 0·003 M., while octanoate and α-ketoglutarate completely block transport at 0·002 and 0·01 M. respectively.

These observations have also been confirmed by clearance methods in the dog (Mudge and Taggart, 1950*b*) and in man

(McDonald, Shock and Yiengst, 1951). In the dog, Tm_{PAH} can be abruptly raised to almost twice its control value by the continuous intravenous infusion of acetate at a rate of about 65 micromoles per kg. per minute. This augmentation occurs without the accumulation of detectable amounts of acetate in the peripheral blood and in the absence of any significant changes in renal hæmodynamics or urine flow. Lesser increases occurred with lactate infusions, while the administration of succinate or fumarate decreased Tm by 20 to 50 per cent.

We have now examined, with slice technique, a great many of the known metabolic intermediates. Of these, only lactate, pyruvate and acetate show any consistent stimulatory effect. It is of interest that pyruvate is always more effective than lactate, and acetate more effective than pyruvate. Thus, we are inclined to believe that it is acetate which acts as one of the important rate-limiting factors in PAH transport, and that lactate and pyruvate function only as good precursors of acetate, or of some common product derived therefrom.

Parenthetically, it should be added that a rather different situation obtains in the potassium transport system (Mudge, 1951). Potassium-depleted slices of rabbit kidney cortex can reaccumulate this ion against a considerable concentration gradient when provided with oxygen and a suitable substrate. In this system, however, lactate, pyruvate, acetate, or any member of the citric acid cycle will support K accumulation equally well. Again, we may conclude that the stimulatory effect of acetate on the PAH transport system is a fairly specific one.

A great many compounds, both natural and synthetic, act as inhibitors of PAH transport. Briefly, they may be classified in the following groups. First are those which limit respiration by acting directly on one or more of the oxidative enzymes of the citric acid cycle or on the cytochrome-cytochrome oxidase system of electron transport. The second group comprises the previously mentioned active nitrophenols and other agents which interfere with the

generation of energy-rich phosphates. Representative of the third group are diodrast and penicillin, compounds which apparently share the same excretory pathway and, therefore, act in a strictly competitive manner. The last group consists of a series of synthetic compounds, the best known members of which are carinamide (*p*-benzylsulphonamidobenzoate) and benemid (dipropylsulphamylbenzoate). Carinamide was originally introduced as an effective agent for delaying the renal excretion of penicillin (Beyer *et al.*, 1947). Both have since been found to suppress the tubular excretion of PAH, diodrast and phenol red as well (Beyer, 1950). While neither is actively excreted by the tubule, each appears to inhibit transport in a truly competitive manner. Benemid and the related compounds have now been examined in a variety of enzyme systems. Of particular interest is the finding that benemid inhibits the reaction whereby benzoate and glycine are conjugated to form hippurate (Beyer, Wiebelhaus, Tillson, Russo and Wilhoyte, 1950). Concentrations of benemid that interfere with hippurate synthesis do not suppress cellular respiration or interfere with phosphorylation reactions.

Is there any reasonable scheme into which one can fit all the foregoing observations? The enzyme studies with benemid might suggest the rather remote possibility that PAH transport involves, first, the hydrolysis of PAH to *p*-aminobenzoate and glycine, and subsequently, their reconjugation. Hippuricase, the hydrolytic enzyme, is known to be present in the kidney of many species, although we have thus far failed to find it in rabbit kidney. In addition, the conjugation system is abundantly present in kidney and, furthermore, has been shown to operate at the expense of phosphate bond energy (Cohen and McGilvery, 1947). However, we believe that this possibility has been excluded by the following experiment. PAH labelled with ^{14}C in the carboxyl group was administered by slow intravenous infusion to a dog and subsequently recovered from the urine. In the event of hydrolysis and reconjugation during transport, the carboxyl labelling should have been diluted by the incorporation of

unlabelled glycine derived from the reabsorption of glomerular filtrate. The specific radioactivity of the original and recovered PAH were, however, found to be identical.

When one reviews the list of inhibitors of PAH transport, particularly those without related respiratory effects, it soon becomes evident that the only structural feature common to them all is the carboxyl group. All the compounds that appear to share the same excretory pathway, with the exception of phenol red, are also carboxylic acids. We have now prepared various derivatives of PAH in which other potentially reactive groups have been blocked by chemical substitution. The fact that they are all still actively excreted by the tubule is in accord with the idea that it is the carboxyl group which must interact with the definitive cellular element of the transport mechanism.

In any speculative attempt to describe the anion excretory mechanism in specific biochemical terms, the following points should be borne in mind. First, we shall assume that the transported compound (A) reacts with a cellular component (X) to form an intermediate compound (AX) which is restrained from outward diffusion through the cell membrane. AX must in turn be capable of undergoing dissociation or cleavage to yield A, the parent compound, as one of its products and X, or a precursor of X, as the other product.

$$A + X \rightleftharpoons AX \rightarrow A + Xi$$

Since active transport, by definition, involves the movement of A against a chemical potential, free energy must be expended in one or both of these reactions. It may be safely assumed that this energy will have been provided in the form of energy-rich phosphate bonds. Our hypothetical scheme should also provide for the interaction of the transported compound and the cellular element via a carboxyl mechanism. Lastly, certain interdigitations with acetate metabolism should be apparent.

Two particular circumstances have led us to wonder about the possible importance of coenzyme A (CoA, CoA–SH) in

the tubular excretory mechanism. The first of these is related to the apparently unique rôle which acetate plays in this system. Time does not permit reviewing the fascinating discoveries in the field of acetate metabolism that have come to light in the last few years (cf. Barker, 1951). It will be sufficient to say that the "active C_2 compound," long sought by the biochemists, has now been clearly established as the acetyl mercaptan of coenzyme A (CH_3CO–S–CoA) (Lynen, Reichert and Rueff, 1951). According to Lynen (1953), acetyl-CoA is formed through the following series of reactions:-

(1) Enzyme + ATP $\rightleftharpoons$ Enzyme-AMP + pyro P
(2) Enzyme-AMP + CoA-SH $\rightleftharpoons$ Enzyme-S-CoA + AMP
(3) Enzyme-S-CoA + acetate $\rightleftharpoons$ Enzyme + CoA-S-acetyl

The "acetate activating" enzyme reacts with ATP to form an adenyl (AMP) complex and inorganic pyrophosphate (reaction 1). By an exchange reaction, the adenyl group is subsequently replaced by CoA to give a CoA-enzyme complex (reaction 2). Finally, by what Lynen has termed an "acetolysis," acetate splits off the CoA as acetyl-CoA and regenerates the enzyme. The acetyl-CoA thus formed can donate the acetyl group for the synthesis of acetylcholine, acetoacetate or citrate, or by exchange reactions with other carboxylic acids yield a variety of other S-acyl derivatives of CoA. Equilibrium reaction studies in several laboratories indicate that acetyl-CoA is an energy-rich compound, comparable in its bond value to the energy-rich phosphates (Stern *et al.*, 1951; Stadtman, 1952).

A second feature of the PAH transport system that directs our attention to CoA is related to the observations with the benemid series of inhibitors. As mentioned previously, Beyer and his associates found benemid to be a potent inhibitor of hippurate synthesis in a respiring or ATP-driven system. Shortly thereafter, Chantrenne (1951) was able to demonstrate that the synthesis of hippurate is performed by a CoA-dependent system. During the past year, we have prepared

benzoyl-S-CoA and have shown that it is indeed the energy-rich intermediate in hippurate synthesis (Schachter and Taggart, 1953). Since benemid does not interfere with the final condensation reaction, in which benzoyl-CoA and glycine yield hippurate, we have concluded that benemid acts by blocking one or more of the reactions leading to the activation of benzoate—that is, the synthesis of benzoyl-CoA.

During the two years since the discovery of acetyl-CoA, a great many other acyl mercaptans of the coenzyme have been identified as important biological intermediates—butyryl-CoA, succinyl-CoA, palmityl-CoA, benzoyl-CoA, to name only a few. Therefore, we are tempted to wonder whether the various carboxylic acids, both those which are transported and those which competitively inhibit transport, may not react with CoA to form the corresponding acyl mercaptans. The rôle of acetate appears to be best explained as involving an obligatory exchange reaction which facilitates either the formation or breakdown of the hypothetical intermediate, *p*-aminohippuryl-CoA. This completely speculative scheme may be depicted as follows:—

(4) CoA-S-acetyl + aminohippurate	$\rightleftharpoons$	CoA-S-aminohippuryl + acetate
(5) CoA-S-aminohippuryl + H_2O	$\rightarrow$	CoA-SH + aminohippurate
Sum (1–5) ATP + H_2O	$\rightarrow$	AMP + pyro P

Reaction 4 is a simple exchange reaction of the type now well known to occur in a variety of CoA systems, while reaction 5 represents a hydrolytic deacylation similar to that which has been described for acetyl-CoA and succinyl-CoA (Gergely *et al.*, 1952). One of the more attractive features of reactions 3–4 is that they provide for the continuous recycling of acetate. Therefore, we might expect acetate to exert an effect on transport in catalytic amounts, as indeed it does. The sum of reactions 1–5 is simply a splitting of ATP.

Another possibility is one in which acetate facilitates the breakdown of *p*-aminohippuryl-CoA, by the following alternative reactions—

(3a) Enzyme-S-CoA + aminohippurate $\rightleftharpoons$ Enzyme + CoA-S-aminohippuryl
(4a) CoA-S-aminohippuryl + acetate $\rightleftharpoons$ CoA-S-acetyl + aminohippurate

Sum (1-4a) ATP + CoA-SH + acetate $\rightleftharpoons$ CoA-S-acetyl + AMP + pyro P

This sequence is perhaps less attractive in that a single enzyme must be involved both in acetate activation and in PAH transport. However, such a sequence of reactions would provide for the transport of PAH at the expense of very little free energy change.

It must be emphasized that the foregoing has been presented in a purely speculative vein. The proposed scheme represents only the crudest sort of working hypothesis. Certain very obvious objections may be raised, even at this stage. For example, it fails to account for the transport of phenol red, unless we make the further assumption that the dye may be bonded to CoA through an S-S type of linkage—an assumption that is not supported by our present knowledge of CoA chemistry. In addition, Wiebelhaus, Kemp and Beyer (1952) have reported that pantothenic-deficient rats appear to be capable of excreting phenol red and PAH in a perfectly normal manner. However, more recently, Wiebelhaus and his associates (1953) reported that the administration of pantothenate to dogs receiving simultaneous infusions of PAH and acetate resulted in a substantial enhancement of Tm_{PAH} above that obtained with acetate alone. Finally, it should be added that we have recently made the surprising observation that acetate is a potent inhibitor of PAH transport in the dogfish kidney. It is apparent that only time and a good deal more information will permit us to fit all these apparently conflicting facts into a really acceptable scheme.

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DISCUSSION

ROBINSON: Dr. Taggart, in your experiments *in vitro*, why are the Q_{O_2} figures so tiny?

TAGGART: One reason the Q_{O_2} appears to be small is that our slice experiments were performed at 25°C. Consequently, the values are one-half, or less, of those which would be obtained at 37°. Secondly, the respiration is expressed in terms of wet weight of tissue rather than the more conventional dry weight. Also, slices of rabbit kidney cortex have a lower Q_{O_2} than do those of either the rat or the dog.

ROBINSON: Yes. The dog is about 2½. I wondered, though, if you did think that made any difference? Because I also find the greatest stimulatory effect with acetate as substrate, but when the respiration is stimulated by acetate the tissue ceases to be normal and swells and takes up a great deal of water.

TAGGART: At what concentration?

ROBINSON: About M/50.

TAGGART: That is surprising, for I am certain that we have never observed an increase in hydration of the slice at such a low concentration of acetate*.

*A subsequent review of the data revealed that abnormal hydration occurred only when the concentration of acetate exceeded M/20.

ROBINSON: Yes, at 37° with rats I've tried M/50, and got a 50 per cent increase in oxygen uptake, and also about a 40 per cent increase in the percentage of water in the slices.

TAGGART: That is a very large change.

ROBINSON: It is very large indeed, and I have been completely mystified by it.

BERLINER: In your first scheme, acetyl CoA reacts with hippurate to yield hippuryl CoA and acetate. Now if this reaction is reversible, it seems to me that the acetate should exclude the PAH from the reaction and should be inhibitory rather than stimulatory.

TAGGART: In the first scheme, it must be assumed that the formation of the hypothetical intermediate (*p*-aminohippuryl-CoA) involves an obligatory exchange reaction between acetyl-CoA and PAH. The relative rates at which the two acyl-CoA's are broken down, either by further exchange reactions or by hydrolytic deacylation, must also be taken into account. It should be pointed out as well that it may be a serious mistake to put the whole burden on coenzyme A. In the brief time since the discovery of the functional rôle of –SH in CoA, it has been shown that glutathione, firmly bound on the enzyme glyceraldehyde phosphate dehydrogenase, functions in a rather similar manner. In addition, thioctic acid (6,8-dithiooctanoate) appears to play a similar rôle in the oxidative decarboxylation of pyruvic acid and α-ketoglutaric acid. Consequently, even if we assume that –SH groups and the corresponding acyl-mercaptans are of importance in PAH transport, it is impossible at the present time to know which –SH compounds are involved.

RUSSELL: Dr. Taggart, what animals did you use? I missed that.

TAGGART: Most of our slice studies have been done with rabbit kidney, but we have done a limited amount of work with slices from the rat, guinea pig, dog, pigeon and dogfish as well.

RUSSELL: Is the pattern the same throughout all these different species?

TAGGART: Essentially yes. The one glaring discrepancy has been mentioned, namely, that acetate inhibits PAH accumulation in the dogfish slice, while in other species it is stimulatory.

MERRILL: Acetate does, though, reduce the ability of dinitrophenol to inhibit transport of PAH, doesn't it?

TAGGART: Yes. If one depresses transport with dinitrophenol, one can at least partially reverse the effect by adding acetate. In that connection, I have been very much interested in certain observations by Schachter and Freinkel in Homer Smith's laboratory. These investigators have shown that raising the plasma level of PAH to very high levels results in a depression of PAH transport, often to the zero level. In that circumstance as well, the infusion of small amounts of acetate promptly restores PAH Tm to supernormal values.

HELLER: Could one go so far as to assume that under certain conditions when PAH transport is inhibited, such inhibition has something to do with changes in the metabolism of acetate?

TAGGART: Since Shannon first proposed two equations to describe

tubular transport, we have tended to think of the problem in these rather simple terms. In all probability, half a dozen or more reactions are involved in the formation of the hypothetical intermediate of transport and several more in the subsequent breakdown of the intermediate. Consequently, there are many possible sites at which an agent may act either to stimulate or depress transport.

HELLER: But have you actually traced such points of interference in any pathological condition?

TAGGART: No, we have difficulties enough with the normal kidney.

A STUDY OF THE MECHANISM BY WHICH TOXIC TUBULAR DAMAGE CHANGES THE RENAL THRESHOLD FOR GLUCOSE

P. P. LAMBERT

OUR physiological knowledge of the renal excretion of glucose rests on work by Shannon and his associates (1938, 1941), H. Smith (1943, 1951) and Walker, Bott, Oliver and MacDowell (1941). More recently it has been successfully applied to the study of the pathological conditions in man which are characterized by a so-called "renal glycosuria."

According to Shannon and Fisher (1938), excretion of glucose occurs when the concentration of sugar in the plasma becomes higher than a definite level, the "threshold." The amount of glucose filtered at this exact value of glycæmia measures the maximal amount of glucose that the tubules are able to reabsorb ("Tm_G" of H. Smith and Shannon). Therefore the relation between the amount of glucose excreted and the plasma levels appears (Fig. 1) as a straight line (SE) starting from the threshold (S). This line is parallel to the line OF measuring the amount of glucose filtered through the glomeruli at increasing plasma levels. Therefore it must be theoretically possible to measure the glomerular filtration rate from the slope of the line relating the excretion of glucose to its plasma level if this rate is constant. Govaerts, Lambert, Lebrun and de Heinzelin (1948) have brought experimental confirmation to this view. If this was true in dogs, in man it soon appeared that the relation between the plasma level and the amount excreted, was a straight line only at plasma levels significantly higher than the presumed threshold. Near to it, this relation has a curvilinear aspect, which means that the reabsorptive capacity of the kidney becomes saturated in a slowly progressive way at increasing blood levels. We call

"appearance threshold or minimal threshold" the level of glycæmia producing the smallest glycosuria. One might call "maximal threshold" the level of glycæmia at which the curvilinear relation (Fig. 2) joins the straight line SE. Finally, we are accustomed to speak of the level of glycæmia obtained by extrapolating the straight line SE to the base line as "the mean threshold."

The best explanation of this phenomenon is to suppose that

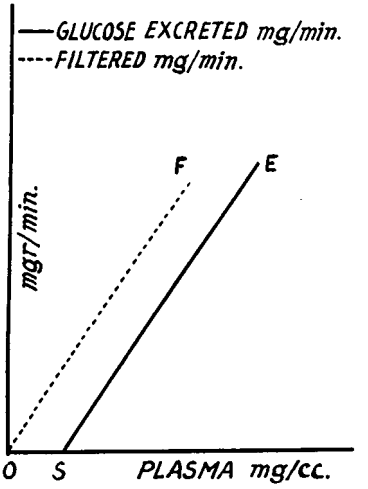

FIG. 1. Theoretical relation between plasma level and glucose excretion if the capacity of all nephrons is equal.

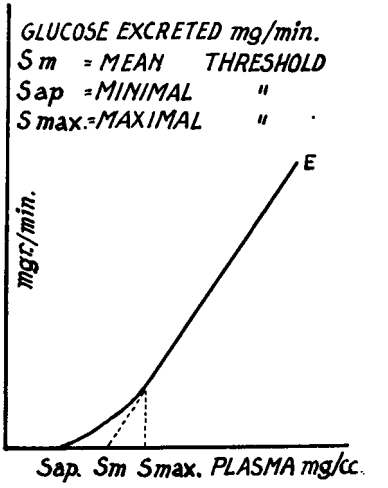

FIG. 2. Curvilinear shape of the relation shown in Fig. 1 around the threshold if the capacity of the nephrons is not equal.

the reabsorptive capacity of the tubules varies from one nephron to the other, as they vary in shape and length. The minimal threshold indicates the level of glycæmia which is able to saturate the reabsorptive capacity of a small number of nephrons, those which are "the weakest" as far as glucose reabsorption is concerned; the maximal threshold the level of glycæmia at which the reabsorptive capacity of the "strongest" of them is saturated; and the mean threshold, which is the threshold of Shannon, is the level of blood glucose

at which the reabsorptive power of all the nephrons would be saturated, if this power was the same in each of them and was equal to the mean of the actual reabsorptive power of all the individual nephrons (Govaerts, 1950). If differences in glomerular activity are responsible for saturation of tubular reabsorption at variable levels of glycæmia, the nephrons with a high glomerular activity will be the first to be saturated at increasing plasma levels (and inversely). It must be pointed out that of these three values, the mean threshold is the easiest to measure. To estimate the two others, it is necessary to increase the glycæmia very slowly and to obtain numerous urinary samples in very short periods of time. The precise shape of the calculated curve will depend on the correct collection of urine samples during periods as short as two or three minutes. The mean threshold may be obtained by measuring the Tm_G at any high value of the glycæmia above the maximal threshold and dividing by the filtration rate.

Before going into the details of the results obtained in several pathological conditions, we think it necessary to define what our experiments have shown to be the normal limits of glucose reabsorption in man.

Sixty-one normal people have been studied until now, 35 women and 26 men. The glomerular filtration was measured, either as the clearance of thiosulphate or of inulin. When thiosulphate was used, care was taken to avoid too low a concentration of the salt in the plasma, as we had earlier observed in this laboratory a secretion of thiosulphate at plasma levels lower than 25 mg. per 100 ml. (Lebrun, 1949). Tm_G, glomerular filtration rate and mean threshold were calculated as the means of the data obtained on three (or four) successive urinary collection periods. By continuous infusion of thiosulphate or inulin and glucose too sharp a fall of the plasma concentration was avoided. The results are collected in Table I.

Small sex differences appear in the values of glomerular filtration and Tm_G as pointed out by Homer Smith and others.

Table I

	Mean glomerular filtration not corrected to body surface area ml./min.	*Mean value of Tm_G mg./min.*	*Mean value of mean threshold mg./100 ml.*
26 men . . .	129.1	312·0	244
35 females . .	108·0	266·2	247

No difference appears in the values of mean threshold. It also made no difference whether the glomerular filtration was measured by the thiosulphate or the inulin method.

Fig. 3 shows the large scattering of these results. The mean threshold varies in normal people (white areas of the figure) between 180 and 320 mg./100 ml.

Data concerning the normal values for the minimal threshold are only available in a few cases. Dr. Verbanck studied nine patients in our laboratory. In these careful experiments the glycæmia was slowly raised by an intravenous drip infusion of glucose. Urine collection periods were as short as possible;

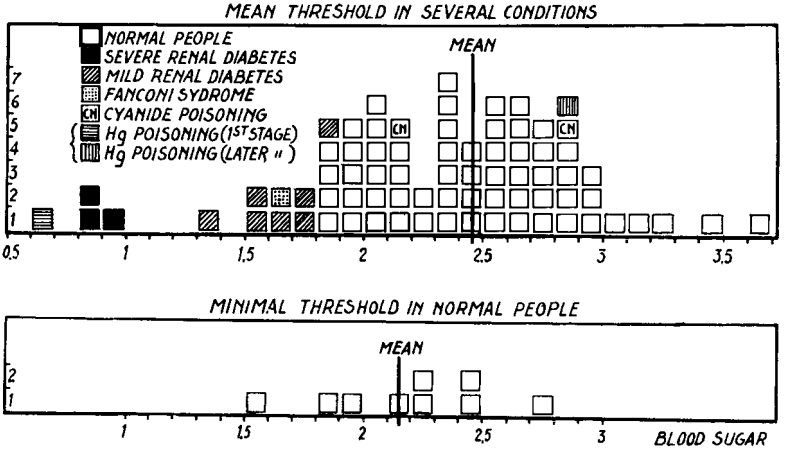

FIG. 3. Distribution of mean and minimal thresholds in normal people and in several pathological conditions.

no longer than two minutes. The samples were immediately analysed for glucose, quantitative estimations being performed later on those samples showing a light positive or a dubious qualitative test. Arterial blood was taken for estimation of glucose at twenty minutes intervals. When glucose was detected in the urine the infusion was stopped and the threshold again estimated on a descending blood curve. Finally the delay time was measured with phenol red. The minimal threshold values show the same scattering as do those of the mean threshold. The mean value for the minimal threshold was 216 mg./100 ml. against 246 mg./100 ml. for the mean threshold. However, it must be pointed out that the minimal threshold was calculated on an arterial blood curve while the mean threshold was on venous blood. In this case the arm of the patient was always kept in hot water to decrease the arteriovenous difference for glucose. In two of Dr. Verbanck's experiments venous blood was taken at the same time as arterial blood. The minimal threshold calculated on the venous blood curve was about 85 per cent of the value obtained on arterial blood (Table II).

Table II

		mg./100 ml.	
		Case 1	*Case* 2
MINIMAL THRESHOLD.	On arterial blood	245	216
	On venous blood	223	185

We did not try to obtain exact measurements of the maximal threshold.

We shall now examine what we know about renal glycosuria in human pathological conditions.

Two conditions may be accompanied by glycosuria without impairment of the metabolism of glucose elsewhere. The first one is renal diabetes, in which the defect of renal function is permanent. In the second group, glycosuria is only a

transitory feature met with in toxic nephropathies and in cyanide poisoning.

Dr. Reubi will examine extensively the problem of renal diabetes so that we shall only say a few words about our personal cases. Our attention will be drawn principally to renal glycosuria in toxic diseases.

From a physiological point of view, two types of renal glycosuria may be imagined. It may result from a lowered minimal threshold without changes in the two other characteristics of the excretion of glucose, Tm_G and mean threshold. In this hypothesis the curvilinear part of the line SE in Fig. 2 is prolonged, rising earlier from the abscissa. Friedman and co-workers (1942) thought that this was characteristic of renal diabetes. However, one can imagine another type of renal glycosuria characterized by a decreased Tm_G and a normal filtration rate. In this case a low mean threshold would be a typical feature of the disease.

The best explanation of the first type would be that the functional capacity of the nephrons is more variable in these kidneys from one nephron to the other than it is normally: either the glomerular load or the tubular capacity varies widely. The second type assumes a decreased capacity of the tubules to reabsorb glucose and makes renal diabetes comparable to the glycosuria due to phlorizin or deoxycorticosterone glucoside (Lambert *et al.*, 1948).

Renal diabetes

In our experience true renal diabetes is always associated with a low Tm_G and a low mean threshold (Govaerts and Lambert, 1949; Nielsen, 1948). We call—as we think Dr. Reubi (1951) does too—"true renal diabetes" a condition generally recognized in young people, characterized by renal glycosuria, without other signs of impaired renal function, and particularly with normal filtration and normal blood pressure. The defect may be observed to a variable degree; some patients being glycosuric when fasting, others only after meals.

We have studied three patients with well established renal diabetes. Their mean threshold was as low as 90 mg./100 ml. (black areas of Fig. 3). Detailed data are reported in Table III.

Table III

Cases	*Glomerular filtration (inulin cl.) ml./minute*	*Blood sugar mg./100 ml.*	*Glucose excreted mg./minute*	*Glucose reabsorbed mg./minute*	*Mean threshold mg./100 ml.*
Sei. (F) .	122·4	212	153·7	105·7	
	117·4	180	114·9	96·3	85
	123·3	174	104·3	108·5	
	M=121·0			M=103·5	
Def. (F) .	106·6	698	635·4	97·5	
	104·0	501	424·4	96·7	88
	105·6	346	281·6	84·3	
	M=105·0			M= 93·0	
Tim. (M) .	131·7	374	380·9	111·5	
	142·1	323	315·0	143·9	90
	156·9	269	285·8	136·2	
	M=143·6			M=130·6	

Six other patients had been sent to us because a small glycosuria had been occasionally observed despite a normal hyperglycæmic blood curve. These patients undoubtedly formed a transition between severe renal diabetes and normal people. Their mean thresholds varied between 134 mg./100 ml. and 190 mg./100 ml. (crossed areas of Fig. 3). The same was true of a patient with Fanconi syndrome and severe osteomalacia of renal origin (dotted area of Fig. 3; cf. Lambert and de Heinzelin, 1951).

We shall now consider what is the type of the transitory renal glycosuria associated with cyanide and mercury poisoning.

Cyanide

(Lambert, P. P., Tagnon, R., Corvilain, J., and Coërs, C., 1950.)

Two young men, aged seventeen and twenty, were brought to hospital immediately after swallowing potassium cyanide. Intravenous injection of sodium thiosulphate rapidly corrected the severe respiratory disturbances; 15 g. were administered as a hypertonic solution (20 per cent). Consciousness reappeared immediately in one case, one hour later in the other. Sodium thiosulphate transforms cyanide into thiocyanate which is non-toxic. A few hours later glycosuria was noted in both cases. On the morning after admission, a renal test was performed as the patients remained glycosuric. Glucose excretion was first determined on a twenty minutes' collection period at a normal level of blood sugar. Glucose was estimated as the fermentative reducing power of filtrates according to Shaffer-Hartman. Thereafter inulin and glucose were injected intravenously and a slow infusion was carried on during the whole procedure. The results were as follows:—

Table IV

Cases	*Collection period*	*Glomerular filtration ml./minute*	*Blood sugar mg./100 ml.*	*Glucose excreted mg./minute*	*Glucose reabsorbed mg./minute*	*Mean threshold mg./100 ml.*
I	I		81	12·4		
	II	114·0	404	210·5	250·1	} 220
	III	103·5	352	137·9	226·5	
II	I		75	4·05		
	II+III+IV	123·2	413	161·9	346·9	281
		rapid injection of $Na_2S_2O_3$ (5 g.)				
	V	125·4	352	95·1	346·3	276

There was no doubt that the patients were glycosuric when fasting. Their minimal threshold was lower than 80 mg./100 ml. However, the values of Tm_G and mean threshold fell in the range of normal values and it was clear that both thresholds were not affected in the same way. Besides, the therapeutic injection of thiosulphate (5 g.) given during the renal test with the purpose of neutralizing the toxic effect of cyanide, did not raise the maximal capacity of the tubules

to reabsorb glucose as one might have expected if the Tm_G had been depressed by the cyanide. These results are comparable with those observed in experimental conditions on dogs by Nicholson (1949). In these experiments, the left kidney was perfused with blood containing M/600 NaCN, the right with normal blood. The urine from the left kidney contained small amounts of glucose; the Tm_G was not measured. Nicholson concludes that "reabsorption of glucose is practically unaffected."

What is the meaning of our observation?

It may be that the nephrons are unequally impaired by cyanide as far as glucose absorption is concerned. We are not completely satisfied with this explanation. Indeed, it seems unquestionable that the trouble must be in the tubular function and not in the glomerular activity, as the rate of filtration is normal and proteinuria absent. If tubular function is impaired in some nephrons sufficiently to decrease the minimal threshold from about 185 mg. to 80 mg./100 ml., one would expect an apparent decrease in the values of Tm_G and mean threshold, as an increased capacity in other tubules is most improbable in cyanide poisoning. One may object that the Tm_G and the mean threshold were not measured later on, after glycosuria had disappeared. However, there is no reason to suppose that, in normal conditions, the Tm_G would have been much higher than at the time of slight glycosuria.

Another explanation may be suggested. One wonders whether the change in tubular function is not an incapacity to reabsorb from the tubular urine the last molecules of glucose when the load is lower than Tm_G, rather than an incapacity to reabsorb an amount equal to Tm_G when the load is much larger.

Mercury

(Gregoire, F., Brauman, J., Lambert, P. P., and de Heinzelin, C., 1953).

A few months ago we had an opportunity to study a young man aged twenty, who—on October 20th—had ingested seven

tabloids of mercury oxycyanide (3·5 g.). On the following day the patient became anuric. He then disclosed the nature of the poison he had taken and was treated with B.A.L. Anuria was complete until the fifth day when the patient was brought to Brussels Hospital. At the time of his admission he was conscious, seemed moderately œdematous, had no respiratory disturbances, no circulatory collapse, no disorder of the bowels, and no rise in temperature. Blood pressure was normal, blood urea 280 mg. per 100 ml., creatinine 19·2 mg. per 100 ml., chlorides 73·3 mEq/l., bicarbonates 14·4 mEq./l. The patient received large amounts of NaCl intravenously for three days. The diet was calculated to give him 1600 calories with no more than 10 g. of protein. On the sixth day after the intoxication he passed 50 ml. of urine; 100 ml. on the seventh, 200 ml. on the eighth day. Thereafter the urinary volume increased rapidly, 685 ml. on the ninth day, and 3470 ml. on the eleventh. Two renal tests were performed: the first one on the eleventh day, when the diuresis rose to 3470 ml.; the second one, fourteen days later, when the patient seemed to have completely recovered apart from a slight glycosuria. At the time of the first test, urea and creatinine in the blood were near to their highest levels; they were almost normal when the second test was performed.

In the first test, the clearance of inulin was established on eight successive urine collection periods and compared to that of urea and endogenous creatinine. Glucose was infused intravenously, the rate of infusion being increased during the whole procedure.

Two facts are worthy of interest. The clearances of inulin, urea and creatinine do not differ significantly (Table V); that of creatinine is somewhat lower than the two others, but this probably results from the presence of a high non-creatininic chromogen in the plasma at the time of marked nitrogen retention. It must also be pointed out that the clearances of the three substances slightly increase during the two last periods. This may be due to the rise of blood sugar. Secondly, as far as the excretion of glucose is concerned, the amounts

excreted, plotted against the plasma concentrations at the mean time of each collection period (Fig. 4a), fall on a straight line; a small deviation is noted for the two last periods during

Table V

OCTOBER 30th. VALUES OF INULIN, UREA, ENDOGENOUS CREATININE CLEARANCES AND OF GLUCOSE GLOMERULAR CLEARANCE AT AN EARLY STAGE OF RECOVERY IN A MERCURY NEPHROPATHY

NOVEMBER 13TH. THE SAME VALUES LATER ON, WHEN RECOVERY IS NEARLY COMPLETE

PERIODS	CL. INULIN cc/min.	CL. CREATININE cc/min.	CL. UREA cc/min.	CL. CREATININE / CL. INULIN	CL. UREA / CL. INULIN	GLOMER CLEAR GLUCOSE	GL. CLEAR GLUC. / CL. INULIN
OCTOBER 30th							
1	6.15	5,27	6.20	0,85	1,00		
2	5.72	5,14	5.71	0,89	1.00		
3	6,93	6,26	7,25	0.90	1.04		
4	7.93	6.88	8,33	0,86	1,05	7 97	1,04
5	7,43	6.91	8.24	0.93	1,10		
6	8,33	6,85	8,66	0,82	1.03		
7	9.39	8.52	10.05	0,90	1,07		
8	9.37	8.75	10.10	0,93	1,07		
M=	7.65	6.82	8,06	0.89	1.05		
NOVEMBER 13th							
1	84,3	124.0	41,8	1.47	0,49		
2	82,2	110.4	37.4	1,34	0,45		
3	80,1	105.5	38.2	1,31	0,47		
4	100,3	133,1	52.5	1.32	0,52		
5	78,4	105.3	47,4	1.34	0.60		
6	86,7	121.0	43.5	1.39	0.50	95.2	1.08
7	94.6	118,6	53,0	1,25	0,56		
8	93,3	107.3	45,9	1,15	0,49		
M=	87,4	115,6	44,9	1.32	0,51		

which all the measured clearances increased. In normal people we have observed with Prof. P. Govaerts that the slope of this line—calculated on a statistical basis—gives a correct value for the glomerular filtration rate when this is constant during the experiment. The same calculation on the basis of the data obtained during four successive periods

(3, 4, 5, 6) gives a reliable value close to the mean of the inulin clearances (7·97 ml. instead of 7·65 ml.). In this calculation, periods 1 and 2 have been neglected as the glycæmia is still too near the threshold, and likewise periods 7 and 8 because of a change in the filtration rate. As a consequence, a small Tm_G may be measured in this pathological condition; its

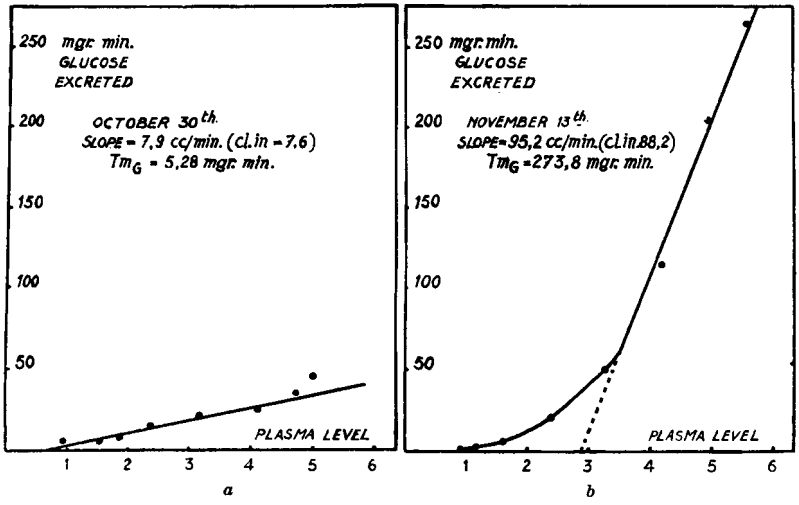

FIG. 4*a*. Relation between the amount of glucose excreted and the plasma level at an early stage of the recovery in a typical case of mercury nephropathy.

FIG. 4*b*. The same relation when the recovery is complete apart from a slight glycosuria.

value is not higher than 5·28 mg./minute when calculated from the glucose data only, as described by Govaerts and associates (1948); 5·5 mg. when calculated from the combined inulin and glucose data in the usual manner (Table VI). The mean threshold is very low, 66 mg./100 ml., whatever the mode of calculation.

As all the clearances measured (inulin, creatinine, urea and glomerular clearance of glucose) are the same, one may think

that their value is a correct measure of the glomerular filtration, at least in those nephrons contributing to the excretion of urine. However, these results do not rule out the possibility of some back-diffusion of tubular urine through partly-

Table VI

CALCULATION OF T_{MG} AND MEAN THRESHOLDS FROM INULIN AND GLUCOSE DATA IN A TYPICAL CASE OF MERCURY POISONING

PERIODS	BLOOD SUGAR mgr. %	FILTRATION RATE cc/min.	GLUCOSE EXCRETED mg/min.	GLUCOSE REABSORBED mg/min.
1st EXPERIMENT - OCTOBER 30th				
1	096	6.15	4,1	1.8
2	146	5.72	5.3	3.05
3	190	6,93	8,94	4,22
4	228	7,93	13.65	4.43
5	310	7,43	20,44	2,59
6	406	8,33	26,42	7.40
7	470	9.39	34.38	9.75
8	502	9,37	42,40	4,63
		M=8,23		Tmg=5,5
MEAN THRESHOLD = 0.66 gr. ‰				
2nd EXPERIMENT - NOVEMBER 13th				
1	95	83,8	0.8	78,79
2	114	81.6	1.14	91,88
3	162	82.5	4.27	129.38
4	238	100,3	20.40	218.30
5	332	77.0	49.90	205.74
6	421	81.2	113,40	228,45
7	498	93.5	203,90	261.73
8	562	93,3	264,18	260.16
		M=86.2		Tmg=239.0
MEAN THRESHOLD=2,77 gr. ‰				

destroyed epithelial walls. This mechanism has been observed by Richards (1929) in frogs. If this were true in our patient at the time of the experiment, one has to admit that water and solutes, as different as inulin and urea, return equally well through peritubular capillaries as they do through glomerular capillaries. It must also be pointed out that Richards' experiments were performed on anuric animals a short time after

mercury administration, while physiological data in man are obtained at a later stage of the disease when spontaneous diuresis has occurred.

If we are unable to rule out some back-diffusion of tubular urine in functional nephrons, it does not change the fact that an active reabsorption of glucose still takes place in these nephrons as shown by a measurable Tm_G. As the mean threshold is low, one must admit that this reabsorptive activity is small.

At the time of the second test the patient seemed to have completely recovered. Blood urea was normal (33 mg. per 100 ml.). Blood creatinine was still a little high (1·33 mg. per 100 ml.). Inulin clearance was 88·2 ml./minute. A small glycosuria persisted, not exceeding 1·6 g. daily.

Technical conditions were unchanged. The results are reported in Tables V and VI and in Fig. 4b.

The discriminating power of the proximal tubule was now clearly demonstrable. The mean clearance of endogenous creatinine was significantly higher than that of inulin, indicating that the tubules had recovered their ability to secrete creatinine in pathological conditions (cf. Miller, Leaf, Mamby and Miller, 1952). Urea clearance is much lower than the clearance of inulin. The ratio creatinine clearance/urea clearance is abnormally high, as already pointed out by P. Govaerts in 1938 (Table V).

The amount of glucose excreted is a linear function of glycæmia at high blood levels. The curvilinear shape of this relation around the threshold is more marked than usual (Fig. 4b). The minimal threshold is as low as 110 mg./100 ml. Tm_G (239 mg./minute) is not far from normal, also the glomerular filtration rate (88·2 ml./minute). The mean threshold is normal, 277 mg./100 ml. when calculated on both inulin and glucose data (Table VI); 287 mg./100 ml. when statistically established from the excretion curve of glucose alone (Fig. 4b).

When these data are plotted on a chart in the usual manner to draw the titration curve, it appears (Fig. 5) that the mean

zone—i.e. for loads between 0·6 and 1·0—has been insufficiently investigated. Therefore it would be unwise to calculate something that would be called a distribution curve. However, we think the facts are conclusive. There is no doubt that the renal glycosuria observed at this latter stage of a typical mercury nephropathy is characterized by a low minimal threshold, without parallel changes in the mean

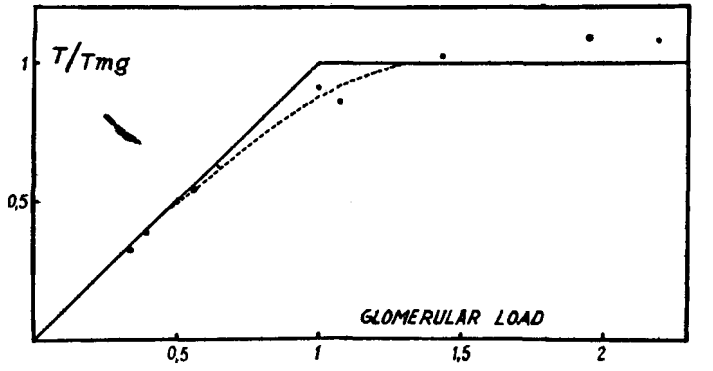

FIG. 5. Titration curve of glucose reabsorption in a case of mercury nephropathy when the recovery is nearly complete.

threshold and the Tm_G, and that this situation is quite different from that observed in true renal diabetes.

Conclusions

Rather than draw definite conclusions from clinical data obtained on a small number of patients, we think it more useful to point out some questions which, in our opinion, are open to discussion and probably require further experimental investigation.

1. We have admitted that the mean threshold is a valuable index when the tubular capacity to reabsorb glucose is to be compared in one patient with another. However, according to Homer Smith the threshold varies inversely with the filtration rate, Tm_G being constant. In man, we have never observed spontaneous variations in the filtration rate above the

limits of experimental error when the same experiment was repeated, for instance, in the afternoon of the same day. Therefore we feel that, in man, both the mean threshold and Tm_G are each a good index of the tubular reabsorptive capacity for glucose, when the experimental conditions are the same for all patients.

2. I expect that Dr. Reubi will discuss thoroughly the glycosuria of renal diabetes. Our opinion is that true renal diabetes—unrelated to any other renal disturbance—is characterized by decreased values of Tm_G, mean and minimal thresholds.

3. Concerning glycosuria in cyanide poisoning, we have pointed out that the easiest explanation is to suppose an unequal recovery of the individual capacity of the nephrons to reabsorb glucose. However, another mechanism seems quite possible. We should be interested to hear suggestions and remarks on this problem.

4. As far as mercury poisoning is concerned, the significance of a small Tm_G at a very early stage in the recovery period is open to discussion by morphologists and physiologists, who would probably disagree. Later on, the renal glycosuria may be imputed to the decreased minimal threshold only.

In our opinion an unequal recovery of the individual reabsorptive capacity of the tubules seems more admissible in mercury than in cyanide poisoning. As glomerular function and tubular function have been disturbed by mercury, it is quite possible that, according to the stage of their recovery, some nephrons still show a functional incapacity of their tubules when the filtration rates of their glomeruli are already normal. Therefore one may expect to find, at this stage of these pathological conditions, two types of nephrons when titration curves and distribution curves are drawn. Our experimental data bring some support to this view. However, the renal glycosuria in toxic nephropathies deserves further experimental investigation.

I wish to acknowledge the participation of many collaborators in the investigations which are summarized in this paper: Prof. P. Govaerts

first, and also Drs. J. Lebrun, M. Verbanck, Fr. Gregoire and Mrs. C. de Heinzelin.

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[Discussion of this paper was postponed until after the paper by Dr. Reubi.—Ed.]

THE KIDNEY
G. E. W. WOLSTENHOLME

GLUCOSE TITRATION IN RENAL GLYCOSURIA

F. C. REUBI

Introduction

RENAL glycosuria has been known for many years. In the past, this condition was considered to be due to a lowering of an ill-defined renal threshold. It is only recently that more elaborate methods of investigation have led clinical physiologists to a better understanding of its mechanism. These methods include the determination of the glomerular filtration rate in man (Smith, 1937) and of the tubular reabsorption of glucose (Shannon and Fisher, 1938). According to present concepts, renal glycosuria results from an imbalance between glomerular filtration and tubular reabsorption of glucose.

During the last ten years, various studies have indicated that glomerular filtration rate is normal in most renal-glycosuric patients (Friedman *et al.*, 1942; Steinitz, 1940). But there is still some disagreement as far as tubular reabsorption is concerned. Thus Friedman and co-workers (1942), and Hamburger and Barbizet (1948) found the reabsorption of glucose to increase with increasing blood sugar concentrations, the maximal rate of reabsorption (Tm_G) being within normal limits. On the other hand Steinitz (1940), Nielsen (1948), Govaerts and Lambert (1949) and Frank and Franco (1951) always found Tm_G values well below the normal range. Corcoran (1948) and more recently Bradley and his colleagues (1950) seem to assume the existence of two different types of renal glycosuria.

Our own studies were started in 1949. Five renal-glycosuric subjects were examined by means of the "titration" procedure devised by H. W. Smith (1943), i.e. glucose reabsorption was determined for levels of blood glucose varying over a wide range. Since this first publication (Reubi, 1950), our investigations could be extended to four additional cases. In

the present paper data on the nine subjects are included, the old and the new cases being treated together.

Methods

Glomerular filtration rate and tubular reabsorption of glucose were determined in the nine patients, effective renal plasma flow (PAH-clearance) in eight, Tm_{PAH} and extraction ratio of PAH in two. Glomerular filtration was estimated from the sodium thiosulphate clearance (Newman *et al.*, 1946), after preliminary work had shown that interference due to high glucose levels was negligible when thiosulphate concentration in blood was maintained between 30 and 40 mg. per cent. Glucose in plasma and urine was determined by the method of Shannon and Fisher (1938). Arterial blood only was used.

Great care was taken to avoid dead space effects and errors due to storage of glucose within the renal parenchyma. For that reason the determinations were not performed at increasing or decreasing glucose concentrations, but at nearly constant blood levels. The following procedure was used:—

An indwelling needle was placed in an antecubital vein and connected with an infusion flask containing fluids of various composition. Blood samples were collected through an indwelling needle from the femoral artery, urine samples through an inlying rubber catheter. Clearance measurements were performed in the usual manner, periods being started only after the sustaining infusion had run in for a sufficient time. In the titration experiments glomerular filtration and glucose reabsorption were first measured without glucose loading. The blood glucose was then progressively raised by infusing adequate amounts of glucose, while plasma thiosulphate was maintained at about 35 mg. per cent. The hyperglycæmia was achieved by giving a concentrated priming solution followed by a more dilute sustaining infusion. After the latter had been given for fifteen minutes, the bladder was emptied and profusely rinsed with water, urine and washings being discarded. Then (usually) two periods were

run at nearly constant blood sugar concentration. After their completion, blood glucose was raised again to a higher level and the same procedure was repeated. Technically unsatisfactory periods are not included in this study.

Results

Data on the nine patients including glomerular filtration rate (C_T), effective renal plasma flow (C_{PAH}), filtration fraction (F.F.), maximal excretory capacity for PAH (Tm_{PAH}), extraction ratio of PAH (E_{PAH}) and maximal reabsorptive capacity for glucose (Tm_G) are presented in Table I. The ratio Tm_G/C_T, normally equal to $2 \cdot 41 \pm 0 \cdot 35$ (Govaerts and Lambert, 1949), has been calculated in every instance. This ratio is decreased in cases one to six and normal in cases seven to nine, so that our patients can be divided into two distinct groups. The ratio T_G/C_T has also been calculated for every single period and plotted against the load C_TP_G. As can be seen from Figs. 1 and 2, the two groups behave differently.

Table I

RENAL FUNCTIONS IN NINE PATIENTS WITH RENAL GLYCOSURIA

Patient	*Sex*	C_T *cc./min.*	C_{PAH} *cc./min.*	*F.F.*	E_{PAH}	Tm_{PAH} *mg./min.*	Tm_G *mg./min.*	*Ratio* Tm_G/C_T
1. M.L.	m	111	443	0·25			204	1·74
2. H.E.	m	152	631	0·24			220	1·31
3. P.S.	m	134					219	1·50
4. F.L.	m	126	710	0·178		50·0	144	1·22
5. R.S.	m	116	620	0·19	0·845	50·5	153	1·40
6. P.K.	m	99	484	0·205			151	1·55
7. E.H.	m	108	394	0·274			275	2·30
8. K.B.	w	109	640	0·17			304	2·68
9. H.M.	m	84	333	0·252	0·878		253	2·55

The following symbols are used in this table: C_T, glomerular filtration rate. C_{PAH}, effective renal plasma flow. F.F., filtration fraction. E_{PAH}, extraction ratio for PAH. Tm_{PAH}, maximal rate of excretion for PAH. Tm_G, maximal rate of reabsorption for glucose. m, man. w, woman.

In the first six patients, the ratio T_G/C_T is around $1 \cdot 0$ at low blood glucose levels. With increasing loads it rises slightly but remains between $0 \cdot 8$ and $1 \cdot 5$ in all patients, up to a load of 600 mg./min. At very high levels of blood sugar it goes down to $0 \cdot 45$ in one subject and up to $1 \cdot 7$ in another, the

average of all determinations being about 1·0 (Fig. 1). From these data it can be concluded that in this group the reabsorption of glucose is markedly reduced whatever the load may be (at values ranging between 100 and more than 800 mg./min.).

In the second group (patients seven to nine), the ratio T_G/C_T is slightly above 1·0 at a load of 150 mg. per cent.

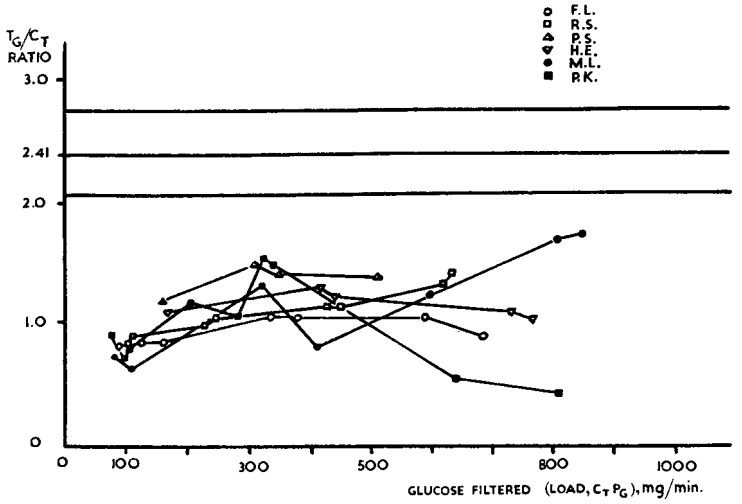

FIG. 1. Ratio T_G/C_T related to glucose load C_TP_G in six glycosuric patients. In this group the ratio T_G/C_T remains far below the normal range of $2·41 \pm 0·35$, even for high values of filtered glucose.

With increasing plasma concentrations of glucose it increases markedly, reaching normal values when the load becomes greater than 500 mg./min. in all patients and greater than 300 mg./min. in one. The highest ratios obtained in these three subjects are 2·68, 2·55 and 2·30 (Fig. 2). Therefore it appears that in this second group the tubular reabsorption of glucose is impaired only at low blood glucose levels. At high levels it is entirely normal.

Interpretation of the results

From the data presented above, it is quite clear that two different types of renal glycosuria may be encountered, one with low Tm_G, one with normal Tm_G.

In the first group, discrete renal functions other than glucose reabsorption may remain unaltered. In our six patients glomerular filtration is normal, varying between 99 and 152

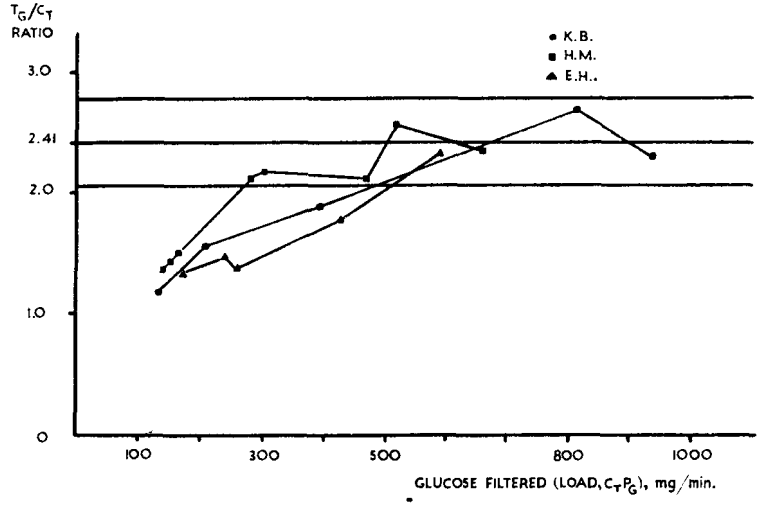

FIG. 2. Ratio T_G/C_T related to glucose load C_TP_G in three glycosuric patients. In this group the ratio T_G/C_T is below the normal range for low values of C_TP_G. But it increases rapidly and almost linearly when the amount of filtered glucose is raised, so that it becomes normal at high levels of blood glucose.

ml./min. PAH-clearance is normal in three and slightly reduced in two, filtration fraction being 0·25 and 0·24 in these two cases. Tm_{PAH} is normal in two cases with normal PAH-clearances and renal extraction of PAH is slightly reduced in one of them. These facts indicate that the excretion of PAH may be normal or somewhat impaired in patients of this group. We wish to emphasize that none of these individuals exhibited symptoms of renal disease. Except for their

glycosuria, they were perfectly healthy. All were males, eighteen to fifty-four years of age. One gave a familial history of glycosuria in an otherwise healthy brother.

As the glucose reabsorption is impaired at any level of blood sugar, this type of glycosuria seems therefore to be due to a relative impotency of the proximal tubules. It is not known whether the disturbance is anatomical or functional in nature. In the literature there are only two reports of postmortem findings in renal glycosuria. In one instance (Grote and Heilmann, 1935) the renal parenchyma was of normal appearance, in another case (Monasterio, 1939) there was marked flattening of the tubular epithelium. The latter's finding suggests that anatomical changes on a congenital basis might account for the glycosuria on some occasions. On the other hand Govaerts and Lambert (1949) believe that the tubules are anatomically normal, as they found in similar cases the reabsorption of phosphates to be within normal limits.

In the second group the interpretation of the data is more difficult. Only one patient has normal thiosulphate and PAH clearances. Another has normal filtration rate and decreased PAH clearance. The third, a patient with mild essential hypertension, exhibits moderate reduction of both clearances and normal extraction ratio for PAH; this patient showed also an abnormal glucose tolerance test, so that he may be considered to have not only a renal glycosuria but also some degree of true pancreatic diabetes. There were two males and one female in this group. They were forty-two, fifty-three and fifty-seven years old. No familial tendency could be detected.

In this group, the most important fact is that glucose reabsorption increases with the loading of the tubules. At least two mechanisms could possibly account for this remarkable finding.

The first is suggested by Shannon (1939) to explain the slight increase of T_G observed in normal dogs when blood glucose concentration is raised. According to Shannon, the transfer process of glucose may be described in accordance

with the law of mass action. It is suggested that glucose enters into reversible combination with some element in the tubule cells present in limited amount, and that the subsequent decomposition of this complex limits the rate of glucose transfer from urine to blood. The equation which relates the arterial plasma concentration *a* (mg. per cent), the rate of tubular reabsorption *Tr* (mg./min.) and the maximum rate of reabsorption *Tm* (mg./min.) in terms of this hypothesis is

$$K = \left(a - \frac{Tr}{V}\right)\left(\frac{Tm - Tr}{Tm}\right)$$

where K is the equilibrium constant and V the volume of glomerular filtrate in 100 ml./min.

The second mechanism possibly involved is suggested by Smith (1943) to explain the increase in T_G occurring with increasing blood glucose levels in hypertensive patients. According to Smith, this fact may be related to an increased dispersion in the glomerular and (or) tubular activity, the single tubules being saturated at different loads as a result of an anatomical or functional glomerular-tubular imbalance.

Our data have been reconsidered in the light of these two hypotheses, T_G being plotted against the load C_TP_G (Fig. 3).

From this graph it is apparent that our three subjects behave very similarly. In order to simplify the graphic representation, we have chosen for the whole group an ideal Tm_G of 300 mg./min. (Tm_G). If a smooth curve is drawn by visual approximation from the values (curve a), its shape is that of a straight line inflected at both ends. This experimental curve was first compared with theoretical tracings obtained from Shannon's formula*

$$C_TP_G = \frac{kC_TTm_G}{Tm_G - T_G} + T_G$$

In Fig. 3 two such curves are presented, which have been drawn by plotting the experimental values of T_G obtained

*In a previous publication (Reubi, 1951) Shannon's formulæ are inaccurately quoted.

in patient K.B. against the calculated values of C_TP_G. The constant k was determined before drawing the curve at two different points of the experimental tracing *a* from the equation

$$k = \left(P_G - \frac{T_G}{C_T}\right)\left(\frac{Tm_G - T_G}{Tm_G}\right)$$

These points were taken at $T_G=105$ ($k=1\cdot15$) and $T_G=205$ ($k=56\cdot5$). The curve based on $k=1\cdot15$ (dotted line *b*) lies

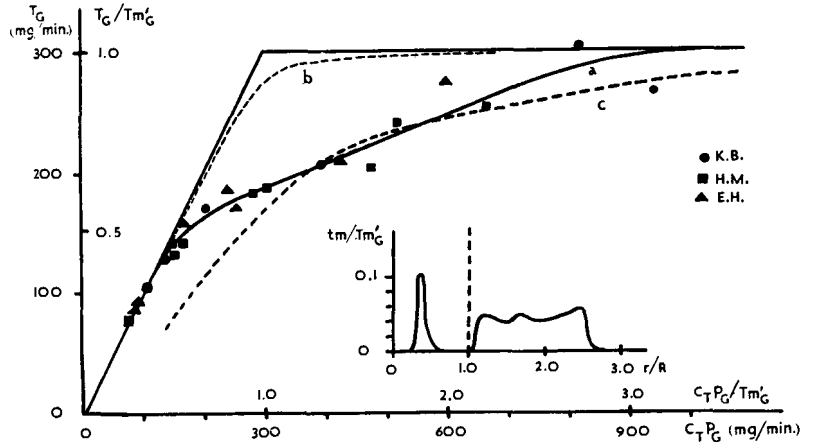

FIG. 3. Glucose reabsorbed T_G related to glucose filtered C_TP_G in the three glycosuric patients of the second group (see Fig. 2). The solid line *a* is the titration curve drawn from the experimental values. The dotted lines *b* and *c* are theoretical curves obtained from Shannon's equation. A frequency distribution curve which relates tm/Tm_G to r/R is also presented on this graph. It was calculated from the titration curve *a* and is typically bimodal, indicating the existence of two categories of nephrons, one with a low and one with a high relative tubular activity.

on the whole far above, and that based on $k=56\cdot5$ (dotted line *c*) far below, the experimental tracing *a*. The two lines *b* and *c* have in common with the curve *a* only the points chosen for calculating the constants k. It is easy to imagine intermediary positions between *b* and *c* for values of k lying between $1\cdot15$ and $56\cdot5$. It is also quite evident that none

of them would have a shape even approximating to the experimental curve *a*.

From this comparison it appears that the mechanism suggested by Shannon does not play a major rôle in the development of this type of glycosuria. At most it may be contributory.

Homer Smith's method of analysing the titration curves seems to provide a more satisfactory clue to this problem. Of course the mathematical analysis of our data *does not prove* that dispersion in the glomerular or tubular activity plays an exclusive part in producing the splay observed. But at least this splay *can be explained* on this basis.

Technical details of the mathematical procedure involved are omitted from this study, the method being accurately described in Smith's lecture (1943). In order to make the comparison with the diagrams of this author easier, we have not changed his notation. Instead of considering the dispersion in the tubular activity, we have, like Smith, plotted the relative glomerular activity r/R against the ratio tm/Tm_G. The frequency distribution curve obtained in this manner from our data is typically bimodal, indicating the existence of two distinct groups of nephrons. Instead of showing a single population with an average activity of 1·0, glycosuric kidneys of this type apparently consist of two kinds of nephrons, one with a relative activity around 0·5, the other with an activity comprised between 1·2 and 2·6. The existence of two groups of nephrons could already have been predicted without mathematical analysis from the inspection of the titration curve (straight line with two inflections).

We do not attach much quantitative significance to the frequency distribution curve, the calculation of which is subject to large errors. These errors are due to uncertainties in the exact location of the titration curve, particularly at the point of the higher inflection where the number of experimental data is small. But if quantitation is somewhat illusory, the main point of this study, i.e. the probable existence of two groups of nephrons, remains entirely valid.

In contrast to these findings, the frequency distribution curves obtained from glycosuric patients with low Tm_G exhibit unimodal appearance. This means that dispersion in this group is not great and that such kidneys are constituted with nephrons of an equally low tubular activity.

Let us consider now the possible causes of the increased dispersion in the group with normal Tm_G.

The glomerular-tubular imbalance is conceivably of congenital origin, anatomical or functional. There might be short tubules attached to large glomeruli and long tubules attached to small glomeruli. In this case one would expect some disturbance in the reabsorption of substances handled similarly by the kidney, for instance phosphates. This possibility has not been investigated in our three cases. The relatively low PAH clearance found in patient E.H. could be another expression of this anomaly.

The disturbance might also be functional in nature, the tubular enzymes being unevenly distributed among the proximal tubules. This hypothesis cannot be easily ruled out, but evidence in its favour is still lacking.

A further possibility is that the increased dispersion might result from acquired pathological changes in the kidney, tubules being more involved than the corresponding glomeruli, and conversely. Since the Tm_G are normal, compensatory hypertrophy would be considered to take place in some renal units, in addition to regressive changes. Such alterations have been described in chronic pyelonephritis and in the kidneys of true pancreatic diabetics. Titration curves with a marked splay are also reported by Smith (1943) in hypertensive patients. We believe that organic changes of this type may explain the glycosuria in our patient H.M.

Conclusions and Summary

From the results reported in this study, it is apparent that at least two different types of persistent renal glycosuria may occur (the transitory form reported by Lambert *et al.*, 1950, after cyanide poisoning is not considered here). The first

type, which we propose to call "true renal diabetes" is characterized by a marked glycosuria and a low Tm_G. It is a congenital anomaly and may show familial tendency. Its cause seems to be a relative impotency, organic or functional, of the proximal tubules, which are involved in an equal manner. The second type, which we should like to call "renal pseudodiabetes," is characterized by a moderate glycosuria, a low Tm_G at low blood glucose levels and a normal Tm_G at high levels. It may be congenital or acquired. It is probably due to an increased dispersion in the glomerular-tubular activity, anatomical or functional. Titration studies with glucose at different blood levels reveal the existence of two groups of nephrons, one with a low and one with a high relative tubular activity.

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DISCUSSION

ROBINSON: I wonder whether this very marked division of the nephrons into two groups, with apparently no overlap at all, has any correlation with the fact that there are two kidneys?

REUBI: I suppose one could answer this question very easily by separating the urines. As a matter of fact, we didn't do that.

DENT: When you said the second group was either congenital or acquired, what reason did you have for saying that? Is there any evidence that either of them was acquired?

REUBI: I think in the first group we have to deal with an anomalous condition, because none of the six patients had any symptoms of renal disease. In the second group we had three patients, one of whom was entirely normal apart from the glycosuria, in the second one could suspect some degree of nephrosclerosis consecutive to essential hypertension, and in the third we didn't know. I think glycosuria in the second group may occur on a congenital basis and also on an acquired basis, but I have no definite evidence that it is so. The dispersion may rest upon special anatomical findings, small glomeruli attached to large tubules, and conversely, or just be a consequence of a pathological arrangement of different units.

DENT: Have you any idea how long they had had it? You said it was a long-lasting glycosuria, but do you know, had some of them had it twenty years?

REUBI: Yes. Renal glycosuria was found in one patient twenty years ago, and we made our investigations on this case two years ago, so he was known for eighteen years at least to have had glycosuria and no other trouble.

BRADLEY: In the course of the initial work on glucose titration, we realized that a glomerulotubular imbalance of some kind might be responsible for renal diabetes. However over a ten-year period extending from 1941 to 1950, we were able to make studies on only five patients in Boston and New York. This paucity of subjects is attributable to the difficulty of persuading healthy people to come into the hospital for a long stay and subject themselves to the rigours of the titration procedure which requires, I believe, at least two separate evaluations of from ten to fifteen periods in each. Our data, like Dr. Reubi's, breaks down into two groups. In one, the glucose Tm is low and in the other it is normal or almost so. Two of our subjects fell into the first group. Both were men in middle life who gave *no* family history of the disorder. One of these may have had chronic pyelonephritis. The second group comprised three young people all of whom had had glycosuria since early childhood. In one an elaborate family history of renal glycosuria extending through three generations was obtained, and in the others the familial history was suggestive if not conclusive. In these three subjects the glucose Tm was normal or nearly so. Successful glucose titrations carried out in two of them revealed considerable "splay." Now I am not certain that we are entitled to set up distribution curves on the basis of these data obtained in separate

individuals. For a fairly uniform group, collected data may be treated in this manner, I believe, because statistical methods assist in minimizing the experimenter's preconceptions. Thus in the initial study, the smoothed titration curve for normal persons is probably valid, but I am no longer so certain about individual curves. The slope of the "smoothed" curve drawn through the points on the T/Tm to Load/Tm plots is all-important in arriving at a distribution. Here the personal equation must be important. All I am willing to say is that in these three subjects a glomerulotubular imbalance of some kind appears to be the basis of glycosuria. I am inclined to believe this is an anatomical defect rather than a biochemical fault but no evidence is available to permit a final conclusion.

REUBI: The familial tendency was in which group?

BRADLEY: It was in the group with normal or almost normal glucose Tm.

REUBI: That is just contrary to my findings, because the familial tendency was only evident in the first group, i.e., the group with low Tm_G.

RAASCHOU: If in a normal person you perform a titration curve with diodrast or PAH after giving the person the diuretic mersalyl (Salyrgan), you will find a great flattening of the titration curve as compared with the normal titration curve; at the same time Tm or Tm_{PAH} are greatly depressed (Brun, Hilden and Raaschou, 1947, *Acta pharmacol.*, 3, 1.).

REUBI: That would fit in with the findings of Dr. Lambert.

TAGGART: Dr. Reubi has been careful to point out that the difference may be either morphological or functional. Some of the observations from the dinitrophenol studies may bear on this point. We did glucose titrations in the dog before and after the administration of DNP; the data from the two experiments were practically identical. When similar titration studies with PAH were performed, very different results were obtained. In the normal dog, PAH Tm was reached when the Load/Tm ratio was only slightly above 1. After DNP, even the reduced Tm was reached only when the Load/Tm ratio exceeded 4. Now, presumably both of these functions are located in the proximal convoluted tubule, and, consequently, it is difficult to reconcile the two sets of data on a morphological basis.

REUBI: That might be a question of enzymes being unevenly distributed among the tubules.

OLIVER: Couldn't it be a matter of spatial distribution along the course of the tubule? I have seen processes, such as glucose absorption and glycogen storage occurring one high up and the other lower down in the proximal convolution. If this were the case, then you certainly could have two different functions in the proximal convolution disturbed independently in different ways.

It is also commonly observed that morphological damage to the proximal convolution occurs in certain places, while other regions remain structurally quite intact. In other words, the "proximal convolution" is neither structurally nor functionally a uniform entity.

BRADLEY: Wouldn't you expect, too, that there would be some defect in glucose metabolism in the cells in organs other than the kidneys?

TAGGART: All this hinges upon knowing something about the nature of glucose transfer, and I'm not sure that we do.

SANDERSON: Is the effect limited to glucose? Have these patients been studied for other possible metabolic reabsorption defects? It would be very odd if glucose were the only thing picked out.

BRADLEY: There is no evidence as far as I know that patients with renal glycosuria show disturbances in electrolyte metabolism, unless there is a profound glycosuria. Several cases of marked dehydration under special stress states have been reported.

EGGLETON: Dr. Reubi, were any of your patients true diabetes mellitus cases, with renal glycosuria?

REUBI: Yes, the last one of the second group was at the same time a true diabetic, because he had a pathological glucose tolerance test. But I think it was probably coincidental and not related.

EGGLETON: In the last number of the *Lancet*, there are a couple of papers by Gray*, who says that in his experience renal glycosuria is much more common with diabetes mellitus than on its own. He tested various plasma concentrations of glucose in, I think, five or six subjects, and they showed no Tm value at all. The amount reabsorbed just increased steadily on increasing load. I was specially interested, because I have found that is what the cat's kidney does.

REUBI: As far as I know there are two extensive studies published on this subject, whether renal glycosuria may be associated at the same time with a clear true diabetes. One is by Marble, from the Joslin Clinic, and he found no relationship with diabetes. The other one is by Roberts, an extensive study of 60 cases, and he found also no relationship. From my own experience, I couldn't tell you anything about it.

DE WARDENER: Can someone explain why, if you have a group with a normal Tm, in whom you explain the glycosuria because some nephrons have a low Tm, you do in fact get a normal Tm at higher glucose levels?

REUBI: It is because other nephrons have a very high activity, higher than normal; in our frequency distribution curve it was about 2·0, compared with the normal. Then while you measure the overall activity of the nephrons, you assume that you have not only a decrease in some units but an increase in others.

BERLINER: One of the assumptions upon which these curves of dispersion are based can be only an approximation. This is the assumption that each nephron loses no glucose until completely saturated. Therefore, although we may think that anatomical dispersion is a major factor, we still have to introduce the other factor—that of failure of the individual tubule to reabsorb its maximum amount until the amount in the tubule is somewhat in excess of that amount.

PITTS: In that connection I would like to ask Dr. Lambert how he defines his threshold of appearance. You can always find a little glucose in the urine if you try hard enough.

**Lancet*, 4th July, 1953.

LAMBERT: We just accepted that the threshold of appearance was attained when 1 mg./min. was excreted.

TAGGART: I have always been amazed by the fact that even the seriously damaged kidney is capable of reabsorbing practically all the glucose presented to it. With damage, many other functions may fail, but glucose reabsorption remains essentially complete.

OLIVER: As a slide I showed this morning demonstrated, the first part of the proximal convolution, which is known by direct observation to be responsible for glucose absorption, is rarely damaged by any means, so there is quite a good structural reason why in many tubular dysfunctions there should still be persistence of glucose absorption. Then the unabsorbed glucose passes on into the medullary segment, and we don't know how much is absorbed by this second half of the proximal convolution—perhaps a considerable amount, perhaps none. I would like to emphasize that I am speaking only of objectively demonstrable facts; the localizations I have referred to were made by isolating and dissecting nephrons and by taking samples of fluid from the renal tubule by means of a micropipette. These data are of a different order of credibility from a guess made on the appearances in histological section or a deductive assumption from a clearance experiment.

EGGLETON: I think even when you do have damage of that first part, glucose reabsorption may still be normal. I have been doing some experiments with licheniformen (one of the antibiotics, a histamine liberator) which has been shown specifically to hit the first part of the proximal tubule; and although in experiments in the cat it will depress phosphate reabsorption, glucose reabsorption is still completely normal. I had begun to wonder whether it is the mitochondria which have been upset. We know that most of the enzymes are present in or around that region, with the exception, I think, of the glycolysis enzyme system. I don't know if Prof. Baldwin is prepared to support me in public: apparently there is talk now of glucose reabsorption involving conversion to lactic acid *en route*.

BALDWIN: That was in intestinal absorption, but if it is true I think the same might hold for renal tubular reabsorption as well.

REUBI: If you consider toxic nephropathies like mercury poisoning, you have to take into account the fact that renal blood flow is so much decreased and probably filtration rate also decreased due to interstitial œdema and to obstruction of some tubules.

TAGGART: I'm sure that is true. What impresses me, however, is not the total quantity reabsorbed, but the fact that reabsorption is so complete.

RAASCHOU: If you stain kidney biopsies from acute renal failure or from a nephrotic syndrome according to Gomori's method you will find no phosphatase activity in the proximal convoluted tubules, but nevertheless these patients present no glycosuria.

TAGGART: Of course, this presumes that the alkaline phosphatase observed with Gomori's method has something to do with glucose reabsorption. I am not sure that we know this to be true, although it is

now quite generally accepted that the hexokinase-phosphatase sequence of reactions underlies glucose reabsorption. There are, however, several bits of evidence which make one wonder whether the scheme should be accepted so readily. Dr. Gerty Cori has recently pointed out that the proximal tubules of patients with the classical glycogen storage disease of von Gierke show an almost complete absence of the specific glucose-6-phosphatase; yet these individuals do not usually have glycosuria. A second bit of evidence comes from the work of Dratz and Handler, who injected radioactive orthophosphate intravenously into cats and subsequently followed the rate of glucose-6-phosphate turnover in the kidney. The turnover of glucose-6-phosphate appeared to be very slow and, moreover, was not significantly changed either by the administration of phlorhizin or by increasing the glucose load. Now, the authors were the first to point out that the glucose-6-phosphate recovered by them might not have been homogeneous—that a very small fraction, related to glucose transport, might have turned over at a very high rate, while the bulk of the glucose-6-phosphate, unrelated to transport, was turning over very slowly. Nevertheless, their findings suggest, at least to me, the very real possibility that glucose-6-phosphate has nothing to do with glucose transport in the kidney. Finally, I should like to return again to our studies with dinitrophenol. If glucose transport is driven by ATP via the hexokinase reaction, one would expect DNP to interfere, since its effect is to diminish the supply of ATP. Such was not the case. Taken individually, these various bits of evidence prove nothing conclusively, but taken together they suggest that we actually know very little about the manner in which glucose is reabsorbed by the tubules.

BLACK: Several times today I think we have been on the brink of mentioning intermittency of function, either of glomeruli or of individual capillary loops, and I suppose we have been frightened to do so because that concept was solemnly buried by Homer Smith about ten years ago in his "Lectures on the kidney."* But I suppose it is not beyond all conjecture to say that it may have been buried alive. I would like to resurrect it if only to stimulate discussion. I suppose that Dr. Reubi's "two populations" could conceivably represent glomerular segregation rather than differences in tubular functions. I wonder whether he has any observation on the influence, say, of caffeine diuretics, or even of artificial pyrexia. If this were a glomerular mechanism rather than a tubular one and some glomeruli were inactive or came into action at different glucose loads, if one were to give a caffeine diuretic then I think one might increase the number of active glomeruli. Have you any observations on those lines?

REUBI: No, I didn't do that because the Tm_G are anyhow normal in the second group, and if they are normal, you cannot assume that the dispersion may result from a lower filtration rate due to intermittency of function. If some glomeruli are inactive, this would decrease the filtration rate and might increase the dispersion; but this would not bring about any glycosuria. All our patients were glycosuric at low

*Smith, H. W. (1943). Lectures on the kidney. Kansas.

levels, and had normal Tm_G's. In my opinion, these findings cannot be visualized on the basis of functional intermittency.

MERRILL: This explanation has been exhumed by Handley who found the filtration rate and Tm_G dropped when his dogs were dehydrated by mercurial diuretics, and conversely, when they were salt-loaded, filtration rate and Tm_G increased.

PITTS: Well, I suppose we can say that we threw one more spade of earth on the grave, because we found exactly the opposite of what Handley found.

OLIVER: We have been doing some simple experiments the results of which are not clear to us, and if someone could offer an explanation it would be very helpful. When we inject small amounts of hæmoglobin into a rat and look at his nephrons after immediate fixation in alcohol, which localizes the hæmoglobin quite sharply, we observe in dissected specimens that the hæmoglobin is distributed only to some of them, in fact to a very few if we have killed the rat within five minutes. Some nephrons have only a part of the proximal convolution filled with hæmoglobin; in others we can see it in the glomerular capsule and perhaps more of it in an isolated stretch of the convolution lower down in the tubule. If we allow the experiment to continue long enough, then in the end this irregularity disappears and more nephrons become completely filled. The only thing I can think of to explain these observations is that the hæmoglobin is passed through the glomeruli in intermittent squirts. Can anyone offer any other explanation of how such an appearance could come about?

BRADLEY: That doesn't show intermittency of filtration, though, because the empty spaces are not empty, they are filled with filtrate.

OLIVER: I mean intermittency of filtration of hæmoglobin; there is apparently continuous filtration of water.

BRADLEY: Yes, but that doesn't imply intermittency in the usual sense.

OLIVER: That is what makes it so mysterious, doesn't it? That in apparently continuous filtration, certain substances in the filtrate pass only occasionally?

BRADLEY: No, I don't think so. This observation agrees with results reported years ago by Khanolkar. He regarded a similar spotty distribution of india ink injected into the renal artery as evidence for intermittency. However, H. L. White subsequently showed that non-uniform dispersion of india ink within the stream of blood perfusing the kidney could account for the phenomenon. Some glomeruli are perfused by blood derived chiefly from the peripheral portion of the arterial stream which contains little or no india ink, and others by blood derived chiefly from the axial stream holding the ink particles in high concentration. As a result, irregular staining was noted. This seems also to explain how hæmoglobin-containing blood might be distributed irregularly through the kidney and even intermittently to any single glomerulus. The hæmodynamic factors are quite complex and it is not unlikely that eddy currents and other discontinuities may occur from time to time.

OLIVER: After intravenous injection of hæmoglobin one would suppose that the blood entering the main renal artery was a perfect mixture.

Part III—Renal Share in the Regulation of Acid-base Balance

RENAL ACID-BASE CONTROL AND CELL PHYSIOLOGY

J. R. ROBINSON

Introduction

THE excretion of acids and bases by the kidney has been studied mainly on whole animals, and it may be of interest to summarize what has been learned by seeing how far it can be explained by physiological properties of the cells which do the work.

Gamble (1937, 1947) and Pitts (1945) showed how weak acids can be excreted partly or wholly as free acid according to their *p*K values, so that these physical constants determine how much base can be restored to the blood by excreting urine of any *p*H. Acids with *p*K values more than one unit below the *p*H of the urine must always be accompanied by more than nine-tenths of their equivalent of base. This need not all be fixed base, however, because ammonia formed within the kidneys provides ammonium ions which were not present in the glomerular filtrate and which allow an equivalent amount of fixed base to be reabsorbed. It follows that the sum of the titratable acid and the ammonium in the urine is equal to the amount of fixed base conserved by the kidneys.

The Excretion of Alkaline Urine

Since alkaline urine can only be excreted with the loss of fixed base from the body, it is fortunate that alkalosis is a rarer contingency than acidosis, which our dietary habits make an everyday affair for most of us. Excess cations in alkaline urine are balanced by bicarbonate, the anion of a volatile acid, just as excess anions in acid urine may be balanced by ammonium, the cation of a volatile base. Pitts, Ayer and Schiess (1949) showed that ordinarily, when the

composition of the plasma controls respiration, bicarbonate is excreted freely in the urine when its concentration in the plasma rises above 27 to 28 mEq./l., and the virtual disappearance of bicarbonate from the urine when its concentration in the plasma falls below this "threshold" has been taken to show how efficiently the kidney can reabsorb this anion (Pitts, 1950). It is not easy to visualize the mechanism of this process, however, for not only is bicarbonate presented to the cells in the glomerular filtrate, but CO_2 is generated inside them as a by-product of respiration, and they contain carbonic anhydrase which rapidly converts this into carbonic acid, an intracellular source of bicarbonate ions. Moreover, the usual concentration (27–28 mEq./l.) of bicarbonate in the plasma does not indicate a true threshold, for bicarbonate is freely excreted in alkaline urine during forced breathing when its concentration in the plasma is much lower. The clue to this discrepancy was given by Pitts (1950, footnote) in the words "The kidney stabilizes the concentration of base in the body fluids at a level some 25–27 mEq./l. above that of the sum of all the fixed non-volatile anions." When bicarbonate is thus regarded as a make-weight and not as something actively handled by the tubules, its excretion during respiratory alkalosis seems less paradoxical. As the *p*H of the plasma rises, more cations are balanced by the ionization of plasma proteins, but since the latter do not enter the glomerular filtrate, the fluid in the tubules will tend to contain an excess of cations and to become alkaline if the reabsorption of these cations is not increased. Carbonic acid produced in the cells will ensure that any excess of unreabsorbed cations over unreabsorbed anions enters the urine as bicarbonate, but this bicarbonate need not have come from the depleted plasma. Like the bicarbonate in the saliva (Sand, 1951) and the ammonium in acid urine, it may have been synthesized in the epithelial cells. The essential act of the kidney in elaborating an alkaline urine is to refrain from reabsorbing cations whilst the reabsorption of fixed anions continues or is enhanced.

The Excretion of Acid and Ammonia

Pitts, Lotspeich, Schiess and Ayer (1948) concluded that the acidification of the urine depends upon active secretion of hydrogen ions by the tubules because other mechanisms could not account for observed rates of acid excretion. Active transport seems to be involved because a urinary *p*H of 4·6 when the *p*H of the plasma is 7·4 implies a U/P concentration ratio for H ions of the order of 600 : 1. The H ions probably come from H_2CO_3 formed within the cells from metabolic CO_2, and the bicarbonate ions produced at the same time presumably accompany reabsorbed base into the plasma.

Only acid urines contain much ammonium, and the relation of ammonium content to *p*H has suggested that the acidity of the fluid in the lumen may be the stimulus which leads the cells lining the tubules to produce ammonia (Briggs, 1934). I found (Robinson, 1954) that isolated kidney slices from rats produced ammonia *in vitro* at about the same rate as Ferguson (1951) had found for the kidneys of intact rats of the same strain. The slices did not form much ammonia in neutral media, but the rate of ammonia formation increased quite rapidly with falling *p*H, provided that the slices continued to respire satisfactorily. Pitts (1950) was probably correct, therefore, to suggest that the "secretion" of ammonia might occur by passive diffusion out of the cells when the fluid in the tubular lumen became acid, even though the physiological response does not reflect the optimum *p*H of the intracellular enzymes. If the reactions by which ammonia is generated are reversible, the rate at which it leaves the cells should be governed by external *p*H (which affects the gradient across the membrane by fixing the activity of ammonium outside the cells) rather than by the capacity of the ammonia-forming enzymes, although this would become a rate-limiting factor if the substrates were present in high enough concentrations to saturate the enzymes.

The output of ammonium, even in urines of the same pH, has been found (e.g. Pitts, 1948), though not invariably

(Ryberg, 1948), to increase during the first hours or days of a sustained acidosis. This adaptation might be explained by a more efficient secretion either of acid or of ammonia. Adaptive changes in the activity of the enzymes which form ammonia have been demonstrated in rats by Davies and Yudkin (1952) in experiments which lasted several months. Faster formation of ammonia would allow more H ions to be added to the tubular fluid in a given time without further lowering its *p*H. On the other hand, more efficient secretion of H ions, producing a lower initial *p*H in the tubular fluid, would lead to more rapid diffusion of ammonia from the cells without an increase in the activity of their enzymes; urine of a given final *p*H would have to contain more ammonium because the tubular fluid from which it was formed had been more acid before ammonia was added to it. Rival theories are commonly both partly correct, and both processes might be involved in the response to sustained acidosis. In either case, if the appearance of ammonium in the urine is a consequence of the acidity of the fluid in the distal tubules, H ions must first be secreted before they can be replaced by ammonium. Hence the amount of H secreted is not merely equivalent to the titratable acid of the urine, but to the sum of the titratable acid and the ammonium, and so to the whole amount of fixed base conserved by the kidneys.

This appears to strengthen the case for regarding the primary process in the acidification of the urine as active secretion of H ions, which are later exchangeable for other cations. But when we consider the mechanism of this secretion and try to relate it to the physiological properties of cells, we have to ask which is the cart and which the horse?—whether H ions are secreted actively or only "secreted"? Does active secretion of H ions allow sodium to be reabsorbed, or is active reabsorption of sodium the primary process which leads H ions to be "secreted" by a process as passive as that whereby acidity leads to "secretion" of ammonia and alkalinity to "secretion" of bicarbonate?

The Mechanism of Acid Excretion

Most cells must transport sodium actively. They contain little of this ion and much larger amounts of potassium, although they are bathed in fluids which are rich in sodium. Tracer studies have shown that their membranes are permeable to both cations, and active transport of sodium outwards across the membrane may account for the large concentration of potassium as well as for the small concentration of sodium inside cells (Hodgkin, 1951). In nerves and other excitable tissues the separation of ions which the sodium pumps maintain across the cell membrane allows impulses to be conducted along it, but this common mechanism may subserve other functions in other cells. If sodium were actively extruded at the striated basal ends of the cells lining the renal tubules, but could diffuse in from the lumen at the opposite pole, it would be transported continuously from the glomerular filtrate back to the blood. Wesson and Anslow (1948) attributed the obligatory phase of reabsorption of water and salts in the proximal tubules to active reabsorption of sodium, and Homer Smith (1952) has suggested further that active reabsorption of sodium in the distal tubules provides "osmotically free water" to dilute the urine when it is hypotonic. Can the same process account for the secretion of acid?

In so far as active reabsorption of sodium tends to leave free anions in the lumen it must set up a gradient of electrical potential which will both limit the rate of reabsorption of sodium and tend to draw other cations out of the cells into the lumen. H ions are not abundant inside the cells, but their mobility is so high, and they can so readily be generated by the ionization of H_2CO_3 formed from metabolic CO_2 that active reabsorption of sodium might lead to its replacement in the tubules by H ions from the cells without the intervention of a separate process actively secreting H ions. Inhibition of carbonic anhydrase, which is known to prevent the acidification of the urine (Pitts and Alexander, 1945) would do so by reducing the supply of available H ions. Even though the sodium pumps continued to operate, the lack of H ions for

exchange would hinder the reabsorption of sodium so that more would appear in the urine, whilst the gradient tending to withdraw cations from the cells would persist, and might now lead to a loss of potassium, which is the most abundant cation inside the cells. Thus the tubular excretion of potassium which was observed (Berliner, Kennedy and Orloff, 1951; Berliner, 1952) when carbonic anhydrase was inhibited during acidosis might also be a consequence of the active reabsorption of sodium. When a mercurial diuretic was administered after the inhibition of carbonic anhydrase, still more sodium appeared in the urine and the apparent tubular excretion of potassium ceased, as would be expected if the mercurial diuretic had interfered with the reabsorption of sodium. Thus instead of supposing that potassium and hydrogen compete for a common channel of excretion, we might picture the acidification of the urine as the normal consequence of active reabsorption of sodium, and the excretion of potassium from the cells lining the tubules as an abnormal consequence which may occur under unphysiological conditions when hydrogen ions are not freely available for exchange. Ammonium does not appear to be exchanged directly for sodium, perhaps because it moves across the cell membrane as ammonia, which would not be influenced by a gradient of electrical potential, but would be fixed by a fluid made acid by prior movement of H ions.

It greatly simplifies our picture of the functions of the renal tubules to attribute the excretion of acid, of ammonia, and sometimes also of potassium, to one primary active process, especially to one like the transport of sodium, which seems to be common to most mammalian cells. The old statement that ammonia and titratable acid together measure the success of the kidney as a conservator of base acquires new force and becomes less empirical if conservation of base is regarded as the primary process. Such a general dependence upon transport of sodium might explain the inability of adrenalectomized rats to excrete acid and ammonia (Sartorius and Pitts, quoted by Pitts, 1950), the diminished

excretion of ammonia by adrenalectomized dogs (Harris, Hartmann, Rolf and White, 1952) and the acidosis which human patients with Addison's disease may develop because of deficient renal formation of ammonia (cf. Jiménez-Diáz, 1936). It might also explain the observation of McCance and Widdowson (1936) that forced breathing did not make the urine alkaline during an experimentally induced deficiency of sodium, and the fact that patients in whom alkalosis is accompanied by sodium depletion may pass acid urine (McCance, 1936). Suprarenal cortical activity is presumably increased in both these cases and this may be why the reduction in renal tubular reabsorption of sodium which would be required to make the urine alkaline does not occur. A gradual response of the kidneys to adrenocortical hormones, released as the body's stores of extracellular base become depleted, might also account for the adaptive increase in ammonia excretion during sustained acidosis if, as has been suggested, formation of ammonia is secondary to acidification, and this in turn is secondary to the reabsorption of sodium.

Finally, if one active, energy-requiring process can be made to explain the renal control of acid-base balance, it may become easier to tackle the important and quite unsolved problem of how the activity of the kidney is adjusted to the requirements of the body as a whole.

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DISCUSSION

Stanbury: Dr. Robinson, I wonder whether you are by any chance accepting a little too readily the concept that in the maintenance of the normal intracellular cationic composition the prime movement is an active extrusion of sodium? I appreciate that this is the classical and currently accepted idea, which is strongly supported by Hodgkin's mathematical treatments of the behaviour of nerve fibre; but on the other hand Shanes, using entirely similar material, has arrived at the opposite conclusion that probably potassium is the prime mover. I raise this point particularly because you invoked the possibility that the mitochondria at the base of the renal tubular cell were in fact the mechanisms actively responsible for that process. During the last year Mudge and I (*Proc. Soc. exp. Biol., N.Y.* 1953, **82**, 675) have had the opportunity to study the electrolyte metabolism of mitochondrial preparations. We found that washed mitochondria did in fact contain a moiety of sodium and potassium, each of which appeared to be non-diffusible, or, if you want an alternative but dangerous term, to be "bound." With an actively metabolizing system we could get clear cut evidence of a metabolically conditioned turnover of the fixed potassium of the mitochondria, but the fixed sodium of the mitochondria did not appear to exchange. In other words, during metabolic activity we could label the "bound" potassium of the mitochondrial particle with radioactive potassium; but, when offered the opportunity to exchange with radioactive sodium, the apparently "bound" sodium of the mitochondria did not take up the tracer. These observations make us wonder if the active factor in the maintenance of cellular composition is a primary active uptake of potassium. I think you will agree that a primary movement of either sodium or potassium could condition movement of the other. Primary sodium movement can account for potassium changes; is it not equally possible that the active establishment of a potassium gradient would simultaneously extrude sodium?

ROBINSON: It could, and it happens at the other end of the cell, presumably. I didn't wish to go into the mechanism, but if you have an active movement of sodium in the direction from tubule towards blood, which is an active reabsorption of sodium, I don't think it really matters a great deal whether it is due to pushing sodium at one end of the cell or pulling potassium at the other. I was really only concerned with the question that if you have an active movement of sodium, which apparently you have, experimentally, it might account for these other things too.

STANBURY: Yes, but you are regarding the sodium movement as primary; if, however, there is not in fact a primary sodium movement, you have to think of two alternative active mechanisms: a potassium secreting mechanism or a hydrion secreting mechanism, of which perhaps our mitochondrial experiments provide evidence for one, and R. E. Davies' experiments evidence, at least in the gastric mucosa, for the other.

BERLINER: I wonder, in considering the movements of these ions, whether we can actually separate the movement of sodium ion in one direction from the movement of a counter-ion in the other direction, so as to be in a position to say that one or the other is primary. It would appear that they must occur equally and simultaneously. Whatever the mechanism of transport of sodium may actually be, it would seem to require the displacement of an equal charge of the same sign. We cannot say that the tubules take up a sodium ion and *consequently* lose hydrogen ion; we have to say that *as* the tubule takes up sodium it loses hydrogen. Depending on how one chooses to look at it, that can be regarded as a primary movement of sodium or a primary movement of hydrogen ion, because they are inseparable. I would agree, however, that the regulatory mechanism seems, in most instances, to be based on sodium rather than hydrogen ion.

LEWIS: Dr. Robinson, can you elaborate the statement you made that patients with Addison's disease do not form ammonia?

ROBINSON: I had read it, and I had been told that sometimes they cannot.

PITTS: Dr. Sartorius investigated the capacity of adrenalectomized rats to produce ammonia and titratable acid. The adrenalectomized animal can produce ammonia. However, its response to an acid stress, such as the oral administration of ammonium chloride, is not as good as that of the normal animal. An adrenalectomized animal, supplied with an excess of sodium chloride, tolerates very well a considerable acid load. However, if the animal is placed on a salt free diet and given ammonium chloride, survival is short. In contrast, the normal animal survives the combined threat of acid loading and salt restriction without difficulty. Thus the adrenalectomized animal can produce ammonia and titratable acid in response to an acid load, but not to the same extent as can a normal animal.

BLACK: In the discussion of Fanconi syndrome yesterday, it was mentioned that they could form ammonia perfectly well but not an acid urine. I don't know if that has any significance—I mean if the

sodium process is primary, then it is difficult to see why those particular patients should be able to react by forming ammonia and not by excreting hydrogen ion.

ROBINSON: Yes, if the ammonia which is formed in Fanconi urine has the same source and the same mechanism of formation, which I rather assumed it might not.

MERRILL: I wonder if Dr. Robinson or anybody else has any information specifically about how the adrenal steroids affect the mechanism by which ammonia is produced. What happens to glutaminase activity in steroid administration?

BERLINER: White and his associates have studied glutaminase activity in adrenalectomized dogs, and found it completely normal. I have assumed, from these observations, that whatever defect there may be in the excretion of ammonia in the adrenalectomized animal it is associated with failure to acidify the urine to the same extent as in the normal.

BALDWIN: There is one thing which I find peculiarly fascinating about ammonia production by the kidney; I think it would be very interesting if somebody would see what happens in adrenalectomized fish. The fish does not have the advantage which we enjoy of being able to turn the waste ammonia or amino groups arising from protein metabolism into urea and I should like to know how the fish deals with the acid-base balance problems which we have to deal with. It cannot, presumably, call on ammonia to neutralize excess acid in the way that we can, because it is maximally producing ammonia the whole time. If one could trace the whole ammonia excretion story from the teleost fish, through amphibia and so on up to the mammal, it might throw a great deal of light on which, if any one, of these things is the controlling factor, or perhaps that it is really the same sort of story as one has in the chloride shift phenomenon. I think Dr. Berliner was suggesting that it is something like this chloride shift phenomenon, only you are dealing now with positively charged instead of negatively charged ions.

There is one other thing that comes to my mind whenever a discussion of this sort appears, and that is how extraordinarily fortunate it is, physiologically speaking, that bicarbonate and chloride ions seem to have exactly the same properties and you can exchange one for the other to a very large extent. If chloride ions and bicarbonate ions did not have the properties they do have, none of these things, neither the chloride shift nor regulation of acidity of urine would be possible.

BRADLEY: Are you implying that the excretion of ammonia in animals that excrete most of their nitrogen as ammonia is the same as the mechanism by which ammonia is secreted in acid-base regulation?

BALDWIN: I am not suggesting anything; I just do not know.

MCCANCE: Is anything known about the concentration of ammonia in the fish's blood?

BALDWIN: It is said to be extremely low. The difficulty with all these blood ammonia determinations is that the moment the blood is shed ammonia rapidly appears from somewhere. I think probably ammonia

does not circulate in fish blood as such any more than it does in ours; it probably goes around as glutamine.

BERLINER: In the marine fish, it appears that the regulation of acid-base balance is largely a function of the gills. The urine of these fish has an almost constant *p*H which cannot be modified by any of the measures which have been tried, including very large amounts of bicarbonate, carbonic anhydrase inhibitors, and acid loads. The *p*H of the urine does not vary, yet the animal gets over the acidosis or alkalosis quite promptly, and one must presume that this regulation is performed by the gills.

BALDWIN: As soon as it leaves the water the kidney has to take over the whole job. That brings us back to the idea that the kidney is primarily not an excretory organ. Its primary job is this business of acid-base balance and the regulation of the intake and output of sodium and potassium ions and so forth. The excretory functions are things which it has acquired secondarily. That is a point of view that perhaps we ought to keep in mind, so that we should study the secretory functions as secondary things. I am convinced myself that we should obtain a great deal of valuable information if we were to tackle the kidneys of the lower aquatic forms, the so-called lower forms of vertebrate life, and try to trace the development of these things. From the structural point of view, of course, quite a lot of work has been done, but physiologically very little.

THE KIDNEY
G. E. W. WOLSTENHOLME

REGULATION OF THE CONTENT OF BICARBONATE BOUND BASE IN BODY FLUIDS

ROBERT F. PITTS, W. JAMES SULLIVAN and PHILIP J. DORMAN

THE bicarbonate-carbonic acid buffer system plays a dominant rôle in determining the hydrogen ion concentration of the body fluids. This dominance derives not from any especially favourable physiochemical properties of the buffer combination but from the existence of two homeostatic mechanisms which precisely regulate the concentrations of the two buffer components. A respiratory mechanism stabilizes the concentration of carbonic acid within limits of 1·2 and 1·4 mEq./l. A renal mechanism stabilizes the concentration of bicarbonate bound base within limits of 24 and 28 mEq./l. Together these two mechanisms account for the maintenance of the blandly alkaline reaction of the body fluids so necessary for proper functioning of body cells.

In the process of stabilizing the concentration of bicarbonate bound base, the kidneys play two distinct yet interrelated rôles. The first is that of conservation of existing stores of base. The renal tubules, presented with bicarbonate bound base in the glomerular filtrate, reabsorb it, almost completely when concentration is normal or low, somewhat less completely when concentration is elevated. Under these latter circumstances, excretion gradually rids the body of the excess and restores concentration to normal. The second is that of restoration of bicarbonate bound base reserves depleted in the neutralization of metabolic acid within the body. Renal excretion of titratable acid and ammonia constitute the restorative process.

I shall limit my discussion to the first of these renal regulatory rôles, namely that of conservation or excretion of existing reserves of bicarbonate bound base.

The nature and the magnitude of the renal problem of conserving existing base reserves are illustrated in the first two horizontal columns of Table I. Roughly 200 litres of glomerular filtrate are formed in the kidneys of man each day. Normally each litre of filtrate contains about 25 mEq. of

Table I

The nature and magnitude of the renal processes involved in conservation and excretion of bicarbonate bound base in man

	RENAL CONSERVATION AND EXCRETION OF BICARBONATE BOUND BASE				
	GLOMERULAR FILTRATE	PLASMA $BHCO_3$	$BHCO_3$		
			Filtered	Excreted	Reabsorbed
	Liters/Day	mEq./Liter	mEq./Day		
NORMAL	200	25	5000	1-2	4998
ACIDOSIS	200	15	3000	0	3000
ALKALI INGESTION	200	26	5200	202	4998

bicarbonate bound base. Accordingly some 5,000 mEq. enter the renal tubules every twenty-four hours. A litre of urine of *p*H 6·0 contains but trace amounts of bicarbonate, namely 1 or 2 mEq. Hence, 4,998 mEq or 99·9 per cent of the quantity filtered are reabsorbed per day. In acidosis, in consequence of the reduction in plasma concentration, less than normal quantities of bicarbonate bound base are delivered into the renal tubules. Even the trace amounts normally excreted are conserved under these conditions.

The problem of excretion of an excess of bicarbonate bound base is illustrated in the lowest horizontal column of this Table. If an individual in normal acid base balance were to ingest 17 g., thus 200 mEq., of sodium bicarbonate, an increase

in plasma concentration of only 1 mEq/l. above the normal would deliver into the filtrate in a day's time this extra 200 mEq. of bicarbonate bound base. Were tubular reabsorption to be maintained at the normal level, the excess ingested could readily be eliminated. These data are hypothetical and serve merely to illustrate principles. How closely do they agree with experimental fact?

In the experiment summarized in Table II, the plasma bicarbonate concentration of a normal adult male subject

Table II

An experiment on a normal human subject which illustrates renal tubular conservation of bicarbonate bound base when plasma level is subnormal, and frank renal excretion with elevation of plasma concentration to supernormal values. Plasma bicarbonate bound base was reduced by the ingestion of a small dose of ammonium chloride on the preceding day. It was elevated by the intravenous infusion of sodium bicarbonate. From Pitts, Ayer and Schiess (1949)

Glomerular Filtration Rate	Plasma		Urine			Bicarbonate				
	$BHCO_3$	pH	FLOW	$BHCO_3$	pH	Filtered	Excreted	Reabsorbed	Excreted	Reabsorbed
ml/min.	mM/L.		ml/min.	mM/L.		millimols per minute			millimols per 100 ml. glomerular filtrate	
123	20.4	7.39	4.73	0.031	4.48	2.51	0.0001	2.51	0.0001	2.04
124	20.4	7.39	4.76	0.024	4.44	2.53	0.0001	2.53	0.0001	2.04
126	24.3	7.43	7.08	1.30	5.95	3.06	0.008	3.05	0.007	2.42
122	25.8	7.44	4.33	12.2	6.78	3.15	0.053	3.10	0.044	2.54
128	27.6	7.48	4.94	33.1	7.21	3.53	0.164	3.37	0.128	2.63
134	32.6	7.51	8.46	83.0	7.58	4.37	0.702	3.67	0.524	2.74
132	35.9	7.54	11.1	99.2	7.64	4.74	1.10	3.64	0.833	2.76
136	38.2	7.56	13.3	105.0	7.65	5.20	1.40	3.80	1.03	2.79

was reduced moderately to a level of 20·4 mEq./l. by the ingestion of a small dose of ammonium chloride on the preceding day (Pitts, Ayer and Schiess, 1949). The urine formed was highly acid, having a *p*H of 4·48 and 4·44 during the two control periods. The quantity of bicarbonate bound base which entered the glomerular filtrate each minute, that is, the quantity filtered, was calculated as the product of the rate of glomerular filtration and the plasma bicarbonate concentration and amounted to 2·51 and 2·53 mEq./min.

during the initial two periods. These data have not been corrected for the Donnan effect, which presumably conditions the distribution of ions across the glomerular capillary membranes. Although neglect of this correction introduces but little error, we have applied it in our more recent work. Since negligible quantities of bicarbonate bound base were excreted, these same amounts, namely 2·51 and 2·53 mEq. were reabsorbed each minute. For reasons which will become more evident later, it is reasonable to express the quantity reabsorbed as mEq./100 ml. of glomerular filtrate. During the control periods, absorption in these terms amounted to 2·04 mEq.

Sodium bicarbonate was then infused to raise the plasma bicarbonate concentration gradually from 24·3 to 38·2 mEq./l. Until the plasma level reached 27·6 mEq., the urine remained acid and the rate of excretion of bicarbonate was insignificant. However, at a level of 27 mEq./l., frank excretion of bicarbonate occurred and reabsorption per 100 ml. of filtrate stabilized at a value of 2·6 to 2·8 mEq. All in excess of this quantity was excreted.

The relationship between the quantities of bicarbonate bound base reabsorbed and excreted and the plasma level of bicarbonate is illustrated in Fig. 1, which graphically summarizes the early work of Pitts and Lotspeich (1946), on the dog. Plasma level was reduced by the administration of ammonium chloride on the day preceding an experiment. It was elevated by infusing sodium bicarbonate during the course of the experiment. At plasma levels of 10 to 20 mEq./l. all of the bicarbonate bound base filtered at the glomeruli was reabsorbed; none was excreted. Over a range of plasma levels of 22 to 28 mEq./l., frank excretion was initiated. Above 28 mEq./l. a relatively constant rate of reabsorption was attained, amounting on an average to 2·6 mEq./100 ml. of filtrate. All filtered in excess of this limited quantity was excreted. It is evident that the renal plasma threshold for bicarbonate bound base lies within the range of 22 and 28 mEq./l. in the normal dog.

As is evident from Fig. 2, a similar threshold relationship exists in man, although it was impossible to extend our observations over as great a range as in the dog (Pitts, Ayer and Schiess, 1949). Were the plasma level to be raised above this normal threshold range by the ingestion or infusion of alkali,

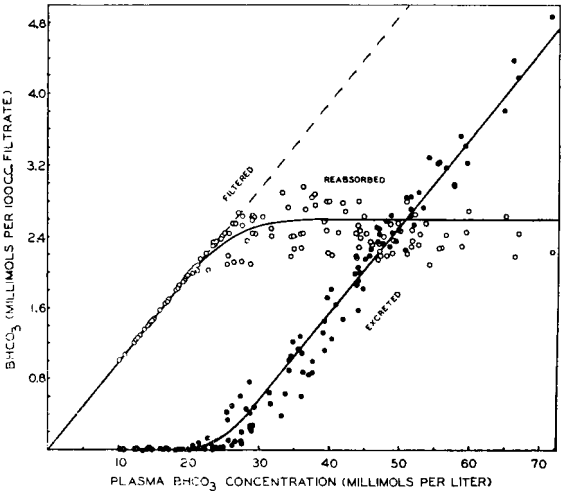

FIG. 1. The relationship in the normal dog between the plasma concentration of bicarbonate bound base and the quantities filtered, excreted and reabsorbed. From Pitts and Lotspeich (1946).

excretion of the excess would gradually return concentration to normal.

Does the relative constancy of reabsorption of bicarbonate at plasma levels ranging from 30 to 40 mEq./l. in man and from 30 to 70 mEq./l. in the dog indicate a true Tm relationship? In other words, is the quantity which can be reabsorbed limited by some fixed rate at which the renal tubular cells are able to transport bicarbonate bound base from tubular urine to blood? The answer to this question is a conditional no.

If, as illustrated in Fig. 3, filtration rate is increased acutely in the dog by the feeding of a meat meal, the ability of the

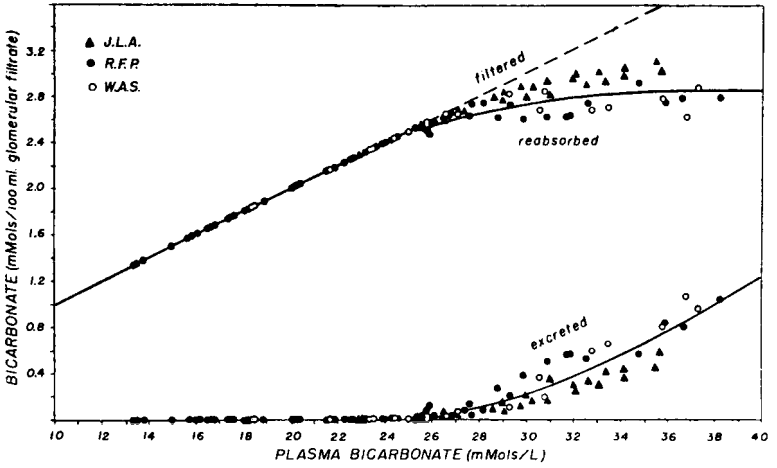

FIG. 2. The relationship in normal man between the plasma concentration of bicarbonate bound base and the quantities filtered, excreted and reabsorbed. From Pitts, Ayer and Schiess (1949).

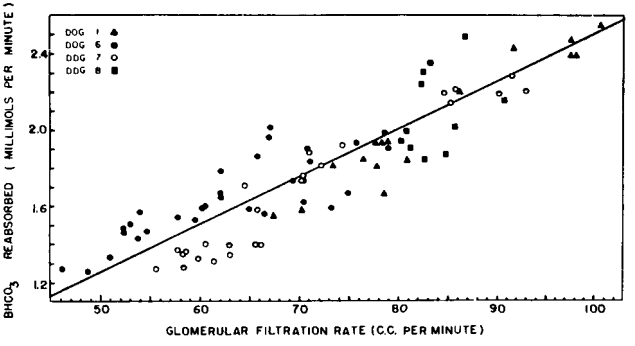

FIG. 3. The relationship in the normal dog between the renal tubular reabsorption of bicarbonate bound base and glomerular filtration rate. Filtration rate was varied by fasting and by feeding meat. Note that reabsorption is expressed in absolute terms of millimols per min. rather than in relative terms of millimols per 100 cc. of filtrate. From Pitts and Lotspeich (1946).

renal tubules to reabsorb bicarbonate bound base increases proportionately (Pitts and Lotspeich, 1946). In each experiment sodium bicarbonate was infused intravenously at such a rate as to ensure frank excretion. An initial series of observations was made with the animal in the post-absorptive state, meat was fed, and at the peak of the resulting increase in filtration rate a second series of observations was made. In this graph, in contrast to the preceding ones, tubular reabsorption of bicarbonate bound base is expressed in absolute terms of millimols per minute. The linear relationship evident between tubular reabsorption and filtration rate would seem to indicate that the renal tubules have no inherently fixed absorptive capacity for bicarbonate bound base as they do for glucose. Rather, reabsorptive capacity increases in exact proportion to the increase in filtration rate. The virtues of such a mechanism are obvious. An increase in filtration rate does not lead to depletion of body stores of bicarbonate bound base, as it would were reabsorptive capacity fixed. Neither does a decrease in filtration rate lead to an expansion of basic reserves. In simplest terms, renal plasma threshold is independent of glomerular filtration rate. However, recent experiments of Thompson and Barrett (1953) indicate that when filtration rate is reduced, absorptive capacity may remain at an elevated level for a brief period, i.e. for several hours.

The nature of this "fly wheel" response is uncertain at present. In fact, we know as little about the mechanism by which tubular reabsorption is correlated with filtration rate as we did some seven years ago when we first described it.

Throughout our work we have been careful to point out the fairly obvious fact that a renal plasma threshold of 22 to 28 mEq./l. for bicarbonate bound base would probably hold only for the normal dog and for normal man, would be reduced with hyperventilation and would be increased with CO_2 inhalation. Within the past year or so there has developed a flurry of interest in an experimental definition of these relationships. Ochwadt (1950) in Germany, Stanbury and

Thomson (1952) in this country, and Relman, Etsten and Schwartz (1953), Elkinton, Singer, Barker and Clark (1953) and Brazeau and Gilman (1953) in the United States have recently outlined their views. Dorman and Sullivan (1953) in our laboratory have also studied these variations in reabsorptive capacity. Their experiments have been performed on dogs anæsthetized with pentobarbital. To avoid the trauma of tracheal cannulation and thus to permit repeated experiments on a given animal, tracheal intubation was performed. In the experiment summarized in Table III, a dog was infused

Table III

An experiment on an anæsthetized dog which illustrates prompt increase in renal tubular reabsorption of bicarbonate bound base on inhalation of carbon dioxide-air mixtures and prompt restoration to normal reabsorption on return to room air. A urine washout period of about twenty minutes was interposed each time the inhaled gas mixture was changed to permit respiratory equilibration. From Dorman and Sullivan (1953).

GAS			PLASMA			BICARBONATE			
BREATHED	URINE	G.F.R.	$BHCO_3$	pH	pCO_2	FILT.	EXCR.	REABSORBED	
	ml./min.		mEq./L.		mm.Hg	mEq./min.			mEq./100ml.
AIR	5.80	65.2	37.4	7.55	44.2	2.44	0.81	1.63	2.50
AIR	6.40	62.5	37.4	7.56	43.2	2.34	0.81	1.53	2.45
CO_2	4.70	64.0	43.4	7.32	86.7	2.78	0.61	2.17	3.40
CO_2	4.60	65.2	44.5	7.31	91.1	2.90	0.62	2.28	3.50
AIR	5.65	59.0	42.9	7.51	55.5	2.53	0.88	1.65	2.81
AIR	5.85	61.5	44.3	7.44	67.2	2.72	0.98	1.74	2.83

with sodium bicarbonate in amounts sufficient to ensure frank bicarbonate excretion throughout. Plasma levels were maintained between 37 and 44 mEq./l. In the first two experimental periods the animal breathed room air. In the second two, a CO_2-air mixture was administered, and in the final two periods, room air was again inhaled. If one concentrates on the last two columns of this Table, it is evident that the quantity of bicarbonate bound base reabsorbed is considerably increased during CO_2 inhalation. This is true whether one measures reabsorption in absolute terms of mEq./min. or in mEq./100 ml. of glomerular filtrate.

What is the stimulus which so acutely alters the renal tubular capacity to reabsorb bicarbonate bound base? You note that inhalation of CO_2 decreases arterial blood *p*H and at the same time increases CO_2 tension. Which factor is significant? Further, is the capacity to reabsorb increased quantities of bicarbonate bound base on breathing CO_2

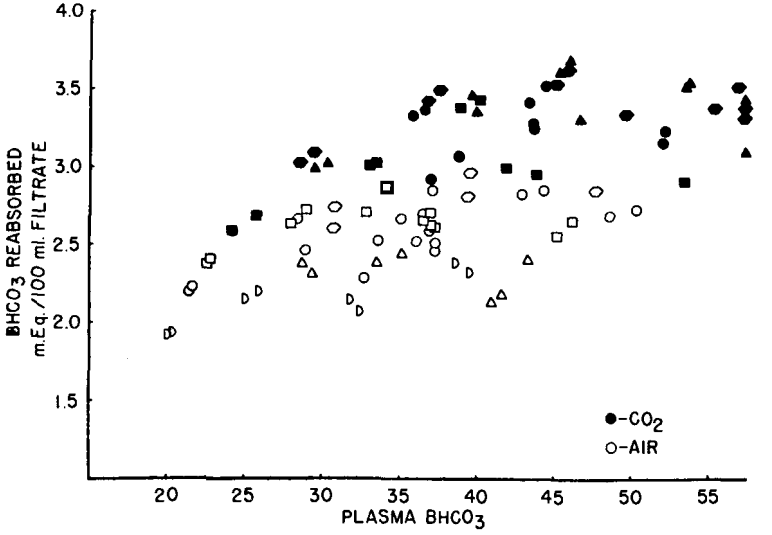

FIG. 4. A comparison of the relationship between renal tubular reabsorption and plasma concentration of bicarbonate bound base in anæsthetized dogs breathing room air and in the same animals breathing 12 per cent carbon dioxide in oxygen. From Dorman and Sullivan (1953).

evident at all plasma levels above threshold? We shall consider this latter question first.

In Fig. 4, two series of experiments are compared. The open symbols represent rates of reabsorption in animals breathing room air, in which the plasma concentration of bicarbonate bound base was gradually elevated to levels of some 50 milliequivalents per litre. The solid symbols represent rates of reabsorption in similar experiments differing only in the

fact that CO_2-air mixtures rather than room air were inhaled. It is evident that reabsorption of bicarbonate bound base was increased at all plasma levels by inhalation of CO_2.

In Fig. 5, all of our data on bicarbonate bound base reabsorption are plotted as a function of plasma *p*H. A fair

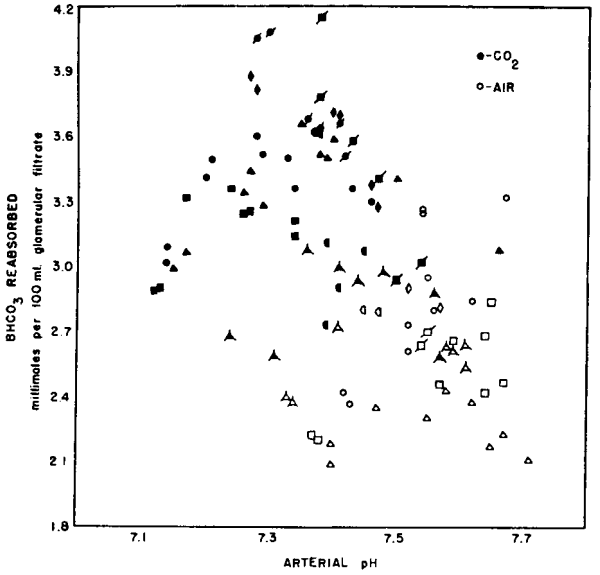

FIG. 5. The relationship between renal tubular reabsorption of bicarbonate bound base and arterial blood *p*H in anæsthetized dogs breathing air or air-carbon dioxide mixtures. In all experiments sodium bicarbonate was infused at rates sufficient to ensure frank excretion. From Dorman and Sullivan (1953).

correlation is evident. That is, with increased plasma *p*H, reabsorptive capacity is reduced; with decreased *p*H, it is increased.

Yet an equally good and perhaps even better correlation is obtained on plotting absorptive capacity as a function of arterial pCO_2 as is evident in Fig. 6. An increase in pCO_2 increases reabsorptive capacity; a decrease in pCO_2 decreases

reabsorptive capacity. Obviously these experiments have not been properly designed to demonstrate which variable, that is pH or pCO_2, is the significant one.

The two experiments summarized in Table IV, and others of similar design, demonstrate that arterial pCO_2, not arterial pH, is the stimulus which enhances reabsorption of bicar-

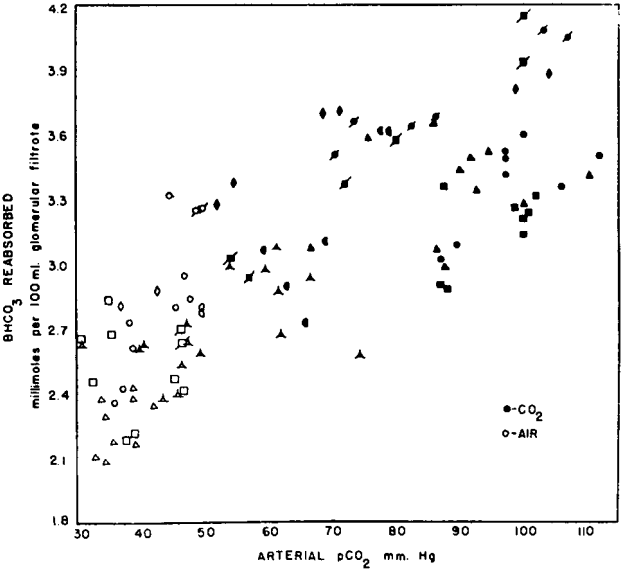

FIG. 6. The relationship between renal tubular reabsorption of bicarbonate bound base and arterial carbon dioxide partial pressure in anæsthetized dogs breathing room air or air-carbon dioxide mixtures. Data from the same experiments as those plotted in Fig. 5. From Dorman and Sullivan (1953).

bonate bound base. In this regard our views are at variance with those expressed by Ochwadt (1950), Stanbury and Thomson (1952), and Elkinton and co-workers (1953). They are in agreement with the views of Relman, Etsten and Schwartz (1953), and Brazeau and Gilman (1953).

In the first three periods of experiment 1, Dog A, the plasma bicarbonate level was raised moderately to 35 mEq./l. Arterial

pH averaged 7·49; pCO_2 averaged 46 mm. Hg. In the second three periods, the plasma bicarbonate level was raised to 94 mEq./l. and 12 per cent carbon dioxide was administered. The acidosis of CO_2 inhalation exactly balanced the alkalosis of bicarbonate administration. Thus pH did not change

Table IV

Experiments on anæsthetized dogs which illustrate the fact that the increase in partial pressure of carbon dioxide in the arterial blood, not the decrease in pH, is the stimulus which enhances the renal tubular reabsorption of bicarbonate bound base. From Dorman and Sullivan (1953).

GLOM. FILT. RATE.	PLASMA			BICARBONATE	
	$BHCO_3$	pH	pCO_2	REABSORBED	
ml./min.	mEq./L		mm.Hg	mEq./min.	mEq./100ml.
DOG A					
SLOW INFUSION $NaHCO_3$; ROOM AIR INHALATION					
50.9	34.8	7.49	47.3	1.44	2.82
51.0	34.9	7.50	46.3	1.44	2.82
46.9	34.2	7.49	46.3	1.31	2.80
RAPID INFUSION $NaHCO_3$; 12% CO_2 INHALATION					
54.4	89.1	7.49	120.9	1.86	3.42
55.4	92.3	7.48	127.9	2.01	3.64
53.0	94.5	7.48	131.1	1.93	3.64
DOG B					
INCREASING INFUSION $NaHCO_3$; 12% CO_2 INHALATION					
100.6	45.8	7.28	100.0	3.60	3.60
108.2	55.4	7.34	106.0	3.64	3.36
89.5	57.1	7.33	112.0	3.13	3.50
100.9	69.0	7.46	100.0	3.34	3.30
98.6	70.7	7.43	110.0	3.31	3.36

significantly. However, correlated with an increase in pCO_2 from 46 to over 120 mm. Hg, reabsorption of bicarbonate bound base increased from 2·8 to 3·6 mEq./100 ml. of glomerular filtrate.

In the second experiment (Dog B) pCO_2 was kept roughly constant around 100 mm. Hg by the administration of 12 per cent CO_2. The plasma level of bicarbonate bound base was raised from 45 to 70 mEq./l. Accordingly, arterial pH

rose from 7·28 to 7·46. Despite this change in *p*H, and no doubt correlated with constancy of pCO_2, reabsorption of bicarbonate remained fixed at an elevated level.

It is evident in chronic pulmonary disease that increased arterial CO_2 tension must, through its action in stimulating renal tubular reabsorption, play a significant rôle in maintaining an elevated concentration of bicarbonate bound base in the body fluids. However, the maximum tubular reabsorption observed in these experiments on acute respiratory acidosis ranged from 3·5 to 4 mEq./100 ml. of filtrate. Plasma bicarbonate would therefore tend to stabilize at concentrations between 35 and 40 mEq./l. Such values are not as great as those seen in long standing, severe pulmonary fibrosis and emphysema. It occurred to us that chronic exposure to high CO_2 tensions might further stimulate tubular reabsorption of bicarbonate bound base, a supposition which has proven correct.

In the experiment summarized in Table V, a dog was maintained for a period of two weeks in a metabolism cage covered with a zippered plastic bag. Ten per cent carbon dioxide in air was blown into the bag at a rate of 20 litres per minute. The bag was opened once a day to feed and water the animal and clean the cage. On the day of the experiment the dog was removed from the cage, anæsthetized with pentobarbital and as rapidly as possible given a 12 per cent carbon dioxide mixture to inhale.

The plasma concentration of bicarbonate bound base was distinctly elevated during the initial two control periods prior to the infusion of sodium bicarbonate, and essentially all of the filtered bicarbonate was reabsorbed. Reabsorption averaged 3·7 mEq./100 ml. of filtrate. With infusion of sodium bicarbonate, reabsorption increased gradually to reach a peak value of 5·6 mEq./100 ml. of filtrate. This considerably exceeded the maximum observed in acute experiments and indicates that further renal adaptation occurs with continued exposure to elevated CO_2 tensions.

Because of the inconvenience and expense of maintaining

Table V

An experiment on an anæsthetized dog which illustrates the fact that continued exposure to an elevated carbon dioxide tension for the preceding two weeks greatly increases renal tubular reabsorption of bicarbonate bound base over a wide range of plasma concentrations. From Dorman and Sullivan (1953).

Dog C. In chamber containing 10% CO_2 for 14 days prior to clearance periods.
No bicarbonate given during this interval.
Respiring 12% - 88% O_2 during experiment.

Period	G.F.R. cc./min.	Urine Flow cc./min.	PLASMA			URINE			$BHCO_3$			
			pH	$BHCO_3$ mEq./L.	pCO_2 mm.Hg	pH	$BHCO_3$ mEq./L.	pCO_2 mm.Hg	Filtered mEq./min.	Excreted mEq./min.	Reabsorbed mEq./min	Reabsorbed mEq./100 cc. filt.
1.	90.5	6.16	7.21	35.5	91.7	6.62	8.68	84.9	3.38	.053	3.33	3.68
2.	90.5	6.13	7.22	35.6	89.7	6.27	4.07	89.4	3.38	.025	3.36	3.71
3.	113	8.06	7.29	44.6	95.6	7.32	39.0	76.0	5.29	.314	4.98	4.41
4.	110	7.50	7.31	44.6	91.4	7.24	30.4	71.2	5.15	.228	4.92	4.48
5.	112	9.80	7.39	54.0	92.0	7.59	71.4	74.9	6.35	.70	5.65	5.04
6.	115	8.66	7.42	54.3	86.4	7.63	73.5	70.3	6.55	.636	5.91	5.14
7.	96.5	9.73	7.49	64.8	87.7	7.73	124	93.9	6.56	1.21	5.35	5.54
8.	99.4	9.66	7.51	65.3	84.4	7.74	125	93.9	6.81	1.21	5.60	5.64

a dog in an atmosphere of 10 per cent carbon dioxide for long periods of time, another dog was placed in this environment for only two days. During this interval 10 g. of sodium bicarbonate were given twice a day by stomach tube. As is evident from Table VI, nearly as great a renal response to respiratory acidosis was observed in two days' time as in two weeks' time. Thus during the two initial control periods, 3·5 mEq. of bicarbonate were reabsorbed per 100 ml. of glomerular filtrate. During the infusion of sodium bicarbonate, reabsorption increased to 5·0 mEq./100 ml. of filtrate. A further analysis of the response to chronic respiratory acidosis seems feasible and we hope to pursue it further.

Our description of the properties of the mechanism concerned with the renal tubular reabsorption of bicarbonate bound base can best be synthesized in terms of the diagram presented in Fig. 7. Because half or more of the quantity of bicarbonate bound base normally reabsorbed by the renal tubules is excreted under the influence of the carbonic anhydrase inhibitor, 2-acetylamino-1, 3, 4-thiadiazole-5 sulphonamide (6063), we feel that hydration of carbon dioxide to carbonic acid is an essential link in the reabsorptive process.

According to our views, carbon dioxide is hydrated to carbonic acid within the tubular cells. This carbonic acid dissociates to yield hydrogen ions which are exchanged for bicarbonate bound base in the tubular urine. Carbonic anhydrase speeds the hydration of carbon dioxide and increases the available supply of hydrogen ions. The action of 6063 in blocking the enzymatically facilitated hydration process reduces reabsorption of base. However, reabsorption continues, albeit at a reduced rate; for hydration occurs despite loss of activity of this enzyme. An increase in pCO_2 *speeds* the hydration process, and consequently increases the reabsorption of base by making available a greater supply of hydrogen ions. A decrease in pCO_2 *slows* the hydration process, and reduces the reabsorption of base.

The mechanism which we propose here is in general accord with the one outlined by Pitts and Alexander (1945) and

Table VI

An experiment on an anæsthetized dog which illustrates the fact that exposure to an elevated carbon dioxide tension for only two days coupled with gavage of sodium bicarbonate similarly increases renal tubular reabsorption of bicarbonate bound base. From Dorman and Sullivan (1953).

Dog A. In chamber containing 10% CO_2 for 48 hours prior to clearance periods.
Gavaged with 10 Gm. $NaHCO_3$ b. i. d. during this period.
Respiring 12% CO_2 - 88% O_2 during experiment.

Period	G.F.R. ml./min.	Urine Flow ml./min.	PLASMA pH	PLASMA $BHCO_3$ mM./L.	PLASMA pCO_2 mm.Hg	URINE pH	URINE $BHCO_3$ mM./L.	URINE pCO_2 mm.Hg	$BHCO_3$ Filt. mM./min.	$BHCO_3$ Excr. mM./min.	$BHCO_3$ Reabsorbed mM./min.	$BHCO_3$ Reabsorbed mM./100 ml. glom.filt.
1.	48.3	4.31	7.15	34.2	99.9	6.75	11.2	80.0	1.73	0.05	1.68	3.48
2.	45.5	5.67	7.17	34.3	99.9	6.76	11.1	80.0	1.64	0.06	1.58	3.48
3.	49.0	8.94	7.25	45.0	106.5	7.35	43.7	83.0	2.31	0.39	1.92	3.92
4.	46.3	7.87	7.30	46.4	96.5	7.36	43.1	80.0	2.25	0.34	1.91	4.13
5.	41.6	7.80	7.40	60.6	99.9	7.63	90.2	90.0	2.65	0.70	1.95	4.69
6.	40.0	7.60	7.43	63.5	99.9	7.63	93.3	90.0	2.66	0.71	1.95	4.87
7.	39.6	12.30	7.45	82.7	123.0	7.67	116.9	103.0	3.44	1.44	2.00	5.05
8.	40.1	13.13	7.48	84.4	116.5	7.69	125.1	106.0	3.55	1.64	1.91	4.76

Pitts and Lotspeich (1946) some years ago in explanation of acidification of the urine and distal tubular reabsorption of bicarbonate bound base. Although in our original work we thought it to be restricted to the distal segment of the renal tubule, we now feel that it may be operative throughout the entire length of the renal tubule. Although there are a number of deficiencies in this formulation, one of the more obvious is

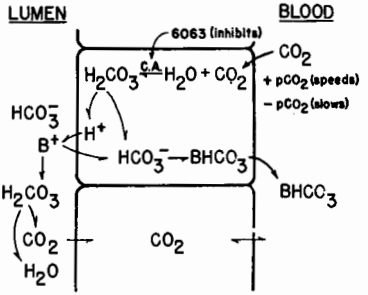

FIG. 7. Cellular mechanism for the reabsorption of bicarbonate bound base. It is probable that this mechanism is operative not only in cells of the distal tubule but in those of the proximal tubule as well.

that it neglects the problem of the cycling of energy into the system.

An experiment summarized in Table VII supports the views outlined in the preceding diagram. In this experiment, a dog was infused with sodium bicarbonate at a rate sufficient to ensure frank excretion throughout. While breathing room air, 3·07 and 3·06 mEq. of bicarbonate bound base were reabsorbed per 100 ml. of filtrate. When CO_2 was inhaled, reabsorption increased to 3·65 and 3·72 mEq. Continuing CO_2 and adding 6063 to the infusion dropped reabsorption to 2·46 and 2·45 mEq. Finally on room air, while the 6063 infusion was maintained, reabsorption dropped to 1·84 and 1·67 mEq.

We would interpret this experiment in the following way. In the normal animal the inhalation of CO_2 increased the

reabsorption of bicarbonate bound base some 0·6 mEq./100 ml. of filtrate. With increased CO_2 tension, cellular hydration of the gas and hence the availability of hydrogen ions for exchange with base are both increased. This physical effect of increased CO_2 tension should be independent of the activity of carbonic anhydrase and dependent solely on partial pressure. It is evident when comparing the fourth group of periods

Table VII

An experiment on an anæsthetized dog which illustrates the fact that the increase in tubular reabsorption of bicarbonate bound base brought about by an elevation in arterial pCO_2 is independent of the activity of carbonic anhydrase. From Dorman and Sullivan (1953).

GAS BREATHED	DRUG GIVEN	URINE	G.F.R.	PLASMA			BICARBONATE			
				$BHCO_3$	pH	pCO_2	FILT.	EXCR.	REABSORBED	
		ml./min		mEq./L.		mm.Hg	mEq./min.			mEq./100ml.
AIR	—	1.80	56.8	36.1	7.43	56.2	2.15	0.41	1.74	3.07
AIR	—	1.76	54.8	36.4	7.43	56.5	2.09	0.42	1.67	3.06
CO_2	—	1.44	54.5	39.4	7.30	82.9	2.26	0.27	1.99	3.65
CO_2	—	1.37	52.3	40.4	7.25	95.0	2.21	0.26	1.95	3.72
CO_2	6063	5.50	48.7	45.1	7.24	108.8	2.31	1.11	1.20	2.46
CO_2	6063	5.50	50.4	45.5	7.21	114.8	2.35	1.12	1.23	2.45
AIR	6063	6.01	50.9	39.8	7.40	66.1	2.13	1.19	0.94	1.84
AIR	6063	5.33	49.6	38.5	7.39	65.9	2.01	1.18	0.83	1.67

on air with the third group on CO_2 that essentially the same increase in reabsorptive capacity, namely 0·6 mEq./100 ml. of filtrate, results from increased CO_2 tension, even though the activity of renal carbonic anhydrase must have been very greatly reduced, if not entirely abolished.

Plasma concentration and CO_2 tension, though very significant, are by no means the only factors conditioning the reabsorption of bicarbonate bound base, for although time does not permit its discussion, there is evidence that potassium ion availability and humoral agents as well affect the reabsorptive system (Berliner, 1952; Roberts, Magida and Pitts, 1953).

In summary, the kidneys normally stabilize the plasma concentration of bicarbonate bound base within narrow limits of 24 and 28 mEq./l. In an individual on the usual acid ash diet reabsorption of the filtered moiety is essentially complete; none is wasted. When an excess of base becomes available, in consequence of the ingestion of an alkaline ash diet or the administration of sodium bicarbonate, plasma concentration rises and the excess filtered is excreted in the urine. A functional increase in glomerular filtration rate is accompanied by an equivalent increase in tubular reabsorptive capacity. Therefore, minor fluctuations in glomerular filtration do not *per se* result either in expansion or depletion of body stores of bicarbonate bound base.

The renal tubular mechanism for reabsorption of bicarbonate bound base is remarkably sensitive to the carbon dioxide tension of the body fluids. Reabsorption increases as pCO_2 increases; reabsorption decreases as pCO_2 decreases. Relative stability of the reabsorptive process in the normal individual is, therefore, in part a reflection of the constancy with which the respiratory system stabilizes the carbon dioxide tension of the body fluids. In chronic pulmonary disease, enhanced reabsorption and increased concentration of bicarbonate bound base in the body fluids compensate to some degree for the acidosis induced by carbon dioxide retention. The renal response to an increase in carbon dioxide tension is prompt, but incomplete, and increases over several days with continued exposure.

Although plasma concentration and carbon dioxide tension are major variables determining completeness of reabsorption of bicarbonate bound base, other factors exert an influence.

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DISCUSSION

STANBURY: Since Thomson and I concluded that it was the plasma *p*H and not pCO_2 that influenced the tubular reabsorption of bicarbonate, I feel that we must bow to Dr. Pitts' exquisite demonstration of the effects of carbon dioxide. We, I fear, gave up after several experiments in man in which inhalation of about 7 per cent CO_2 for periods of about three hours at normal plasma bicarbonate levels was without any apparent effect on the urine. In fact, if we began our experiments at something like 9 a.m. we got the customary matutinal alkaline tide and bicarbonate excretion increased progressively from 9 a.m. to 12 noon, despite continued CO_2 inhalation. My colleagues Longson and Mills (*J. Physiol.*, **122**, 81) have taken this up in greater detail and exposed themselves repeatedly over many hours to concentrations of CO_2 admittedly only between $5\frac{1}{2}$ and $6\frac{1}{2}$ per cent. Again they found that the diurnal excretory rhythm persisted unmodified with a matutinal increase in bicarbonate excretion during the inhalation. Seeing this fact and yet admitting that an effect of pCO_2 on bicarbonate reabsorption was likely to be responsible for the high plasma bicarbonate levels in emphysema, we gave the problem up! Dr. Pitts' demonstration makes it very clear that CO_2 can indeed produce these effects. In respect of the diurnal changes of hydrion excretion we may find an answer in the one factor that Dr. Pitts has left to Dr. Berliner; we have arrived at the tentative conclusion that the excretory changes in hydrion are probably consequent upon the diurnal excretory changes in potassium.

PITTS: Incidentally, what have you found to be the diurnal changes in alveolar CO_2 tension?

STANBURY: Mills has done that (*J. Physiol.*, **122**, 66) and he has made measurements in a state of wakefulness, because throughout a period of twenty-four hours' wakefulness the diurnal excretory changes in hydrion and potassium excretion undoubtedly continue as they do when you are asleep. He finds the changes in alveolar CO_2 tension are extremely small, of the order of about 3 mm. Hg in the twenty-four hours. That is the order of change that occurs spontaneously in the wakeful subject breathing ordinary room air.

MILNE: Is it not true to say that the success of the dog experiments and the failure of the human experiments is entirely dependent upon the very excellent compensation by hyperventilation to the inhalation

of concentrations of CO_2 up to 5 or 7 per cent? Up to that level you get tremendous hyperventilation and the partial pressure of CO_2 rises surprisingly little. No human volunteer will go into a 10 per cent or a 12 per cent CO_2 chamber and that is the reason for this exquisite demonstration of the renal effects of inhalation of CO_2 in the dog and the failure of similar experiments in the human volunteer.

PITTS: Yes, and no dog will tolerate it unless he is anæsthetized with pentobarbital.

BERLINER: We have found that if we take a dog in normal acid-base balance and make it inhale 10 per cent CO_2, there is surprisingly little change in the *p*H of the urine or in bicarbonate excretion. Do you think that one can distinguish, in interpreting these data, between an increase in the CO_2 tension of cells and a change in the *p*H of cells?

PITTS: No. I am actually implying, and I should have stated it directly, that there must be an increase in hydrogen ion concentration in the renal tubular cells. In fact, this increase accounts for the greater availability of hydrogen ions to the exchange mechanism. I think my point would simply be that it is not the *p*H of the external medium, it is the *p*H of the interior of the cell which is the important variant. And the *p*H of the interior of the cell does not necessarily correlate at all with the *p*H of the surrounding fluid environment, but it does correlate quite well, I think, with the pCO_2 of the external environment, at least in acute experiments.

BERLINER: The reason I ask is that I think that this system where the hydrogen ion is made directly available by hydration of carbon dioxide is a little too simple. It does not leave enough room for other effects, which do not appear to be exerted through an action on the rate of hydration of CO_2. As I shall mention later, several other substances which have no effect on that reaction also produce a similar inhibition of urine acidification. We are inclined to think that the common denominator is the *p*H of the cell and not specifically the direct hydration of CO_2.

PITTS: I am in thorough agreement. I think our scheme is much oversimplified. In response to a point which Dr. Robinson made, we have avoided any statement as to which ion, i.e. sodium or hydrogen, is actively transported. We think that one is operated upon and the other is exchanged passively. But I will admit that I have been very much taken with his views that sodium is actively transported. I presume he feels that the primary transport of sodium may be accompanied by the passive movement of hydrogen, potassium or ammonia. However, I admit that the reverse may be true.

ROBINSON: I have one observation which I have never fitted in, which might point the other way, and that is a chance one because in using more buffer I was unwittingly adding more potassium to my medium—the production of ammonia by kidney slices in acid media was inhibited by potassium. I don't know what it means.

BERLINER: We have made similar observations *in vivo* and we think it means that there is a rise in the *p*H of the cells.

BALDWIN: There are, of course, two cases in which the circulation

of base is at least as important, and perhaps more important than it is in the mammalian kidney, and that is in the excretion of uric acid by birds and by insects. In both cases you have a still liquid urine, containing no solid uric acid and apparently markedly alkaline, entering the cloaca or the hind gut, and as the liquid passed down there is a definite replacement of base ions by hydrogen ions. Alkaline urates are on the whole very much more soluble than free uric acid and it is as alkaline urates that uric acid is excreted in the first place by an aglomerular kidney. The base is reabsorbed and replaced by hydrogen, and as the urine passes down it becomes progressively more and more acid until solid uric acid separates out. That seems to me to be an exaggerated form of the sort of phenomenon that you have been telling us about. It might be very profitable to study this system in the bird in order to throw more light on the mechanism of this exchange.

ROBINSON: Has the bird's kidney got carbonic anhydrase?

BALDWIN: I don't know. I don't think it has been looked for. You get a circulation of base again in the developing hen's egg; uric acid formed in the tissues is carried over into the allantois; it goes over apparently as an alkaline urate, then the base comes back again and the uric acid is deposited in a more or less crystalline form.

BRADLEY: Are the kinetics of the reaction between CO_2 and H_2O such that a small rise in pCO_2 actually speeds it?

BALDWIN: I think so.

PITTS: Certainly the pH of an aqueous solution of CO_2 depends on the partial pressure of CO_2. That would be my reason for saying that the availability of hydrogen ions should be related to the pCO_2, independent of the activity of carbonic anhydrase.

TAGGART: When you speak of the absence of carbonic anhydrase, do you think you have completely inhibited it? You may have 100 per cent of the normal activity in one situation and perhaps 90 per cent in the other.

BERLINER: I should guess that the activity of carbonic anhydrase is reduced by very much more than 10 per cent, but it is probably not completely eliminated.

BALDWIN: I seem to remember that at the concentration of carbonic anhydrase present in mammalian red cells, there is an acceleration factor of about 200 times in the rate either of formation or decomposition of carbonic acid. So, to see what would be the effect of getting rid of carbonic anhydrase, you would have to inhibit the thing extremely efficiently—you must knock it stone dead, so to speak.

PITTS: I think it must be 95 per cent or more inhibited before you can demonstrate any effect so far as the red cells are concerned.

THE KIDNEY
G. E. W. WOLSTENHOLME

THE RELATIONSHIP BETWEEN POTASSIUM EXCRETION AND URINE ACIDIFICATION

ROBERT W. BERLINER, THOMAS J. KENNEDY and JACK ORLOFF

THE observation that the urine becomes alkaline when potassium chloride is administered goes back at least some twenty years to the work of Loeb and his associates (1932). A relationship between potassium depletion and the development of alkalosis was noted over fifteen years ago by McQuarrie, Johnson and Ziegler (1937); the phenomenon has been extensively studied by Darrow and his associates (1948, 1950). For some years, it seems hardly to have been recognized that these events might be related; among the investigators of metabolic balances and tissue electrolytes, the rôle of the kidney was almost completely disregarded, while those interested chiefly in renal mechanisms paid little or no attention to the metabolic observations. For some time we were decidedly in the latter category. In a subsequent attempt to generalize from specific observations concerning urine acidification and potassium excretion (Berliner, Kennedy and Orloff, 1951), we made certain assumptions, particularly with regard to the pathogenesis of hypokalæmic alkalosis, which do not appear to have been justified. Nevertheless, it would appear that the alkalosis of potassium depletion, although more complex than we proposed, is dependent upon the relationship between potassium metabolism and urine acidification and that the latter relationship can be described as a competition of potassium ions and hydrogen ions for some component of a secretory mechanism which is common to both. I should like, today, to review some of the data on which this concept is based and try to bring up to date our views on the subject.

The mechanism by which bicarbonate is reabsorbed and the urine acidified, which has been discussed by Dr. Pitts, has one simplifying feature—the entire process can be attributed to a single transport mechanism exchanging hydrogen ions for sodium ions. We are not so fortunate in dealing with potassium excretion since, as you know, both reabsorptive and secretory processes must be dealt with. This circumstance could make well nigh impossible any interpretation of potassium excretion in terms of the renal mechanisms involved. However, whenever it has been possible to trace a change in potassium excretion to the secretory or the reabsorptive process, it has turned out to be the secretory mechanism which was involved. On the basis of a number of bits of minor evidence, none conclusive by itself, we are thoroughly convinced that the potassium contained in the glomerular filtrate makes little or no contribution to the urinary potassium. For those who may not be so convinced, we nevertheless recommend, as a very useful working hypothesis, the view that the filtered potassium is reabsorbed high in the tubule by a mechanism of unknown properties and that potassium destined for excretion is secreted by the tubules further down. The latter process has been shown to be an exchange of potassium ions from the tubule cells for sodium from the tubule fluid (Berliner, Kennedy and Hilton, 1950*a*).

Time will not permit a discussion of the factors which appear to play a rôle in determining the rate at which potassium is excreted. Suffice it to say that they are several. Of these I should like to consider, in detail, only one—namely, that which is concerned with the relationship between hydrogen ion and potassium secretion. Here, as a basis for proceeding, we may start with the hypothesis that these ions are in competition in the process by which they are transported, consider what predictions would be made from this hypothesis and the extent to which the experimental observations conform to these predictions, and finally, examine the extent to which other factors may be involved in the relationship between these ions.

If potassium and hydrogen ions are in competition at some point in their transport, we would expect that each change in the concentration of one at the site of this competition would, other factors remaining constant, be accompanied by an inverse change in the secretion of the other.

Of the four combinations, that in which the primary change is a depression of hydrogen ion secretion is most amenable to

Table I

EFFECT OF CARBONIC ANHYDRASE INHIBITOR 6063 ON URINE ACIDIFICATION AND POTASSIUM EXCRETION

EFFECT OF #6063 ON URINE ACIDIFICATION Dog H. Wt. 26 Kg.						
TIME min.	*URINE FLOW* ml./min.	*URINE pH*	*EXCR. Titr. Acid* μEq./min.	*EXCR. HCO_3^-* μEq./min.	*EXCR. Na^+* μEq./min.	*EXCR K^+* μEq./min.
-77	*Start Infusion of Creatinine 25 mg./min.; Sodium Phosphate (pH 5.8) 200μM./min.; Sodium Chloride 1mM./min.; Water 8 ml./min.*					
-57	*Priming Infusion: Creatinine 2.0 gm; Hydrochloric Acid 40 mEq.*					
0-21	0.81	5.58	105	0.2	85	68
21-43	0.77	5.41	154	0.2	85	61
43-64	0.94	5.38	164	0.3	116	52
73-79	*Infusion #6063 (10 mg./kg.)*					
87-102	8.06	7.82	0	920.0	1654	264
102-117	6.21	7.80	0	750.0	1315	248
117-133	4.17	7.73	0	456.0	935	184

study and has been examined most extensively. Hydrogen ion secretion can be markedly suppressed by the administration of carbonic anhydrase inhibitors (Pitts and Alexander, 1945; Berliner *et al.*, 1951). As shown in Table I, this inhibition of urine acidification is accompanied by a marked increase

in the excretion of potassium. Here a moderately acidotic dog was infused with a solution of sodium phosphate to increase the excretion of titratable acid. During the control periods, the urine was acid and contained moderate amounts of titratable acid. After the administration of the carbonic anhydrase inhibitor 6063, the effect on hydrogen ion secretion

Table II

EFFECT OF SALYRGAN AND 6063 ALONE AND TOGETHER ON URINE ACIDIFICATION AND POTASSIUM EXCRETION

Time	C_{cr}	Excretion of			
		Cl^-	HCO_3^-	Na^+	K^+
min.	ml/min	μEq/min.	μEq/min.	μEq/min.	μEq/min.
-69	Start infusion of creotinine, phosphate (pH 7.4), saline.				
0-38	74	143	22	296	68
41	Salyrgon i.v.				
64-94	70	1160	59	1370	60
97	*6063 i.v.				
108-138	54	1133	375	1737	68
139	BAL i.m.				
154-203	63	113	362	564	146

is shown by disappearance of titratable acid and its replacement by large amounts of bicarbonate. There is a marked increase in the excretion of potassium.

That this increase in the excretion of potassium is specifically due to an increase in secretion rather than a decrease in reabsorption has been shown in experiments like that shown in Table II. Each datum represents the average of that obtained in three clearance periods. After the control periods, the mercurial diuretic, Salyrgan, was administered. Its effects are shown by the increase in excretion of sodium and chloride; there was a slight increase in bicarbonate excretion,

a minimal depression of potassium excretion. When the carbonic anhydrase inhibitor is administered, the depression of hydrogen ion secretion is indicated by the increase in bicarbonate and sodium excretion, but so long as the mercurial is present, the usual increase in potassium excretion does not occur. Finally, when the mercurial effect is abolished by BAL we observe the enhanced potassium excretion which characteristically follows administration of carbonic anhydrase inhibitors. Now if the effect of these inhibitors were to interfere with reabsorption of potassium, it is difficult to imagine how this could be affected by the mercury so as to depress excretion; the increased potassium excretion must, therefore, be due to enhanced secretion which, in turn, can be inhibited by the mercurial diuretic.

Table III

EFFECT OF LITHIUM CHLORIDE ON POTASSIUM EXCRETION AND URINE ACIDIFICATION

Time	Excreted K^+ μEq/min.	Urine pH	Excreted HCO_3^- μEq/min.	Excreted T.A. μEq/min.
-62	Start Infusion			
0-43	31	5.65	0.75	107
46	Lithium i.v.			
48-99	174	6.93	113	31
102	Salyrgan i.v.			
122-152	59	6.95	118	28
152	BAL i.m.			
178-214	128	6.95	68	29

That this is not an effect produced only by carbonic anhydrase inhibitors is shown in Table III in which lithium chloride was used to produce inhibition of urine acidification.

Interestingly, it was the observation of Foulks, Mudge and Gilman (1952) that potassium excretion was markedly increased by infusion of lithium chloride that suggested that it might inhibit urine acidification. Similar effects can be produced by the administration of sodium maleate (Berliner, Kennedy and Hilton, 1950*b*). Neither the lithium ion nor maleate salts are inhibitors of carbonic anhydrase.

It is hardly surprising that the increase in potassium excretion when hydrogen secretion is inhibited fits the hypothesis of competition, since it is largely on these observations that the hypothesis was based. However, before going on to consider the other aspects, it should be mentioned that the depression of hydrogen ion secretion in respiratory alkalosis is accompanied by an increase in potassium excretion, as first shown by Prof. McCance (McCance and Widdowson, 1936) and more recently by Stanbury and Thomson (1952), and by Singer and his associates (1952). The effects of metabolic alkalosis are not included here for the reason that it is uncertain whether it really belongs in this category.

The next most easily produced change in this system is a primary increase in potassium concentration and excretion. The increase in urine *p*H which has been known to follow the administration of potassium chloride has already been referred to. There is nothing specific about the chloride salt as similar changes have been found with the phosphate, sulphate, thiosulphate and ferrocyanide (Berliner *et al.*, 1950*a*). An example of the latter is shown in Fig. 1. This experiment was begun by infusion of a solution of sodium ferrocyanide until a more or less steady state was reached. The sodium was then progressively replaced by equimolar amounts of potassium. Note that as potassium excretion begins to rise there is a sharp rise in the *p*H of the urine, a manifestation of a decrease in hydrogen ion secretion since the filtered bicarbonate is actually falling. The extent of the fall in plasma bicarbonate is worth noting since, as Roberts, Magida and Pitts (1953) have pointed out, the bicarbonate is not lost in the urine. It is apparent, however, that the

effects of administering potassium are in accord with the hypothesis.

A primary increase in hydrogen ion secretion can be produced by an inhalation of gas mixtures containing carbon dioxide at concentrations of 5 per cent or more (Brazeau

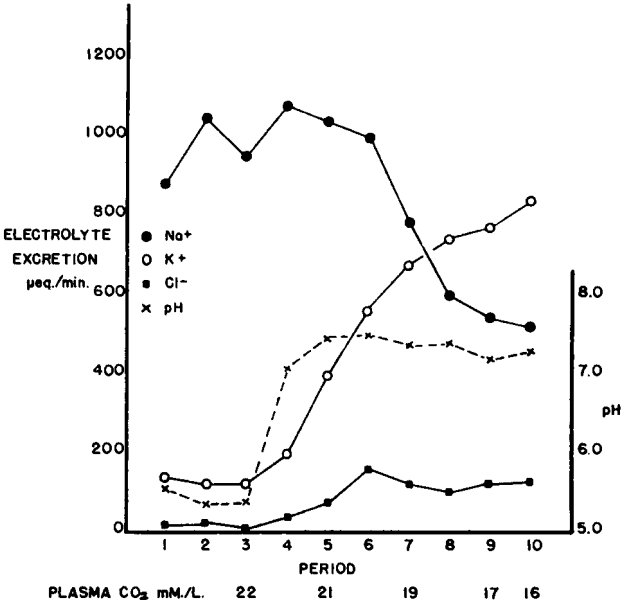

FIG. 1. Electrolyte excretion during infusion of sodium ferrocyanide and replacement of the sodium by potassium.

and Gilman, 1953; Dorman and Sullivan, 1953). A decrease in the excretion of potassium in respiratory acidosis has been described in man by Elkinton and his associates (1953) and in the sheep by Denton and co-workers (1952). The effects of metabolic acidosis are more complicated and it is by no means certain that there is an increase in hydrogen ion secretion under these conditions—the increased acidity of the urine may represent only the effect of a decrease in the filtered load

of bicarbonate. Further, there are usually additional factors—particularly an increase in total electrolyte excretion when metabolic acidosis is produced, as by the administration of ammonium chloride. In this circumstance, it is well-known that potassium excretion is increased. However, we have found that if acidosis is produced in such a way as to give no

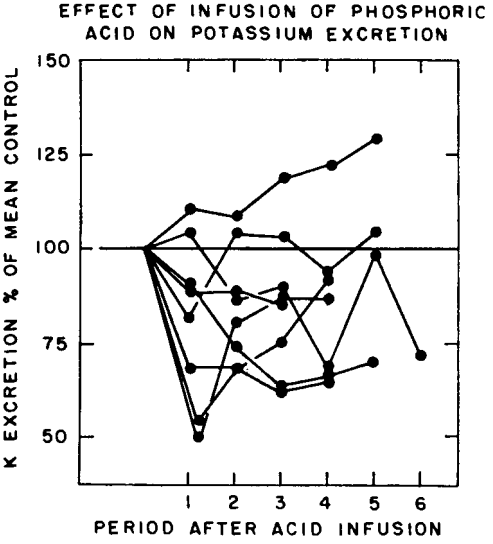

FIG. 2. Changes in potassium excretion during infusion of phosphoric acid in place of equimolar amounts of neutral sodium phosphate.

increase in total electrolyte excretion, potassium excretion does not increase, but on the contrary is usually slightly depressed. This is illustrated in Fig. 2 which shows the changes in potassium excretion in dogs subjected to the following procedure:—Each dog received an infusion of neutral sodium phosphate until a reasonably steady state was achieved. The line representing 100 per cent is the average of the potassium excretion in at least three periods of this approximately steady state. To avoid confusion the control values

have been omitted, but in general, they showed small fluctuations but no specific trends. The sodium phosphate in the infusion was then replaced by an equimolar amount of phosphate as phosphoric acid and, after an hour to allow the development of some degree of acidosis, the periods plotted were collected. The situation is one in which the only known change is the development of a mild metabolic acidosis. In one of the eight experiments there was an increase in potassium excretion, in one essentially no change, in the remainder a definite fall. There is nothing in these data to suggest that acidosis *per se* increases potassium excretion. This is not intended to imply a suppression of potassium secretion secondary to an increase in hydrogen ion secretion, since it is not known that hydrogen ion secretion *is* increased nor that the intracellular fluid reflects the acidosis of the extracellular fluid.

Finally, we must consider the effect of removal of potassium from the mechanism on the secretion of hydrogen ion. Since mercurial diuretics inhibit secretion of potassium, the obvious step is to examine the effect of mercurial diuretics on urine acidification. This has been done and there is no consistent effect. In addition, when potassium salts are administered during the action of a mercurial diuretic, the suppression of acidification occurs although there is only a slight increase in potassium excretion. This would not appear to be in accord with the competition hypothesis. We have, however, rationalized this to our own satisfaction by assuming that it is the concentration of the particular ion which is important, and not its turnover. There is no reason to believe that the mercurials decrease the concentration of potassium in cells. However, it is quite probable that hydrogen ion concentration decreases when the metabolic production of these ions is interfered with. Or, if the rôle of carbonic anhydrase in the kidney corresponds to that proposed by Davies and Roughton (1948) for gastric acid secretion, the specific effect of inhibition of this enzyme would be a rise in cell *p*H.

If it is the concentration that is important, the potassium concentration must be reduced to examine this alternative. This has not been possible in acute experiments. That chronic depletion of potassium is accompanied by alkalosis is well recognized. The mechanism by which this alkalosis develops is complex, but it must be conceded that the persistence of this alkalosis can mean only that there is a relative increase of hydrogen ion secretion over the normal, or the relatively slight excess of bicarbonate in the extracellular fluid would be excreted and the alkalosis would disappear.

It would seem that the hypothesis of competition between potassium and hydrogen ions is a fairly adequate description of the relationship between them. The only exception, in the action of mercurial diuretics, is fairly easily rationalized. However, is this the sole basis of the observed association of changes in potassium balance and acid-base equilibrium? The answer would appear to be a definite *no*.

In proposing the concept of potassium-hydrogen competition, we assumed that alkalosis of the extracellular fluid meant alkalosis of intracellular fluid as well and thus that the alkalosis of potassium depletion must be due to loss of acid from the body (Berliner *et al.*, 1951). Subsequent work has shown that this need not be so. Cooke and his associates (1952) found that the recovery of rats from hypokalæmic alkalosis was accompanied by an increased excretion rather than retention of acid; and Black and Milne (1952) found that there was no loss of acid in the urine as they themselves became alkalotic from potassium depletion. From muscle CO_2 analyses in potassium-depleted alkalotic rats, Gardner, MacLachlan and Berman (1952) estimated the intracellular *p*H to be lower than normal. We have found that the alkalosis of potassium-depleted rats can be reversed by the administration of potassium salts although the animals have been nephrectomized (Orloff, Kennedy and Berliner, 1953). The observation of Roberts, Magida and Pitts (1953) of a loss of bicarbonate from the extracellular fluid but not to the outside when potassium salts are infused is presumably similar. These observations

appear to indicate that potassium-depleted cells are more acid and those with an excess of potassium more alkaline than normal. In this case, the question arises: is the apparent competition between potassium and hydrogen ions a manifestation of a reciprocal relationship between their concentration within cells? If this were the case, and if the rate of secretion of each ion were a direct function of its intracellular concentration, most of the observed relationships between their urinary excretions would be expected. This question cannot, at present, be answered with certainty. However, there is no evidence of an uptake of potassium by cells as their *p*H (presumably) rises in respiratory alkalosis, nor is there evidence of potassium loss from cells in respiratory acidosis, yet the changes in potassium excretion are reciprocal to those of hydrogen secretion. There are grounds for believing that more is involved than an inverse relationship of cell concentration.

One additional point should be mentioned concerning the association of potassium depletion and alkalosis. Reference has been made chiefly to the alkalosis which develops with potassium depletion. However, it is also known that alkalosis produced by excess alkali intake or by continued loss of gastric juice leads to potassium depletion, and it is quite easy to demonstrate a marked increase in potassium excretion when bicarbonate is administered. When we assumed that intracellular fluid underwent the same changes in acid-base balance as extracellular fluid, the explanation seemed obvious. Now it is not so clear. The observation of Roberts, Magida and Pitts (1953) that there is apparently a shift of potassium into cells when bicarbonate is administered suggests an explanation.

However, if the primary event were a movement of potassium into cells, the situation would be paradoxical in that the ultimate effect is a decrease in the potassium content of most body cells. Even in acute experiments a fairly large negative cellular potassium balance may be produced. Nevertheless since the bulk of evidence suggests that muscle cell potassium

is determined largely by the relationship between intake and renal excretion, it is conceivable that elevation of renal cell potassium or *p*H might lead to sufficient loss of potassium to deplete the body as a whole. In any case, this may represent a possible approach to a reasonable explanation of the phenomenon.

Acknowledgement

Tables I and II from Berliner, R. W. (1952), *Fed. Proc.*, **11**, 695.

FIG. 1 from Berliner, R. W., Kennedy, T. J., Jr., and Hilton, J. G. (1950), *Amer. J. Physiol.*, **162**, 348.

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DISCUSSION

MILNE: I should like to question whether the inverse relationship between potassium and hydrogen ion is always due to competition between these ions. I shall present data this afternoon which I think would be better explained by the intracellular availability of these ions rather than a competition between them. For instance, after the development of hypokalæmic alkalosis, you say that this is maintained by a competition between potassium and hydrogen ions. Might not the explanation equally well be that owing to the intracellular acidosis there is an increased availability of hydrogen ion within the cell, an increased secretion of hydrogen into the tubular lumen, and therefore a titration out of the excess bicarbonate which is filtered by the glomerulus?

BERLINER: I tried to indicate that in hypokalæmic alkalosis we could not distinguish competition between potassium and hydrogen ions from an inverse relationship of their intracellular concentrations. I can't be certain that we should ever talk about competition as such. But we do encounter other situations, for instance in respiratory acidosis, where we have no evidence that the potassium concentration in cells goes down, yet potassium excretion *is* reduced. In that case, it would appear that the increase in hydrogen ion concentration has specifically depressed potassium secretion, although potassium concentration has presumably not diminished.

PAYNE: I should like to make some inquiry as to the movement of potassium in and out of the cells, in response to alteration in environment. One observes—my work is entirely clinical—that in the acute acidosis of an infant, serum potassium rises, although we know that the child is losing potassium from the body. If you make a child like that alkalotic rapidly by giving a lot of alkali, the serum potassium will fall to very low levels not by excretion but by going back into the cell. This must affect the interpretation of the urinary changes. Now on the question of excretion of acid, we have studied two types of renal acidosis, one where you have an acidosis in the blood, due to a loss of bicarbonate in the urine and an alkaline urine, and the "Fanconi" type, where you have a loss of bicarbonate and a very considerable loss of potassium. In the latter, if you give large quantities of alkali as sodium, you get an acute hypokalæmia demanding large quantities of potassium to control it. In the infantile renal acidosis, on the other hand, there is no potassium loss at all, for although there is an alkaline urine and a loss of bicarbonate, and you have to give large quantities of alkali in order to bring the bicarbonate up to a reasonable level, you still do not

lose potassium; the blood potassium remains level and the excretion of urinary potassium remains practically unaltered. So there is quite obviously in one case an association and in the other a dissociation between the alkaline urine and acidosis of the body and the loss of potassium.

BERLINER: I have the impression that some patients with renal tubular acidosis do lose potassium and some do not. I have not a sufficient idea as to what the defect in the tubules might be to offer a reasonable explanation for the difference. It is true, I believe, that, *in vitro*, muscle cells tend to take up potassium more easily from an alkaline than from an acid medium. The distribution of potassium between the cells and the extracellular fluid is quite possibly conditioned by that phenomenon. The total amount in the body, however, depends on the behaviour of the kidney.

DENT: Isn't it a question of degree, that the infantile ones can sometimes pass slightly acid urine, whereas the Fanconis cannot?

PAYNE: No, because I get more acid urines in the Fanconis than in the infantile acidoses.

SANDERSON: I have one observation, Dr. Berliner, which may be of interest here. In the course of some experiments on normal humans with Mersalyl, we obtained a relationship similar to what you have shown on your chart, namely that when giving an injection of Mersalyl to a normally hydrated, normally salt-containing man, the urine becomes acid and the potassium excretion falls. But we found that if we maintained our subjects for a few days on a salt-poor diet (not actually a salt-free diet) and then gave Mersalyl, the potassium excretion in the urine increased, but the *p*H change remained the same.

BERLINER: We have found that the effect of mercurials on urine *p*H is unpredictable, and we doubt that mercurials have any specific effect on urine acidification. The evidence does not really support the view that mercurials interfere with sodium transport by the tubules. We are inclined to agree with others who have recently come to the view that it is the chloride transport that is inhibited by mercurials; the potassium effect is probably entirely independent. As to the effect on potassium excretion, we have made the same observations as Dr. Sanderson, that potassium excretion rises when it has been low before the mercurial is administered and goes down when it has been high. This has caused a great deal of confusion and discussion. It is our belief that the only specific effect of mercurial diuretics in potassium transport is the inhibition of secretion; when a rise in excretion occurs, we believe it is due to an increase in the amount of sodium delivered to the mechanism which exchanges potassium for sodium.

BALDWIN: I have been wondering—I hesitate to say this in the presence of a tape recorder—if this apparent competition between hydrogen and potassium might just conceivably be a competition between the two of them for possession of the intracellular proteins. As a general rule the intracellular proteins are rather on the alkaline side of their iso-electric *p*H, which means they will be negatively charged, and in the ordinary course of events they are probably bound

mainly with the predominant intracellular ion, potassium. When hydrogen ions come along—they are extremely mobile, of course—they might well shift into the cells, probably into all the cells of the body with the exception of the red cells, and displace potassium. So that when you have a large population of hydrogen ions, you might expect the potassium concentration to rise. This would explain at any rate some of the findings we have been hearing about this morning, and of course there are occasions when things happen exactly in reverse. I am wondering whether there may be perhaps a sort of second order effect as opposed to a first order.

BERLINER: I see no reason for keeping that off the record.

ROBINSON: With a *p*H that does not appear to go below 7, I do not see a very large population of hydrogen ions outside the cells to rush in.

BALDWIN: Perhaps you do not see a large population of hydrogen ions for the simple reason that they get mopped up, largely by intracellular protein, as fast as they are formed.

ROBINSON: Yes, but I see them being formed in the cells, where there is carbonic anhydrase, much more easily than in the extracellular fluid.

BERLINER: We have gone astray in assuming that hydrogen ions could rush in and out of cells very easily. I think the evidence now suggests that the intracellular *p*H is to some extent independent of that of the extracellular fluid, at least under some conditions.

ROBINSON: I would like to ask for one further piece of information, Dr. Berliner. The slide which showed the effect of an infusion of lithium chloride did not give data for sodium excretion, and I would like to ask you whether lithium salts, and the maleate which you also mentioned, inhibit the reabsorption of sodium, as do carbonic anhydrase inhibitors.

BERLINER: Yes, sodium excretion goes up quite markedly—to the extent that the fall in titratable acidity and the increase in bicarbonate excretion is not covered by the increment in potassium excretion.

PITTS: Where does lithium enter into this picture?

BERLINER: I do not know. It may get involved in the transport mechanism without being very effectively transported by the cell.

DENT: One does not in general terms expect the first member of a series of the periodic classification to bear very much resemblance to subsequent members, so there is no particular reason why lithium should interfere with sodium and potassium, whereas there is every reason why sodium and potassium should interfere with each other. Fluorine is quite different from chlorine, bromine and iodine, and so on. That is why I am rather puzzled as to the theoretical basis for using lithium—most lithium salts are not even ionized.

BERLINER: You may remember that some four or five years ago lithium came into vogue as a salt substitute, and caused a lot of trouble. Consequently a number of studies of the handling of lithium in the body were done. Among these was a study of lithium excretion in the dog by Foulks, Mudge and Gilman. They found that potassium excretion increased markedly when lithium chloride was administered. We thought that if potassium excretion goes up, there must be an inhibition of urine acidification. Our experiments were done to test this hypothesis.

STANBURY: It may be of interest to draw attention to the reciprocal changes in hydrion and potassium excretion which occur with the rhythmic variations in renal function in the course of twenty-four hours. These are *spontaneous* changes occurring without the drastic stimuli

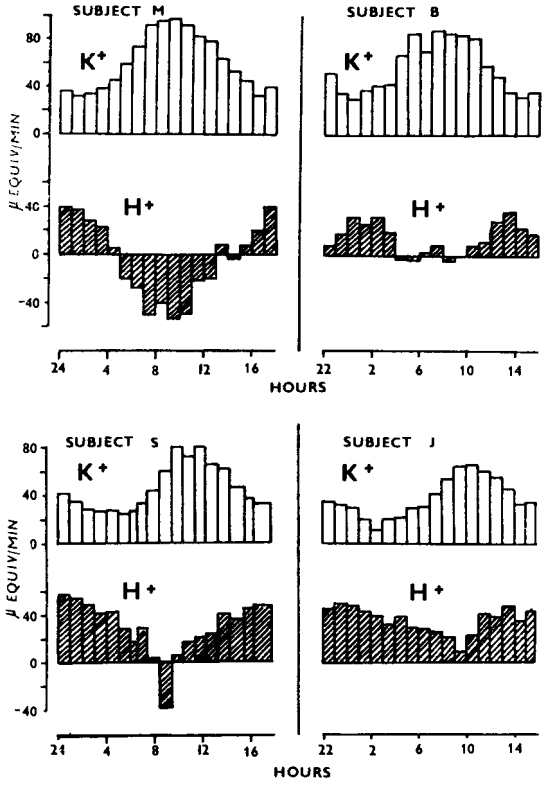

FIG. 1 (Stanbury).

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usually employed for demonstration of the reciprocal relationship between K^+ and H^+ excretion.

Fig. 1 shows observations on four subjects studied throughout a twenty-four hour period; the last eighteen hours of the period are charted. In each subject a generally inverse relationship is apparent between potassium and hydrion excretion. The same relationship is even more obvious in the forty-eight-hour observations of Fig. 2. The

subject remained awake throughout the first night and at about midnight there was a short lasting trough in his rate of potassium excretion, which was associated with a sharp peak in hydrion excretion. On the second night he slept normally, and then there was a long shallow trough in potassium excretion and a sustained plateau in hydrion excretion. Data from one of the subjects of the first experiment are charted in Fig. 3 where a multiple harmonic curve has been fitted mathematically to the potassium figures (open circles). The close relationship between potassium and hydrion (closed circles) excretion is apparent. The interesting point is that as much as 82 per cent of

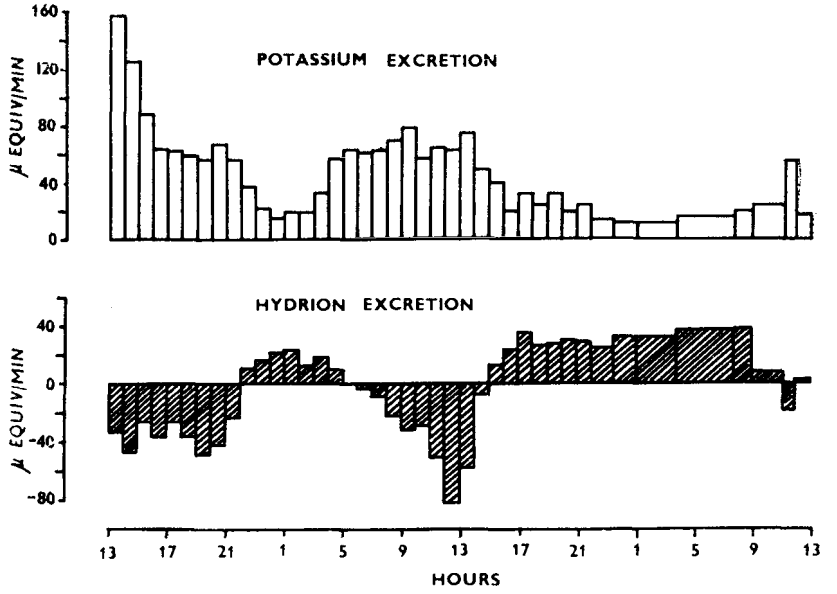

FIG. 2 (Stanbury).

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the squared deviations from the mean hydrion excretion can be accounted for mathematically from the curve fitted to the potassium changes.

MCCANCE: What diet was this subject taking?

STANBURY: Some were starving throughout a period of twenty-four hours, with a regular fluid intake; others took either glucose water or a biscuit hourly. It doesn't really make much difference whether the subject eats or not; the same relationship is usually apparent.

BULL: Dr. Stanbury, do *p*H changes affect the potassium binding on mitochondria?

STANBURY: Only when they exceed the usual physiological range, that is below 7·0 and in excess of 7·6 or 7·8. Then there is evidence of a release of mitochondrial potassium from its bound position. It is, however, associated with concurrent disturbance of the respiratory activity of the mitochondria. There is simultaneous depression of Q_{O_2},

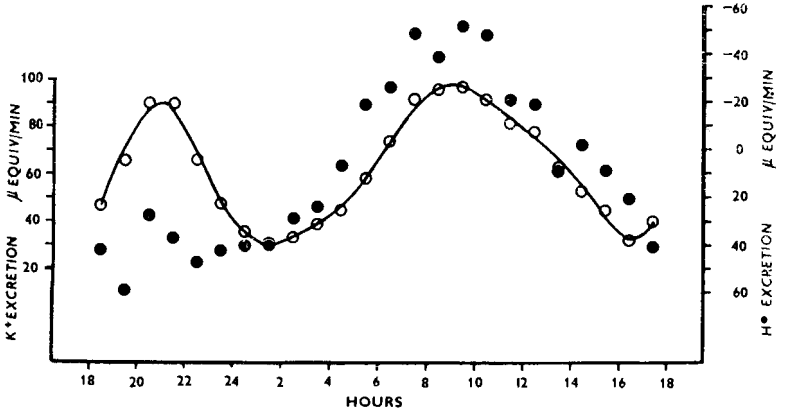

FIG. 3 (Stanbury).

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so whether we could relate it specifically to the *p*H rather than to the concurrent effects on respiration is an open question. It has to be studied in much more detail.

RENAL RESPONSE TO MASSIVE ALKALI LOADING IN THE HUMAN SUBJECT

P. H. SANDERSON

THE work I want to describe this morning arose out of a study of alkali therapy in peptic ulceration. It was planned to compare the results of ordinary "gastric" diet and intermittent alkali with those of continuous administration, through a fine-gauge stomach tube, of milk with added sodium bicarbonate. The object in the latter case was to maintain the gastric *p*H at 4 or over, and it soon became clear that while relatively small amounts of bicarbonate—20 to 40 g. a day—were sufficient for this in nearly all cases of gastric ulcer, duodenal ulcers required doses of 80 to 100 g. a day. Clearly it was important to know whether such large doses were safe, and if so, what changes occurred by way of homeostasis in the face of such a large and continued sodium and alkali load.

Fig. 1 shows the changes in plasma electrolytes, blood urea and weight in a male ulcer patient aged forty-three, receiving the largest dose of alkali we have given—140 g. of sodium bicarbonate (about 1660 m.Eq.) in 3 litres of milk daily for three weeks. The plasma CO_2 and sodium both rose by about 7 m.Eq./l., the former to some 40 m.Eq./l. The chloride fell slightly, while the potassium fell to about 80 per cent of its initial value and recovered when the alkali was withdrawn. The blood urea remained almost unchanged until the end of the alkali period, when it rose abruptly. The weight increased by nearly 7 kg. and began to decline when the alkali was stopped. There was some œdema of the ankles on the tenth day of alkali administration, but this had gone by the sixteenth day. At the height of the alkalosis the Chvostek and Trousseau signs were both negative, and apart

from some weakness of the arms and tenderness of the biceps muscles on the sixteenth day the patient was in excellent health and spirits throughout. The urine was free of albumen during the whole of this experiment.

These changes in plasma electrolytes were the most extreme

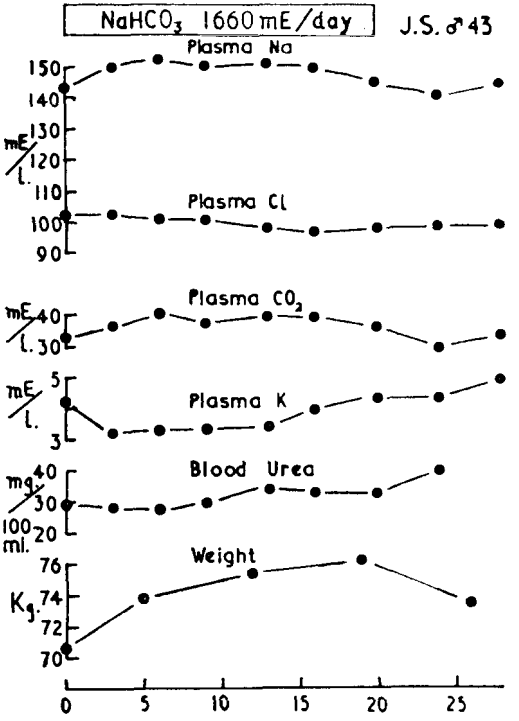

FIG. 1. Effect on plasma electrolytes, blood urea and weight, of the ingestion of 1660 milliequivalents (140 gm.) of sodium bicarbonate daily for three weeks.

that we have seen, with one exception, but in 21 other experiments with smaller doses of alkali, usually about 1000 m.Eq. daily, we obtained results which were qualitatively the same although not so gross in extent. The plasma CO_2 levels with these smaller doses were generally of the order of 35

m.Eq./l. Evidently man is well adapted to cope with an alkali intake of this order, at any rate for periods of up to three weeks.

Moreover, in our cases the symptoms which, since the observations of Hardt and Rivers (1923), have been thought to indicate alkalosis, namely, nausea, dizziness, headache and mental disturbance, were entirely absent. Two patients had attacks of diarrhoea, and in both of these an infective origin was likely, though not proved; one patient complained of abdominal fullness. The remaining patients had no complaints and were, on the contrary, very glad to be free of their ulcer pain, which usually disappeared in the first twenty-four hours of continuous alkali treatment.

Many cases have been reported in the past of severe renal damage accompanying alkalosis, with marked nitrogen retention. Damage of this degree did not occur in our cases; the blood urea levels rose a few milligrams per cent in ten cases, but all but one of these were receiving the ordinary ward diet in addition to 3 litres of milk. Their nitrogen intake was therefore increased by a maximum of about 16 grams a day, and the small rises in blood urea are more likely to be due to this than to any renal lesion. Fig. 2 shows the blood urea variations in six patients who received milk and alkali alone without any ordinary meals; their nitrogen intake varied between 13·5 and 16 g. daily, probably a little more than the ward diet and certainly not less. It can be seen that the tendency is for the blood urea to fall while the alkali is being given. When the alkali was withdrawn, the nitrogen intake being kept constant, a pronounced rise in blood urea occurred in four of the five cases in which the observation was made. This rise was too great to be accounted for by the contraction of body fluids indicated by the loss of weight at this stage, and it seemed possible that changes in glomerular filtration rate might be responsible. Fig. 3 shows the changes observed in eight cases in which either inulin or endogenous creatinine clearances were measured. There is a tendency for these clearances to rise while alkali is being given; and in every case

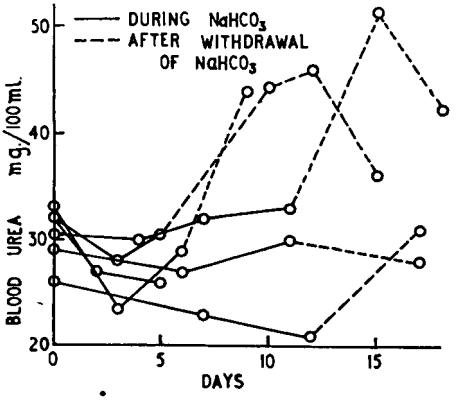

FIG. 2. Effect of alkali ingestion (1,000 milliequivalents = 84 gm. of sodium bicarbonate daily) on the blood urea. Note the tendency for the level to fall during alkali administration and to rise after its withdrawal.

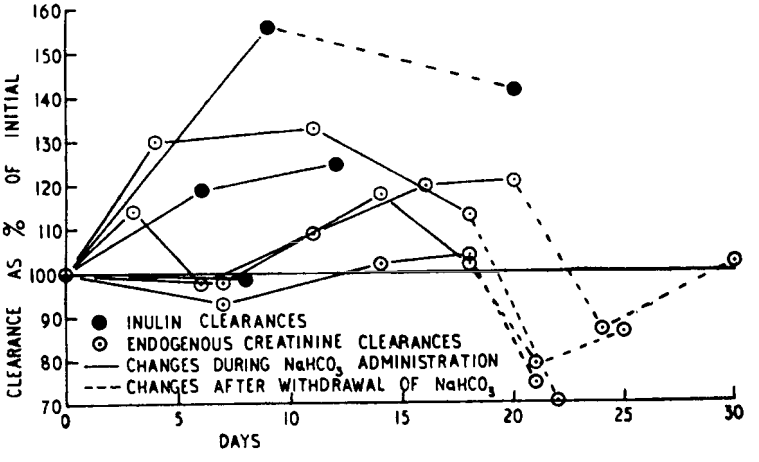

FIG. 3. Changes (expressed as percentage of the initial value) in inulin or endogenous creatinine clearances during and after alkali administration (1,000 milliequivalents = 84 gm. of sodium bicarbonate daily).

in which observations were made after the withdrawal of alkali, the clearances show a sharp fall, often well below the initial figure. Where later observations have been made, a return towards this figure is seen.

This seemed an adequate explanation for the changes in blood urea. However, Leaf and Couter (1949) in some similar

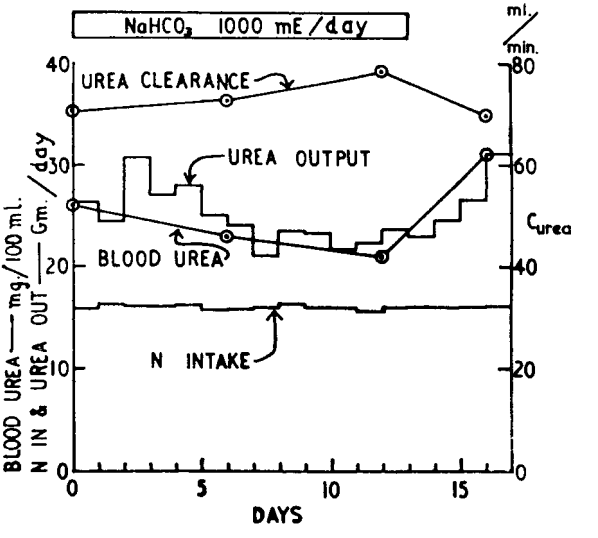

FIG. 4. Changes in urea output, blood urea and urea clearance in a man receiving 1,000 milliequivalents (84 gm.) of sodium bicarbonate daily. Changes in both urea clearance and urea output (the nitrogen intake remaining constant) are involved in producing the observed fluctuations in blood urea.

experiments in man, using about 500 m.Eq. of $NaHCO_3$ or NaCl per day for periods of three days, noted a fall in urea output when sodium intake was raised, and postulated the following chain of events:—increased sodium load —→ diminished secretion of suprarenal cortical hormones —→ diminished tissue breakdown —→ reduced urea output. Fig. 4 shows a study of this point in a subject maintained on a constant intake of nitrogen (milk only) and receiving 1000

m.Eq. (84 g.) of $NaHCO_3$ daily for part of the experiment. It can be seen that the urea output declines slowly during the administration of alkali, and rises after its withdrawal; the blood urea shows similar, but more marked, changes, so that the changes in urea clearance correspond to the changes in glomerular filtration rate indicated in Fig. 3. Evidently both effects, changes in filtration and changes in tissue breakdown, play a part.

Acute alkalosis induced in this manner evidently does not cause major renal damage. The question whether it can cause slighter degrees of damage is still being examined; of nine cases, receiving from 500 to 1660 m.Eq. $NaHCO_3$ daily, one (who also had advanced bronchial carcinoma) developed slight but persistent albuminuria, one had unexplained hæmaturia for two days, and the rest had no albuminuria. The severe renal damage and nitrogen retention occasionally seen in clinical alkalosis seem less likely to be due to alkalosis *per se* than to the dehydration and consequent renal ischæmia which almost invariably accompany it. A case illustrating this point is shown in Fig. 5. The patient, a man of fifty-two with severe pyloric stenosis and active duodenal ulceration, was admitted after some eight weeks of abdominal pain and vomiting. During the administration of $NaHCO_3$ a considerable alkalosis developed, but the blood urea fell. The patient at this time looked and felt well. When the milk and alkali was stopped, he was put back on to solid food. He immediately began to vomit once more; the plasma CO_2 rose again, but to a less extent than with alkali administration, whereas the blood urea rose sharply to over 120 mg./100 ml. The patient looked and felt extremely ill; in the three days following cessation of alkali he lost 5·3 kg. in weight, and then appeared grossly dehydrated. Solid food was stopped; the milk drip, without alkali, was started again; vomiting ceased, and the plasma CO_2 and blood urea began to return to normal.

The large gains in weight seen in our patients indicate that large amounts of water, and presumably corresponding amounts of electrolytes, were being retained. We investigated

this point by some simple balance experiments. Similar experiments, with smaller doses and lasting for shorter periods, have been performed on adults by Leaf and Couter (1949) and on infants by Gamble and his associates (1951). If a patient is fed with the milk drip alone, the problems of analysis of

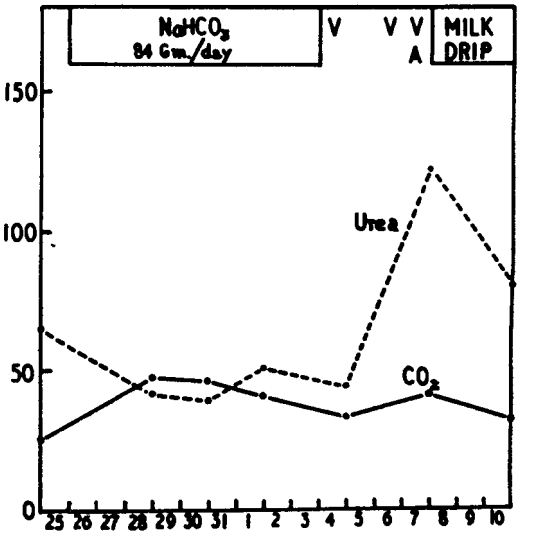

FIG. 5. Blood urea (mg./100 ml.) and plasma total CO_2 (m.Eq./l.) in a man with pyloric stenosis receiving 1,000 milliequivalents (84 gm.) of sodium bicarbonate daily. On the days marked with a "V" the subject vomited; "A" represents the aspiration of over a litre of gastric contents. Administration of bicarbonate caused severe alkalosis but lowered the blood urea; vomiting caused a relatively trivial alkalosis but a marked rise in blood urea.

intake become enormously simplified, and it is possible to calculate quite accurate daily electrolyte balances without special metabolic technique other than accurate urine collections. Fig. 6 shows the cumulative balance data in a case investigated in this way. The most striking feature is the very large positive sodium balance, together with a similar, but much smaller, positive chloride balance. When the sodium

load is removed, both these accumulations are rapidly lost and the *status quo* is restored in about five or six days. Changes in potassium balance are trivial by comparison, although they are, as might be expected, opposite in sign to the sodium changes.

If the conventional calculations, assuming all chloride

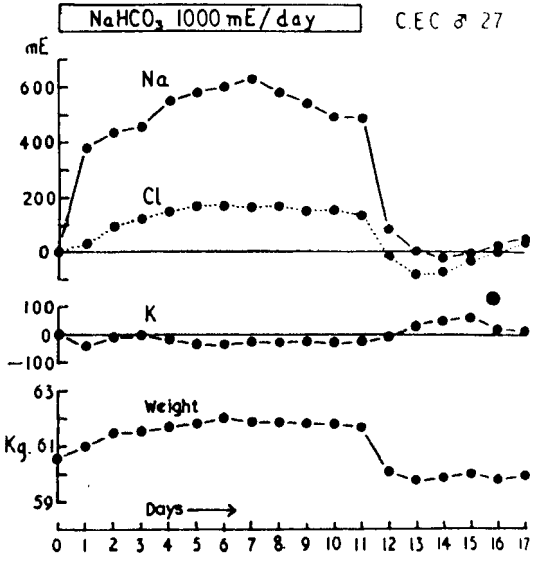

FIG. 6. Cumulative Na, K and Cl balances and weight changes in a patient receiving 1,000 milliequivalents (84 gm.) of sodium bicarbonate daily. Note the large retention of sodium, the smaller retention of chloride and the relatively trivial changes in potassium balance.

exchange to be extracellular, are carried out, movement of large amounts of sodium, in this case some 350 m.Eq., into the cells is indicated. If the opposite assumption, that no sodium enters the cells, is made, then chloride and water must move out of the cells. The only way to decide which assumption (if either) is correct would appear to be to measure the changes in extracellular fluid volume. This we are now undertaking,

using the inulin space as an index. So far only one case has been investigated.

To sum up: patients given large amounts of sodium bicarbonate in milk by continuous drip for up to three weeks tolerate it extremely well. The symptoms ascribed in the past to alkalosis are not seen, nitrogen retention does not occur and renal damage of any sort is probably quite uncommon. The more probable explanation of the major nephropathies seen in clinical alkalosis is gross dehydration with ischæmic lesions, either recoverable or permanent. The possibility that a long-continued alkalosis without dehydration could cause renal damage cannot be excluded. Large amounts of sodium are retained on this regime; the location of the retained sodium is as yet uncertain.

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DISCUSSION

BALDWIN: If alkali had been administered in, so to speak, big lumps rather than by the slow continual drip, would the damage be more or less?

SANDERSON: I don't think I can answer that. The reason why we gave it by drip was primarily to get continuous neutralization, and to see if that was an important therapeutic factor. I don't think you could get these amounts down except by stomach tube; the patients would not tolerate it. If they could tolerate the same amount in intermittent dosage, probably the renal mechanism would stand up equally well.

DENT: I believe the renal damage, on such clinical evidence as we have, is due not to the alkalis but to the calcium which nearly always accompanies it. Nearly all the observed cases of renal damage following large alkali intake have also had a high continuous calcium intake. In this instance the three litres of milk per day contains nearly 4 gm. of calcium in a very readily absorbable form. I should think the blood calcium must have risen to 12 or 13 or higher.

SANDERSON: We have estimated serum calcium in four cases and found no change.

DENT: In addition, the output in the urine would be very high and in the presence of an alkaline urine they would be very likely to form

stones—I should think the hæmaturia was probably due to stones or a bit of calcium phosphate.

SANDERSON: We cystoscoped and didn't find any.

DENT: In such a short duration experiment it would be in the form of gravel. I think that if you look into this milk and alkali intoxication, and try and sort out the clinical evidence, the ones who have had renal damage have always had a high calcium intake, and moreover there are some cases described with a high calcium intake without alkali—some of the ulcer patients in the literature have drunk a gallon of milk a day as sole therapy for several years, and there is just the same degree of renal damage as if they had had alkalis. If the alkali is also taken, it may possibly contribute to the renal damage by predisposing to stone formation.

BALDWIN: If it is due to calcium, would it not be a good idea to drip in citrate along with bicarbonate?

DENT: It is metabolized much too quickly once it gets into the blood.

PAYNE: Unfortunately, citrate increases the absorption of calcium experimentally.

DENT: I think one should just provide a low calcium intake and give alkali by itself.

SANDERSON: The trouble, of course, is that calcium carbonate is a very favourite prescription for ulcer. Most of these patients have been taking enormous amounts of it.

LEWIS: You don't necessarily have to have nephrolithiasis; nephrocalcinosis can occur.

SANDERSON: These were people who were only treated for three weeks.

LEWIS: If, for instance, after testosterone implants for multiple secondaries in bone, you find the patient has developed uræmia and you take the implant out, they get well again. So presumably it is a reversible condition.

SANDERSON: Yes. Dr. Dent, you investigated that boy with renal sarcoidosis, do you remember? As I recall, it was possible to push his blood urea up and down simply by altering his calcium intake.

DENT: Yes, that is so. This illustrated well the reversibility of calcium intoxication of the kidney, and actually Dr. Bull did something very similar, so that in one case by varying the calcium intake and in another case with cortisone (this has also been done by Dr. Howard at Johns Hopkins), the nitrogen retention was diminished.

LEWIS: What do you regard as the mechanism of that?

DENT: I just call it biochemical—I really haven't the faintest idea. It is not morphological, at least not as has been described here by Dr. Oliver. We (with Drs. Nabarro and Flynn) had the opportunity with another sarcoid to do a biopsy of the kidney, during the removal of a stone apparently due to a long-continued high calcium output with a high blood calcium. This kidney, which had been associated with a blood urea up to 60 mg. per cent and showed defective renal function on other tests, gave an almost normal section. When the patient was given cortisone, his blood calcium fell, his blood urea fell, and he now

appears to have normal renal function. But when he was dysfunctioning the biopsy showed his kidney to be apparently normal.

RAASCHOU: We have examined two cases of nephrocalcinosis by the aspiration kidney biopsy method (*J. clin. Invest.* 1952, **31**, 727). In the first case (hypervitaminosis D_2) we found calcium deposits in the medulla and normal glomeruli; in the second (hyperparathyroidism) calcium deposits were found both in the medulla and in the cortex and hyalinized glomeruli.

In this connection I should like to mention that in Copenhagen last autumn there was a tremendous epidemic of poliomyelitis with a large number of bulbar paralytic cases. In Blegdamshospitalet it was found later on, when these patients were cured of their respiratory paralysis, that they had kidney stones.

STANBURY: Were they treated by artificial respiration? Is it conceivable that as part of their therapy you exposed them to long-sustained alkalosis?

RAASCHOU: Yes, they were treated by tracheotomy and artificial respiration through a tube in the trachea. Indeed, it was difficult to teach the students to ventilate the patients at a normal rate and often we found that the patients were hyperventilated.

LEWIS: What about the calcium in the tubules, Prof. Oliver?

OLIVER: That depends on what method one uses to demonstrate calcium. If von Kossa, hæmatoxylin and alizarin stains are used the calcium may be missed entirely. The only way one can see it certainly is by micro-incineration. We did some experiments, administering calciferol and parathormone to rats, and in the early stages of the experiment the sections looked perfectly normal by von Kossa or hæmatoxylin or alizarin; one could not see any calcium. But when we used micro-incineration—we did not do it on sections but took out the nephrons and micro-incinerated them individually—then gross accumulations of calcium were found, particularly in the proximal convolutions, which by ordinary methods of staining had looked quite normal. So I am sure there is a pre-visible stage, as you might say, of calcification, this term applying to an increased calcium content in the protoplasm of the renal cells; it is only later when actual tissue damage occurs that calcium is visible by the ordinary methods. The process is of course then irreversible. This is what the pathologist usually sees in the form of blue chunks in various parts of the kidney. I suspect he has missed the important pre-visible stage of calcium accumulation which occurs after various factors have disturbed calcium metabolism.

STANBURY: Dr. Sanderson, did you by any chance measure the changes in plasma phosphate? I noticed that you get a depression of plasma potassium and Dr. Pitts has made the same observation during infusion of sodium bicarbonate. We get it with hyperventilation, but *pari passu* in hyperventilation with the fall in potassium you get a very significant fall in phosphate. I wonder whether it has been done?

SANDERSON: No.

PITTS: What was the total fluid intake in one of these cases?

SANDERSON: Four to five litres a day.

PITTS: In what concentration do you give bicarbonate, and was it tolerable?

SANDERSON: It is dissolved in three litres of milk. Sodium bicarbonate is the only suitable antacid because it is completely soluble. If you put in something that is not soluble, like magnesium hydroxide, it just blocks the drip. The patients are given also free access to water, and generally have a fluid intake of about four litres.

BORST: They must drink much more, otherwise it would be impossible to excrete that enormous amount of sodium. When the intake is 1600 mEq. they cannot excrete it without larger amounts of water.

SANDERSON: With that dose the intake was about five litres.

BORST: Even more; we tried to load our patients with a combination of sodium chloride and sodium bicarbonate and sometimes the trouble was not the enormous amount of salt but the intake of fluid that had to be given together with the salt, in order to prevent dehydration by osmotic diuresis.

BULL: In support of Dr. Sanderson's suggestion that these people do have episodes of circulatory insufficiency, I have seen a patient who had alkalosis due to vomiting. When the dehydration was corrected he remained oliguric for a while and went through all the typical phases of a tubular necrosis. I wonder, in some of these chronically contracted kidneys with calcium deposits, whether the calcium is not just the tombstone of nephrons that are knocked out in this way by tubular necrosis.

SANDERSON: I think probably both things occur. I reported a case of renal damage due to alkalosis quite a time ago now (Sanderson, P. H., 1948, *Clin. Sci.* **6**, 207). Professor Oliver has sorted out the changes taking place in these acute ischæmic ones, and looking back on it now I am sure that that was what this case was. This patient took about five or six weeks to come back to anything like decent renal function and he wasn't right even then. It was definitely a slowly recovering lesion, very similar in its general behaviour to the ischæmic kidney.

BULL: The particular patient I mentioned is living on a glomerular filtration rate of below 10 ml./min. and has been going along on that for about twelve months now. It is just not rising at all.

DE WARDENER: Dr. Oliver, have you ever had occasion to dissect out the kidney of a patient who has had tubular necrosis and recovered from it?

OLIVER: No. Dr. Bull has sent me some material but we haven't had a chance to get around to it yet.

DARMADY: From a morphological point of view I think the nephron is different from normal cases. We have had an opportunity of dissecting some of them following intestinal obstruction and one following death during alkalosis, and the nephron does not appear to be the same; it is not a focal thing, it has a much more localized appearance, affecting the lower part of the proximal tubule, and really falling more in line with the type of thing that one sees in specific poisoning cases.

SANDERSON: Those were cases in which there had been a lot of dehydration from vomiting?

DARMADY: Yes.

Part IV—General Problems of Electrolyte Excretion

MECHANISMS OF SODIUM RETENTION

JOHN P. MERRILL

THE factors implicated in the mechanisms of sodium retention are so numerous and from many points of view so controversial that a thorough analysis is well beyond the scope of a short paper such as this. Therefore, what I shall attempt to do here is merely to review some of the factors that have been implicated, in order that they may serve as a background for some other data, to be presented later in the session, on electrolyte excretion and possibly volume control and to mention briefly some experiences of our own relating to the rôle of the adrenal cortex in these mechanisms.

In congestive heart failure—the clinical example, *par excellence*, of sodium retention in the pathological state—the rôle of the kidneys has been implicated for some time. Several years ago, Dr. Arthur Merrill formulated this rôle in a fashion which has become a point of study and controversy ever since (Merrill, 1946; Merrill and Cargill, 1948). Briefly, he found, in patients with congestive heart failure, a reduction in renal plasma flow with somewhat less of a reduction in glomerular filtration rate, giving an increased filtration fraction. With various forms of mild exercise, the filtration rates of the majority of the cardiac patients studied fell well below what he chose as a critical level of 70 ml./min., while those of normal individuals did not. His interpretation of these results was that, with a decrease in the sodium load filtered and a constant rate of tubular reabsorption, the net result was sodium retention and œdema. He stated that there seemed to be a mechanism for reducing renal plasma flow when the cardiac output was insufficient for tissue demand. While the correlation with decreased glomerular filtration rate was substantiated by many other investigations, it became

apparent that filtration rate *per se* was not the whole story. A number of investigators have pointed out that while acute falls in filtration rate may diminish the rate of excretion of sodium, there is frequently a definite and marked time lag between the restoration of filtration rate to normal levels and a resumption of control rates of sodium excretion. This might occur whether the drop in filtration rate was caused by tilting, by vascular obstruction, or by a pneumatic cuff about the thigh. Other and longer term studies showed no relationship between recovery from congestive heart failure and change in filtration rate. One such study, carefully carried out, concluded that the most striking defect remedied during treatment was that of venous unsaturation or presumably hypoxia, and the authors postulated that hypoxia might stimulate tubular absorption of sodium, possibly by an adrenal mechanism without a change in filtration rate (Briggs *et al.*, 1948). Conversely (Surtshin, Rolf and White, 1953), when filtration rate is markedly elevated by growth hormone injections over a period of ten to seventeen days, there is no increase in sodium excretion or change in plasma sodium. It is apparent from these data that while acute changes in filtration rate may influence sodium retention, with sustained changes other factors are brought into play. This point of view is nicely summarized by the experiments of Mueller (Mueller *et al.*, 1951). In this study, constriction of one renal artery of a dog was accomplished and separate functions measured on the two kidneys. With light constrictions, the ligated side showed a marked decrease in sodium and water output without detectable falls in PAH or inulin clearances. With greater constriction, there was a decrease in the renal dynamics and an even further fall in sodium and water output. When the unligated kidney was then removed, the output of the ligated kidney rose to the pre-operative output of the two normal kidneys although the filtration rate remained well below that of the two normal kidneys. Three interesting points are made in this experiment which seem to fit the facts derived from other investigations. First, since

in a prolonged experiment sodium retention may occur as a result of decrease in glomerular filtration rate which cannot be detected by our present methods, it is entirely possible that the chronic decrease in sodium output is on this basis. Second, the presence of another normally functioning organ prevents the occurrence of plasma or serum changes which might occur as the result of the dysfunction of the contralateral organ and which then might modify tubular absorption. Third, when the normal organ is removed, the tubules in the ligated kidney can adjust, presumably to changes in the plasma, so that equilibrium again occurs as the results of increased sodium excretion: all of this, without a change in filtration rate. Similarly, experiments designed to decrease the cardiac output (Post, 1951) or to improve it with intravenous digoxin therapy (Eichna *et al.*, 1951) have shown a better correlation between cardiac output and sodium excretion than with the renal dynamic factor.

The necessity for maintaining an adequate intravascular volume has also been postulated as one of the causes of sodium retention. In such a view, congestive heart failure, hæmorrhage, shock, and dehydration have all in common the necessity to replenish intravascular volume by the renal retention of sodium and water, usually in the absence of marked changes in filtration rate. It has been shown that in experimental dehydration, marked sodium retention occurs hand in hand with a decrease in plasma volume and *vice versa*. However, in two reports (Welt and Orloff, 1952; Petersdorf and Welt, 1953) the expansion of plasma volume in normal individuals by hyperoncotic albumin produces *no* increase in sodium and water excretion, but rather a decrease. Since hyperoncotic albumin will move fluid from the interstitial space into the intravascular compartment, this discrepancy might possibly be explained by shifting the emphasis from the intravascular compartment to the total extracellular fluid. In this regard (Strauss *et al.*, 1952) it is interesting that the infusion of hypotonic saline and bicarbonate will *increase* sodium and chloride excretion in the face of decreasing levels

of serum sodium and chloride. The latter results were observed in the recumbent position with no change in creatinine clearances. With quiet standing, the effect of posture is to decrease sodium excretion (Epstein *et al.*, 1951) and even to obliterate the increased sodium excretion that follows a mannitol osmotic diuresis (Goodyer and Seldin, 1953). This postural decrease in sodium excretion cannot be abolished by the infusion of albumin with a resultant increase in plasma volume. To explain such a result in the face of increased plasma volume, which is usually associated with increased sodium excretion, one school of thought has postulated a redistribution of this volume, such that it does not make its presence known to a theoretical volume receptor, located somewhere in the head, whose job it is to regulate renal sodium excretion on the basis of "effective volume." It has been mentioned that the cirrhotic patient with ascites might be such an example. Here we find an abdomen full of fluid, but a dehydrated upper thorax and head so that the volume receptor is not aware of the fluid pooled below it—thus a diminished Na excretion. In an attempt to make the volume receptor aware of what is going on below, one group of investigators (Viar *et al.*, 1951) has inflated a pneumatic cuff around the neck of normal individuals and to some extent abolished the drop in sodium excretion found in the sitting position, without markedly modifying filtration rate or cardiac output. For further support for the locale for this volume receptor, it is pointed out that very marked retention of sodium and chloride with hyperosmolarity of the plasma can result from primary intracranial disease and from surgical and experimental procedures dealing with the frontal lobe and hypothalmus. These data, however, have been derived largely from normal individuals, or at least patients without congestive heart failure who may well represent an entirely different problem.

A factor which combines both the concepts of dynamic change and volume change is that of increased venous pressure. While in one set of experiments (Blake *et al.*, 1949) increasing renal venous pressure very markedly decreased

sodium excretion without change in dynamics, the evidence relating this to the clinical syndrome of congestive failure is not clear (Selkurt, Hall and Spencer, 1949).

Under normal circumstances, the plasma sodium level or tonicity and body sodium stores may determine whether sodium is to be excreted or retained. To some extent, however, it depends on how this tonicity is obtained. The individual made hypotonic with a water load will *excrete the water* to achieve normal tonicity, while the patient made hypotonic from sodium depletion will retain the sodium. In some instances, it has been pointed out that such normal tonicity may be sacrificed for the sake of volume. Of course, sodium excretion will also be influenced by other solute loads. The effect of potassium loads upon sodium excretion and the striking sodium retention that may result from potassium depletion will undoubtedly be dealt with later at this meeting.

Particularly important from the surgical standpoint are the effects of the nervous influences upon the renal handling of sodium. Deep anæsthesia in itself is thought to depress the sodium excretion. It is of interest that deep ether anæsthesia causes a greater fall in PAH and inulin clearances in the normal kidney than in the denervated kidney (Surtshin, Mueller and White, 1952), and that the same effect is noted, with response to renal dynamics in sodium excretion, when comparing the response of normal and denervated kidneys to positive pressure respiration. Sodium excretion may also be modified by chemical interruption of nervous pathways, although this may not apply to high spinal anæsthesia in normal man. It should not be forgotten that in the experimental animal (Blake, 1951), and presumably in the human, emotional stress probably acting through nervous pathways can produce sodium retention which is not correlated with decreased filtration rate.

The remarkable sodium retention and hypernatræmia as well as hyperchloræmia that may result from central nervous system disease has already been mentioned. In addition, this system may work in reverse, and a "salt wasting syndrome"

has been described in central nervous system disease. Intracranial pathology of many sorts as well as experimental frontal lobe and hypothalamic lesions may cause marked sodium retention with resulting hypertonicity of body fluids, in the absence of renal disease. In our own experience the syndrome of marked sodium retention and hypernatræmia occurring during the course of diuresis, following anuria, has been seen many times. These patients all have in common some degree of disturbance of the central nervous system.

The rôle of the adrenal cortex in sodium retention is too well known to need stressing. In assessing the action of the adrenal, however, these points must be kept in mind: first, that the action of the adrenal steroids upon any organ system is conditioned by the resting state of the organ at the time. An example of this, which has clinical application, is the fact that rats of comparable body weight given adrenal steroids may develop renal and gastro-intestinal lesions if not stressed, while rats artificially stressed under the same conditions have no difficulty with the same dose of steroid. In the latter instance, presumably, the cortical hormone goes to meet a need. Second, in terms of the renal response to adrenal steroids the hormones enhance the flexibility and rate of reaction but do not institute it and are not necessary for it to proceed at a slow rate. What this means in terms of sodium excretion is that the adrenalectomized animal, maintained on adequate hormone, may respond normally to an acid load by covering the anion with hydrogen ions and ammonia rather than sodium. Without adequate hormone, the rate of increased excretion of ammonia and titratable acid in response to this load is slower but eventually the urine *p*H may be nearly the same as the normal. Finally, the adrenal steroid effect is not, of course, limited to the renal mechanism. The transfer of fluid and electrolytes between the cellular and extracellular phase may have an effect upon sodium retention which is independent of the direct effect of the steroid upon the renal tubule. Needless to say, it is the influence of the adrenal upon sodium retention which has

frequently been postulated in those experiments in which sodium retention independent of changes in filtration rate have been shown to occur following the various procedures discussed previously. With regard to the unilateral ligation of the kidney, the fact that *both* kidneys are exposed to hormone effects whereas only one shows sodium retention need not bother us, since it has already been pointed out that the action of the steroid may depend upon the condition of the tissue on which it is brought to bear.

Other hormone effects of somewhat lesser importance are the sodium retaining properties of the œstrogenic hormones and to some extent testosterone. The antidiuretic hormone of the posterior pituitary has been stated by some to cause an increase in sodium and chloride excretion, but this is questioned by others (Murphy and Stead, 1951), who feel that under the influence of pitressin, sodium and chloride concentration may increase but that the rate of excretion is decreased possibly as a consequence of increased tubular reabsorption of water.

The relation of anoxia to renal circulation and the excretion of electrolyte has been studied by a number of observers. In general the data (MacDonald and Kelley, 1948; Axelrod and Pitts, 1952; Berger *et al.*, 1949) indicate that the effect of anoxia on the human kidney and on the animal kidney is to increase renal blood flow as well as to increase the excretion of electrolytes and water. Interestingly enough, however, hypoxia in the experimental animal has also produced adrenal stimulation and a need for increased steroid therapy in adrenalectomized animals (Giragossintz and Sundstroem, 1937; Dohan, 1942), and indeed it has been shown (Pitts, 1951) that both anoxia and ACTH will abolish the increased sodium excretion caused by a mercurial diuretic.

Because many of these mechanisms have been deduced from animal work or from data derived from normal humans, it might be interesting to make a few more observations about the problem from the clinical point of view. Although there is ample evidence from animal work that the rôle of the adrenal

cortex, in influencing renal dynamics, is mediated purely through its effect on sodium and water retention, a recent report has suggested that in humans it may have a direct effect in increasing filtration rate and plasma flow. As one reviews the data from this paper, however, it is suggestive that these experiments were also accompanied by sodium retention and plasma volume expansion. We have had the opportunity of studying this problem in the rather unique situation presented by a hypertensive patient with congestive heart failure, who had been bilaterally adrenalectomized for hypertension. Under these conditions, where the sodium balance was carefully controlled, treatment with massive doses of cortisone over an acute period of twenty-four hours produced no changes in the filtration rate or plasma flow.

Another fascinating problem from the standpoint of the adrenal steroid-sodium retention relationship, is that of the nephrotic. The nephrotic patient may show marked sodium retention in the face of normal or even supernormal filtration rates. Of great interest in studying the adrenal relationship to all this, is the diuresis which may follow the cessation of a course of ACTH therapy and to a lesser extent after cortisone and may even occur during the course of therapy. The majority of observers have found this to be associated with an increase in filtration rate, but it may occur in the absence of these changes. Interestingly also, the ability of the nephrotic to handle a sodium load is greatly improved during the course of a steroid induced diuresis. A frequent cause of failure of this form of therapy is the presence of renal functional impairment in the late stages of the nephrotic syndrome, manifested as part of chronic glomerulonephritis. Here it has been postulated that a marked decrease in filtration rate with a decrease in sodium load delivered to the tubules renders insignificant the adrenal component in sodium regulation. Even in these situations, however, a combination of factors which tend to increase filtration rate and to decrease hydrogen exchange for sodium (such as the use of albumen and aminophyllin concurrently with carbonic anhydrase inhibitors)

may produce small but definite increments in sodium excretion. As a rule, however, a marked impairment of filtration rate with nitrogen retention, mitigates against success of adrenal steroid therapy.

It was therefore of interest to us to observe the course of a patient with the nephrotic syndrome following bilateral

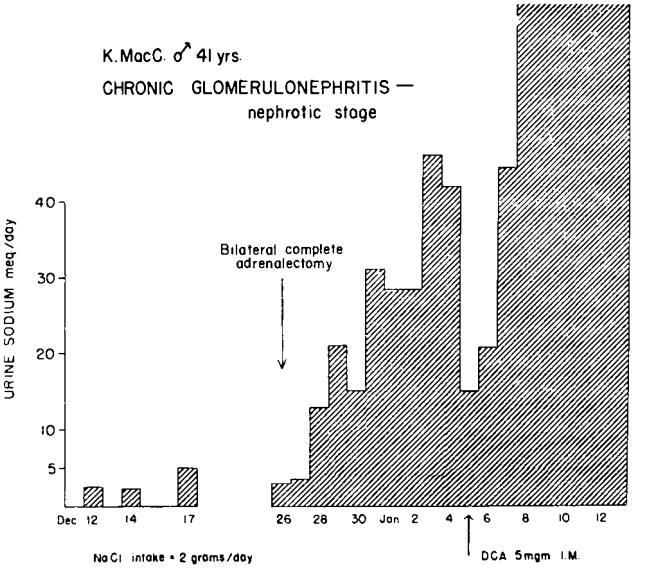

FIG. 1. Sodium diuresis following adrenalectomy in patient with chronic glomerulonephritis, hypertension and uræmia.

complete adrenalectomy for malignant hypertension, developing during the course of his disease. This patient had been previously treated by multiple courses of ACTH and cortisone, plus the adjuncts mentioned above. He had failed completely to respond. His filtration rate (inulin) was 20 ml. per minute; renal plasma flow 170, with a low filtration fraction, characteristic of the chronic nephritic. Blood urea nitrogen was elevated to 30 mg. per cent. Following bilateral adrenalectomy, there was a massive sodium diuresis, noted in Fig. 1,

with almost complete loss of œdema. That this sodium handling was under the influence of the adrenal cortex is indicated by the deoxycorticosterone acetate (DCA) effect noted.

The adrenal cortex has also been implicated in the sodium retention which follows major surgery. Here it is postulated that the trauma of surgery calls forth an out-pouring of "mineralo-corticoids." In Fig. 2, however, one can see the effect of bilateral adrenalectomy upon sodium excretion in a

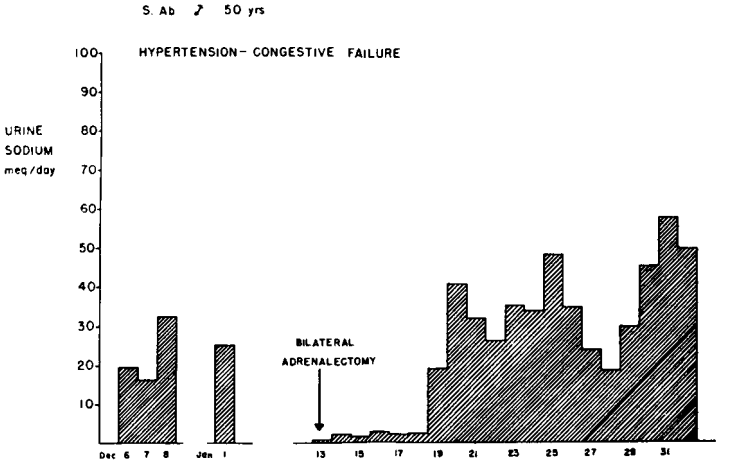

FIG. 2. Postoperative sodium retention in adrenalectomized patient. (Thorn *et al.* (1952), *Ann. intern. Med.*, 37, 972)

hypertensive patient with congestive heart failure. This patient was given no DCA and his dose of cortisone was totally inadequate to explain the sodium effect shown. Possibly these results may be explained by dynamic factors or factors other than those mediated by the adrenal cortex. It should not be forgotten, however, in the light of this observation as well as of the remarks made previously about the rôle of the adrenal cortex, that Ingle has pointed out that the decreased sodium excretion and increased nitrogen excretion which follows traumatic fractures in rats, may take place in the adrenalectomized animal if a small, but constant, dose of steroid is

given. In other words, from this clinical example, we may also perhaps imply that the presence of the adrenal cortex is not necessary to institute such a marked and sudden change.

Finally, it may be of interest to present another clinical example of sodium retention which has been influenced, we

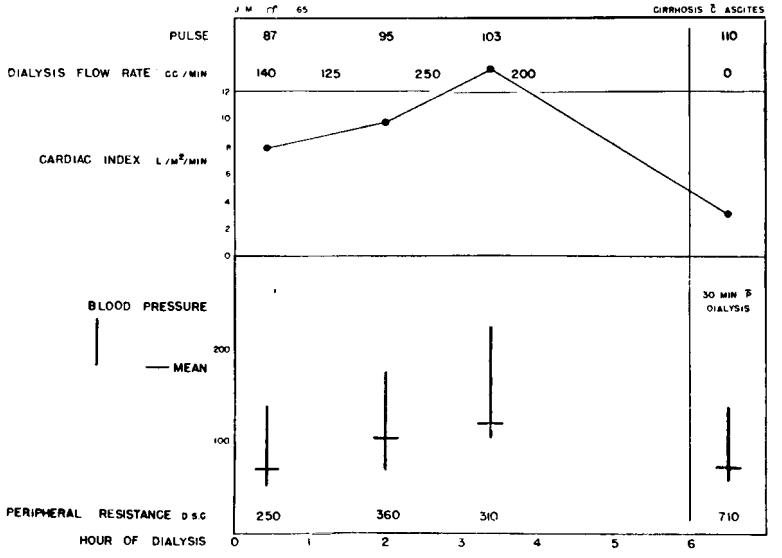

FIG. 3 Increase in cardiac output in cirrhotic patient during dialysis with artificial kidney

believe, by a factor not ordinarily postulated as important in this syndrome. Fig. 3 shows the course of a patient with advanced cirrhosis of the liver and massive œdema. This patient was treated with the artificial kidney in an attempt to reduce œdema by dialysis against a low sodium bath made hypertonic with glucose. His œdema was markedly reduced during the course of this therapy, but in a fashion for which we were totally unprepared. For during the four hour period of the procedure, he had a massive diuresis with the loss of

2700 ml. of urine containing 169 m.Eq. of sodium, the concentration of the latter rising from 4·6 to 80 m.Eq./l. at the peak of diuresis. During this period of time, the serum chloride remained constant, the serum sodium dropped slightly, the hæmatocrit rose and the body weight dropped as might be expected. With the rise of sodium and chloride excretion, there was a concurrent drop in urine urea nitrogen and potassium. This is significant, we believe, in view of the failure of Tarail (1951) and ourselves to induce sodium diuresis in the steady state cirrhotic with hypertonic glucose. A possible explanation for this might be the marked rise in cardiac output, which is shown in Fig. 3, and which we have found frequently to accompany this procedure. You will note that the initial cardiac index was high so that absolute decrease in output cannot be implicated in initial sodium retention. Although in this instance renal dynamics were not evaluated, in a similar situation in another patient, filtration rate changed very little, although there was some increase in renal plasma flow.

These observations do not of course simplify the problem of sodium retention, but they do emphasize, as is true of so many disease studies, that the influence of any single factor must be carefully evaluated in the light of the particular circumstances in which it appears, before one can draw conclusions as to the rôle of that factor in the genesis of sodium retention generally.

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DISCUSSION

BORST: I should like to recall Starling's theory that the renal output of water is closely related to the circulation. Starling emphasized the importance of this relationship for the stabilization of the circulation at an adequate level. The theory was advanced in 1896, when the important rôle of sodium chloride was not yet known. In 1938 it was found that patients with hæmorrhage from peptic ulcer often completely retained sodium and chloride. The excretion of NaCl was independent of the level in the blood and a retention could be found in the presence of a normal urea clearance. The excretion always started when the post-hæmorrhagic blood dilution was completed. The fact that retention of sodium chloride and water was also found in all other conditions that are accompanied by an impairment of the circulation led to the conclusion that circulatory insufficiency was the common factor in all conditions accompanied by a reduction in the output of extracellular fluid. It was regarded as a fundamental compensatory mechanism and not as the result of an impaired kidney function. The possibility that it was mediated by an increased adrenocortical secretion was considered, but this soon appeared inconsistent with the facts. We are still of the opinion that the "circulatory" retention of extracellular

fluid is a separate mechanism, not necessarily mediated by a reduction in glomerular filtration rate and not by adrenocortical hormones.

If we intermingle the three mechanisms influencing the sodium excretion the problem is made more complicated than is necessary. They can be easily distinguished, each has its characteristic excretion pattern. The "circulatory" retention of sodium is accompanied by a moderate retention of potassium, whilst the adrenocortical hormones which reduce the sodium output always initially increase the excretion of potassium. The effect of the adrenocortical hormones on the excretion of extracellular fluid is opposed and held in check by the "circulatory" mechanism. This explains why in normal people the suppression of the excretion of extracellular fluid during sustained treatment with ACTH or deoxycortone is only moderate and lasts not more than a few days.

To-morrow data will be given of observations by Dr. ten Holt on patients who responded to changes in the circulation with large alterations in the renal output of sodium chloride and water, in spite of an almost constant endogenous creatinine clearance. Subjects with and without adrenals reacted in the same way (Fig. 17, p. 280, Fig. 18, p. 281.)

BERLINER: How do you visualize that this mechanism has been mediated?

BORST: I do not know how and I think that from the clinical viewpoint that side of the problem is not the most interesting. Probably we should have been nearer the truth if we had not been able to determine inulin clearances and the level of adrenocortical hormones in body fluids. Now it is as if the problem of the retention of extracellular fluid is only a matter of glomerular filtration rate and adrenocortical function and no attention is paid to the fundamental mechanism that operates independently of changes in glomerular filtration rate and even in the absence of an adrenal cortex.

It may be that the experiments of the American physiologists Selkurt, Blake, and White and their colleagues* provide a solution. When the renal artery is constricted moderately, sodium output may fall markedly in spite of a constant renal plasma flow and glomerular filtration rate. It is as if two homeostatic mechanisms are linked together, the narrowing of the renal artery is compensated for by vasodilatation and simultaneously the tubular reabsorption of sodium chloride and water increases. So the elimination of waste products is kept at the normal level, whereas salt and water are retained, which adds greatly to an improvement in the general circulation. In both mechanisms the kidney is the receptor and the reacting organ. In this respect we should remember the work of Prof. Winton, who demonstrated that in the isolated kidney, the blood flow remains almost at the same level in spite of great variations in pressure.

BRADLEY: You are directing the focus of your attention at the renal

*BLAKE, W. D. *et al.* (1950). *Amer. J. Physiol.*, **163**, 422.
SELKURT, E. E. (1951). Transactions of the 3rd conference Josiah Macy, Jr. Foundation, Renal function, p. 103.
WHITE, H. L. (1950). Transactions of the 2nd conference Josiah Macy, Jr. Foundation, Renal function, p. 127.

circulation? Is there a relationship between the renal circulation and the output of sodium?

BORST: It may be. The fact is that in all conditions favouring an excessive circulation we find an increased output of water and sodium characterized by a diphasic diuresis. Conditions which tend to depress the circulation are accompanied by a selective retention of water and sodium chloride.

BRADLEY: Do you mean the cardiac output?

BORST: The cardiac output, yes, you can say that; however, it is not

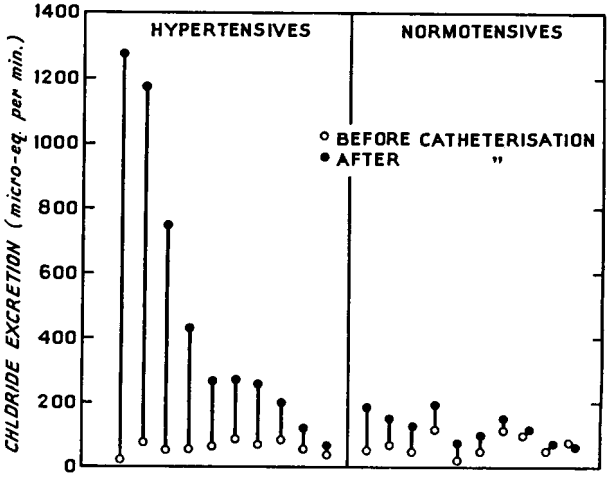


FIG. 1 (de Wardener). Chloride excretion before and after catheterization in dehydrated hypertensives and normotensives.

(From Miles, B. E., and de Wardener, H. E., *Lancet*, **2**, 539, 1953.)

so simple and therefore, I prefer the word "circulation." To-morrow I will comment on this problem.

BRADLEY: How do you account for the effect of emotion or of adrenaline in decreasing sodium output when cardiac output is increased?

BORST: In my paper an example will be shown of diuresis due to emotion. This is not rare. The diuresis is exactly of the type which follows injection of salt-solution, which suggests that it is due to an excessive circulation and not to a suppression in the release of antidiuretic hormone.

DE WARDENER: May I just show two slides? Fig. 1 shows the effect of catheterizing ten normal and ten hypertensive women after twenty-four hours' dehydration. I think the effect is pretty obvious. There is an increase in chloride excretion, particularly marked in the hypertensives and also to a much slighter extent in the normotensives. That

this is emotional, I think Fig. 2 shows us; it also compares the effects of a surgeon and a physician! It will be seen that urine flow rose as I came into the room. Then the catheter was put in and there was a great increase in urine flow which settled rather swiftly; finally I got a

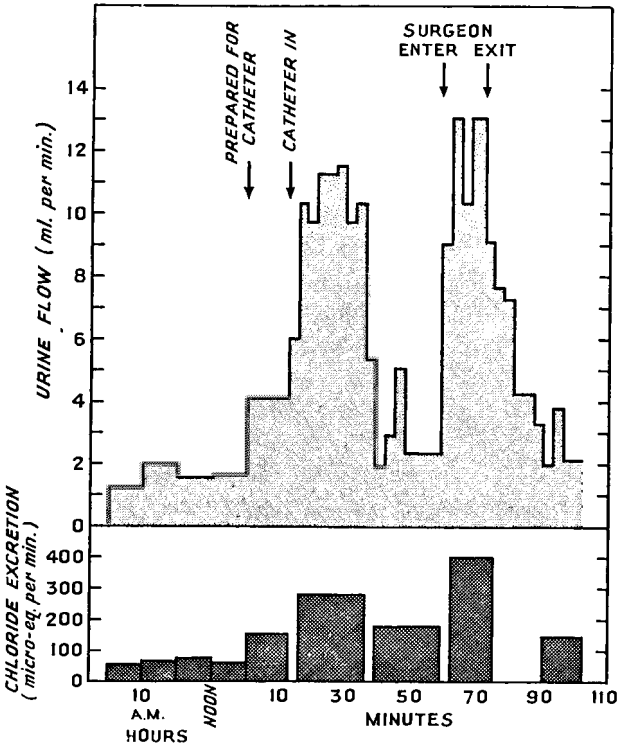


FIG. 2 (de Wardener). Chloride excretion (hatched) and urine flow (stippled) in a patient who drank a glass of milk hourly from 7 a.m. onwards. The figure shows effect of (1) catheterization and discussion of patient's blood pressure; and (2) short conversation with surgeon about possible operation.

(From Miles, B. E., and de Wardener, H. E., *Lancet*, 2, 539, 1953.)

surgeon to come in and discuss an operation for three minutes when the urine flow again increased rapidly and settled down again as he went out. It also shows the chloride excretion going up at the same time. This patient was actually having a glass of milk every hour and was not in a dehydrated state.

BORST: We have had similar experiences.

POST-OPERATIVE RETENTION OF WATER AND SODIUM

L. P. LE QUESNE and A. A. G. LEWIS

MOST of the facts which we have to report have been observed before, but we felt that the situation was too complicated to enable any clear conclusions to be drawn as to post-operative events, that observers had studied too many things simultaneously, and that it was really impossible to say whether it was the sodium or the water that was primarily retained in the days immediately succeeding a major operation. We therefore planned a series of cases with, as far as possible, a fixed intake of both water and electrolytes, so that the problem would be stripped down to its bare essentials. We decided to concentrate on the measurement of body weight and urinary volume, specific gravity and sodium. We did measure other things at the same time, but we wanted to keep our eyes clearly fixed on those changes, to find out precisely what does happen to water and sodium in the five or six days following a surgical operation.

For this purpose we fed a synthetic diet down a Ryle's tube for approximately four days before each operation for about sixteen hours out of the twenty-four, so that the patient was allowed a few hours up and about in the ward. The diet, which was of "Casinal," arachis oil and glucose, provided 2,600 calories but no sodium or potassium unless these were added in known amounts, which could therefore be kept fixed or varied without difficulty. Another advantage of this plan was that the fæcal sodium could be neglected, because the patients passed almost no fæces. When an enema was given on the third post-operative day it usually produced a small result with a very low electrolyte content. We therefore ignored fæcal losses, which were negligible compared with the changes we were observing. We also ignored sweat losses,

because the patients were not febrile, and there was no clinical evidence of sweating except perhaps for a short time immediately after the operation.

The cases were given 10 g. of salt (170 mEq.) per twenty-four hour period and 4 litres of water. The point of this was to give a high volume of urine daily with a fairly low specific gravity and a moderate sodium content as a background against which the changes could be observed. Moore (1953) has recently said that this work needs repeating without overloading the kidney with salt and water. However, the kidney can deal with this load before the operation: we are interested in how it deals with it afterwards. If you do not give much sodium and water you are not going to observe any retention. The water and electrolyte were given intravenously after the operation, with 150 g. glucose (providing 600 calories).

We studied nine cases without any potassium intake and seven with an intake of 100 mEq. a day throughout (except that on the day of operation this intravenous intake was dropped to 50 because of the low urine volume). The patients were also given a pint of blood while on the table. For the estimate of the blood loss we must take the surgeon's word. We did not make any exaggerated attempts to estimate it; it has been done before. Mr. Le Quesne is sure that none of these patients bled very much, and that half a pint of blood was an ample replacement for anything they had lost. It seems probable that most of them finished up with slightly greater blood volume than when they started. In allowing for the sodium loss, therefore, it was simpler to take some round figure which was obviously of the order of magnitude of the loss, and we chose 30 mEq., which seemed to us a reasonable estimate. It may have been a little more than this in some cases, it may have been a little less, but it does not alter the significance of the results.

The patients had morphine before the operation, and usually pethidine afterwards. Intravenous thiopentone was given, combined with an inhalation anæsthetic.

We observed, in every one of 15 patients weighed on the day after operation, a gain in weight, the average being 1·5 kg. in twenty-four hours. Fig. 1 shows the urine specific gravity and the urine volume which were associated with this gain in weight. The urine volume falls immediately after the operation, remains very low into the next day, and then rises again. The urine specific gravity shows a reverse change; urine chloride concentration parallels specific gravity with the U/P concentration well over 1 for the greater part of the time. Urine chloride excretion, however, falls when the anæsthetic is given, and stays low for the whole of that twenty-four hours, rising to the previous level during the next day. These are representative changes, and they have been found in every case, not only in gastrectomy but in a case of colectomy and in one of herniorrhaphy. In every case there is a gain in weight, which may be very considerable, associated with high urine specific gravity and low urine volume.

This retention of water occurred without necessarily any sodium retention. We took two of these cases and gave them no sodium on the day of operation except that in a bottle of blood: both were in negative sodium balance that day (Fig. 2). There is a weight gain of 2 kg. in spite of a negative sodium balance in case 11. The urinary specific gravity shows the same changes as before.

In other words, this retention over the twenty-four hours after operation is a *water* retention, and we have called it "primary water retention." It has nothing whatever to do with sodium retention, and occurs in its absence. The average duration of this water retention, in the recent cases which have been specially studied by Mr. Le Quesne from this point of view, was thirty-two hours. The longest lasted into the third day, in a case of colectomy (Fig. 3). During this period the patient was noted to be confused. She was suffering from nausea, and was clearly under the influence of a mild water intoxication. There was a slight rise in jugular-venous pressure, and there was slight pitting œdema over the sacrum by the third day. When the diuresis occurred the

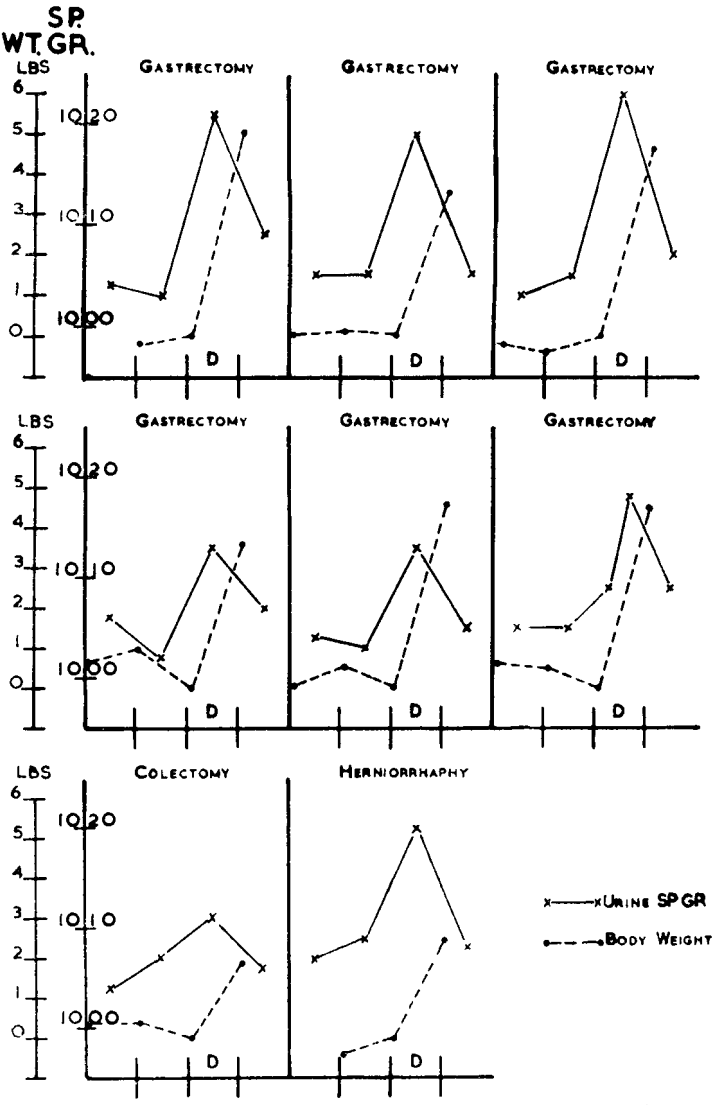


FIG. 1. Data from eight cases, showing the weight gain during the twenty-four hours following operation, and the urinary specific gravity changes on that and the following day. The weight gain in these eight cases averages 3·8 lb. (1·7 kg.), representing a retention of nearly 2 litres of water. (Le Quesne, 1953.)

transformation was obvious. Fig. 4 shows the water balance, with a litre a day allowed for insensible loss. Water retention occurs on the first and second day; the next day there is a

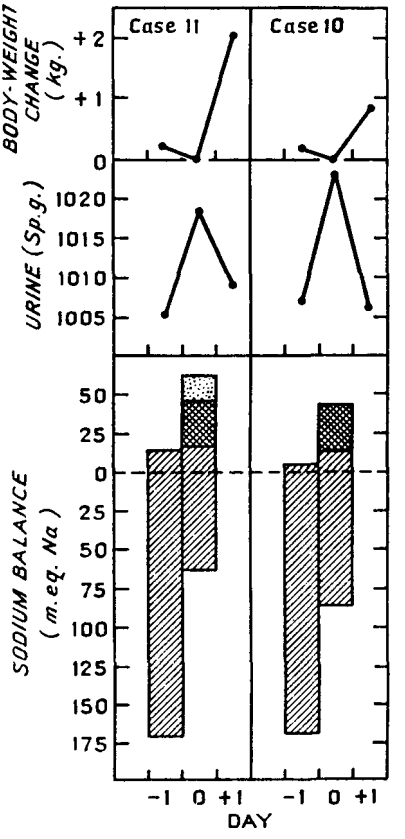
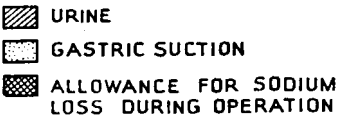


FIG. 2. Data from a case of colectomy and a case of gastrectomy, showing the sodium balance, urine specific gravity, and body weight. In all cases intake is plotted downwards from the base line. Output is plotted upwards from the intake, so that a negative balance shows above the line and a positive below it. Despite a negative sodium balance on the day of operation these patients showed the weight gain of primary water retention. (Le Quesne and Lewis, 1953.)



Key to Figs. 2, 9, 10 and 11.

diuresis. The weight fell by 1·7 kg. during the course of the third day. The urine volume rose at the same time; the plasma sodium, hæmoglobin and chloride rose as the water was excreted. The sodium balance is also shown. This is a case of pure water retention producing a water intoxication on the second and third day after operation.

We believe that this retention of water, which may in some cases be prolonged for two or three days and produce water intoxication, is due to the action of the antidiuretic hormone. We have a number of reasons for saying this. We have been able to demonstrate a greatly increased output of antidiuretic

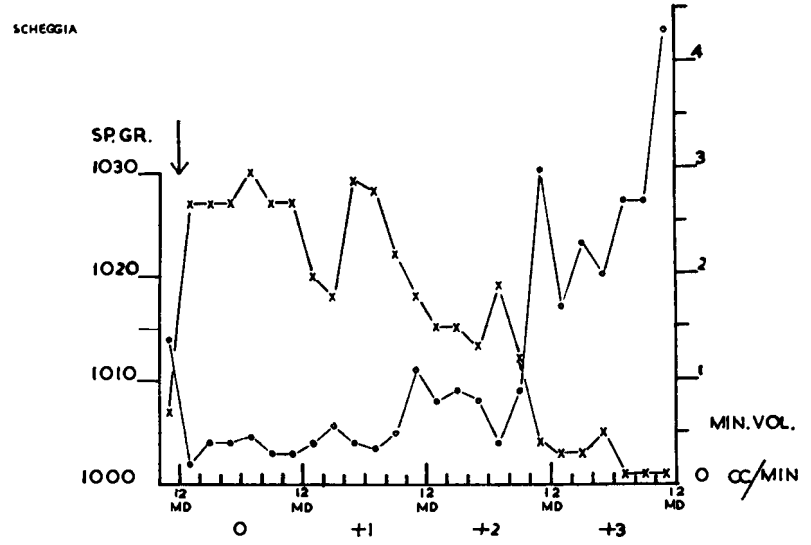


Fig. 3. Data from a case undergoing sigmoid colectomy, who developed signs of water intoxication forty-eight hours after operation. The arrow indicates the operation, and the graphs record the minute volume of urine (circles) and urine specific gravity (crosses) of successive four-hourly urine specimens. In this case the changes of primary water retention last for seventy-two hours after operation. (Le Quesne, 1953.)

hormone in the urine. Dr. Eisen, working with us in Professor Keele's Department, has shown it in the Van Dyke-Ames alcohol-anæsthetized rat, and we have been able to show it on intravenous injection of the human urine into the dog with diabetes insipidus. There is therefore a greatly increased output of antidiuretic hormone during this period. The responsible factor is not the normal osmotic change, because

the extracellular fluid in all these cases is actually hypotonic next morning. Moreover you can give glucose during the period of operation so as to preclude any rise in extracellular fluid osmotic pressure and still get this release. We believe that the major factors in the release of this hormone are the

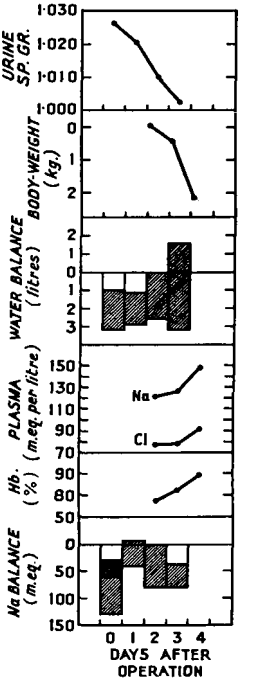


FIG. 4. Upper balance shows water intake charted below base line with output built up from this: output consists of urine volume, plus an allowance of 1 litre per twenty-four hours for insensible loss. The figure also shows specific gravity of pooled twenty-four-hour urine specimens, together with body-weight changes during last forty-eight hours. Lower graphs show plasma figures; dilution due to water retention is clearly seen, followed by return to normal levels after the diuresis. Lower balances show sodium exchanges: water retention was accompanied by negligible alteration in sodium balance, which clearly played no part in whole disturbance; cross-hatched area on day of operation represents allowance made for losses during operation. (Le Quesne, 1954.)

pain, anxiety and trauma associated with the operation. These are all factors which may produce a release of antidiuretic hormone. We have studied a great number of students and normals in the last four years, and we believe that an antidiuretic response to emotion is common. We have seen it in painful experiments, and we have seen it in anticipation of them. We have seen it in the malaise produced by nicotine and morphine, and following anxiety during the course of an

experiment. We think that the anæsthetic contributes in surgical patients. It was first pointed out by Beecher and his group (Burnett *et al.*) in 1949 that anæsthetics probably stimulated the output of antidiuretic hormone. We think that drugs such as morphine and pethidine also contribute to this. Morphine is a very interesting stimulus. We did a good deal of work on this at the Middlesex Hospital last year.

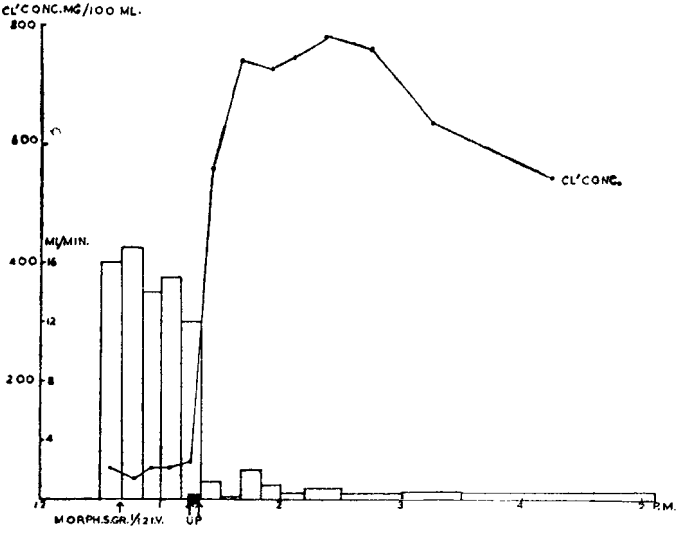


FIG. 5. The antidiuresis provoked by standing after an injection of morphine. (Lewis, 1953.)

It is difficult to assess the results finally, and we have not published them, but I would like to show you some of the results we have had in a group of normal subjects, to demonstrate the type of antidiuresis that may occur.

Fig. 5 shows the results in a normal subject lying down, and having a high water diuresis of 16 ml./min. This was allowed to proceed for an hour, in the middle of which he was given intravenously 5 mg. of morphine sulphate. The urine flow remained high and the chloride remained low until he got up

and walked around for five minutes, and then lay down again. He felt a bit dizzy, and though he did not vomit, there was a certain amount of malaise. The urine flow fell until the evening, and the urine chloride concentration rose. There was a prolonged release of antidiuretic hormone. It is very difficult to believe that it was all produced by the slight malaise which occurred after walking about for five minutes

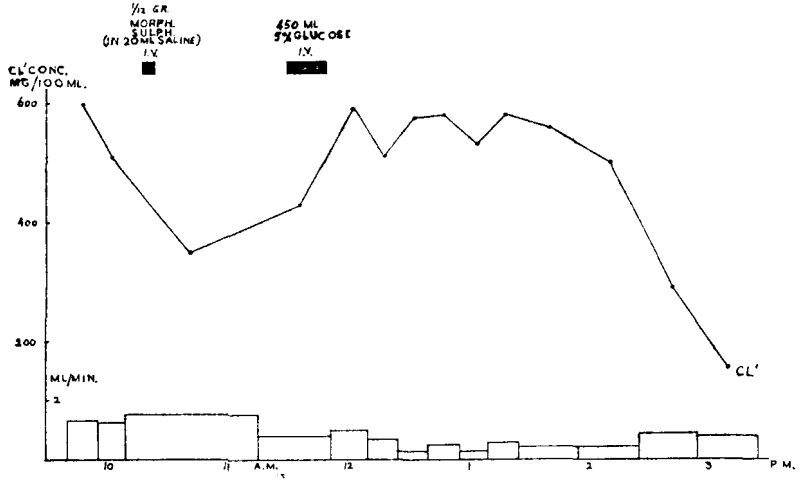


FIG. 6. The antidiuresis provoked by an experimental procedure (rapid intravenous infusion of glucose solution) after an injection of morphine. (Lewis, 1953.)

earlier in the day. We feel that the probable answer is that the effect had been augmented. Dr. Mary Pickford (Duke *et al.*, 1951) has shown that when morphine is injected into the supraoptic nucleus of dogs there is a release of antidiuretic hormone. We have rarely seen it in human subjects without malaise—we *have* seen it, but I have no doubt that the two effects are reinforcing one another.

In Fig. 6 the subject was actually given morphine before diuresis began. He was given rapidly a bottle of glucose intravenously with the intention of establishing a diuresis. At

this point he received a bigger needle prick than he expected. He was obviously a bit perturbed. Instead of diuresis and a rise in urine volume, the reverse is actually seen, lasting three hours.

Now, we believe this result is important, because one is inclined to think that when a patient has been given ¼ gr. of morphine there is no need to worry about his emotional

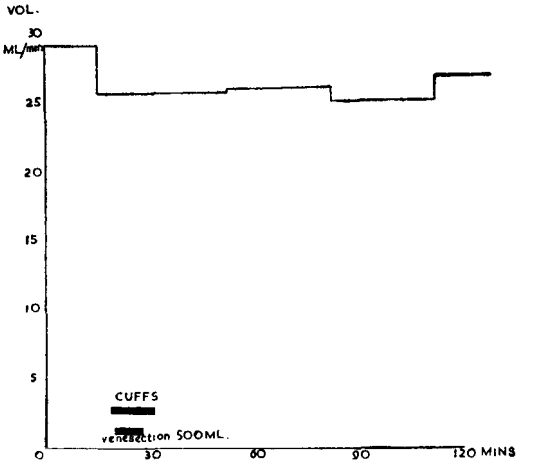


FIG. 7. The effect of reduction of blood volume (by rapid venesection after applying congesting cuffs to the thighs) on urine flow in water diuresis. (Lewis, 1953.)

reactions, but in actual fact the somatic response may be unmodified, or even augmented.

This antidiuretic release is not related to loss of circulating blood volume. In Fig. 7 the results are shown from another subject who was given a large water load. Congesting cuffs were then applied round the thighs, at a pressure equal to the diastolic, which was equivalent to producing a venesection of about 700 ml. A rapid venesection was performed through a large needle of a further 500 ml., so that the circulating blood volume was reduced by about 1200 ml. in a short period

of time. There was a slight fall in urine volume, due to the slight reduction in the filtered load by the fall in filtration rate as a result of the cuffs and venesection.

So much for the water retention. We feel that it is due to the antidiuretic hormone, released, not after the normal physiological stimulus, but after pain, anxiety, the anæsthetic, and the prolonged action of morphine, and not directly related to blood loss or to any volume change at the time of operation.

We observed a standard pattern of *sodium* retention (Fig. 8). On the day of operation there was invariably a retention of about 100 mEq. of sodium. We thought it was probably due to hæmodynamic factors such as those that Dr. Bradley and his group (Habif *et al.*, 1951) have been studying, but they found that these changes were very short-lived, and subsided at the end of the operation. Apart from the effect of the anæsthetic, we felt that adrenal cortical activity must be partly responsible for this sodium retention, because on the same day there was a coincident high output of potassium, higher than the nitrogen output, which is evidence of adrenal activity. The urinary sodium concentration fell in a very characteristic pattern in the three or four days after operation. It tended to fall to a minimum about the third or fourth day, when a further sodium retention occurred. We considered that this was also due to adrenal activity. It coincides with the sweat sodium fall noted some years ago by Johnson *et al.* (1950). We are also making salivary studies to get further evidence. If this second retention is partly adrenal in origin, it is difficult to explain why there is a big output of over 100 mEq. sodium the day after operation, separating the two phases. We would like to leave that for discussion, but possibly a compensatory mechanism on the day after operation has temporarily overcome the factors making for sodium retention.

This late sodium retention, although accompanied by water retention, is a primary sodium retention, because if sodium is not given (Fig. 9), so that there is a negative sodium balance, there is no weight gain. Evidently there is no water

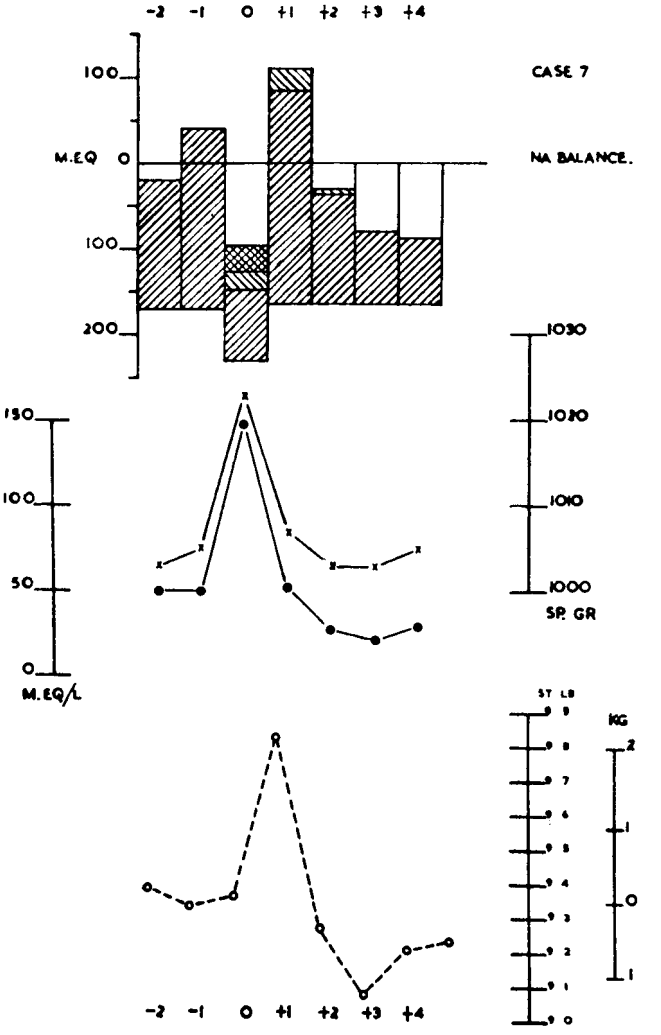


FIG. 8. Full data from one case receiving 170 m. equiv. sodium but no potassium throughout, showing, from above downwards, the changes in sodium balance, urine specific gravity, sodium concentration, and body weight, before and after operation (O). (Le Quesne, 1953.)

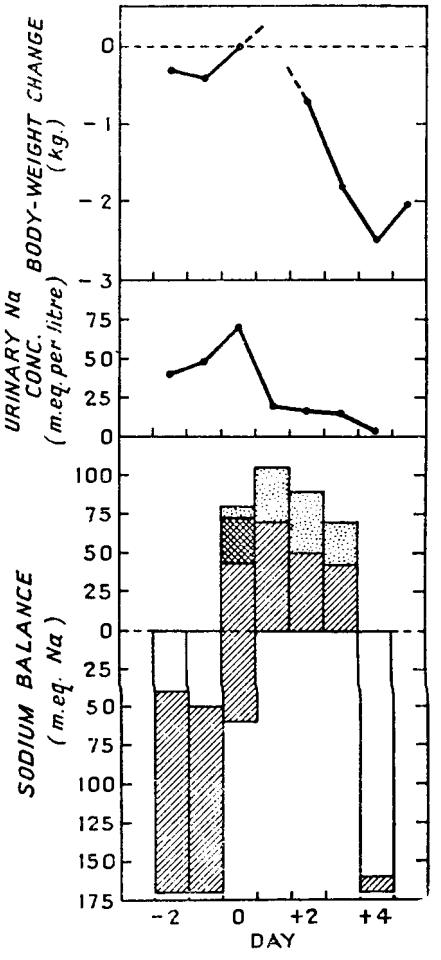


FIG. 9. Full data from a case in which no sodium was given after operation apart from that in a blood transfusion. Although the water intake was maintained throughout at 4 litres per day, the weight fell steadily, showing that water is not retained after the first twenty-four hours in the absence of sodium retention. This patient was not weighed on the morning after operation. (Le Quesne and Lewis, 1953.)

retention here without sodium retention: the latter is the primary event, and we call it "late sodium retention" to distinguish it from the early phase.

What is the effect of giving potassium? Fig. 10 shows three

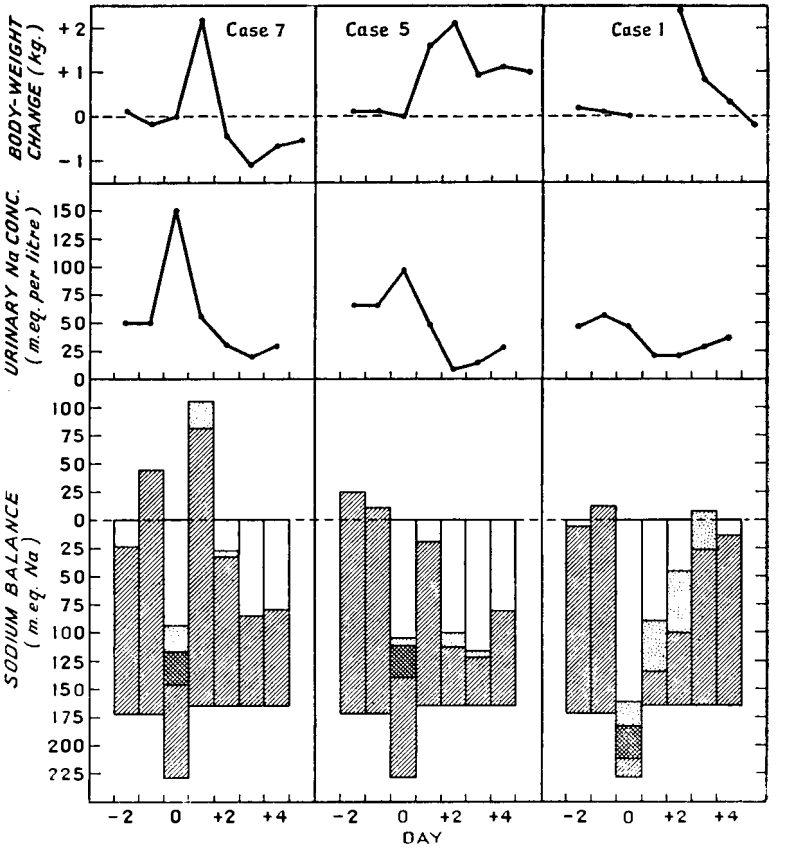


FIG. 10. Data from three cases receiving no potassium during the period of study. In each the weight is adjusted to zero on the morning of operation. In the first, early and late sodium retention are separated; in the second they are distinct, while in the third they have coalesced. In the last two cases there is continuous post-operative salt and water retention. (In a five-day period on an intake of only 100 g. glucose, the normal subject loses nearly 200 m.equiv. sodium.—Gamble, 1951.) (Le Quesne and Lewis, 1953.)

patterns of sodium retention when no potassium is given. In all there is sodium retention on the day of operation. The first case shows the late sodium retention clearly separated

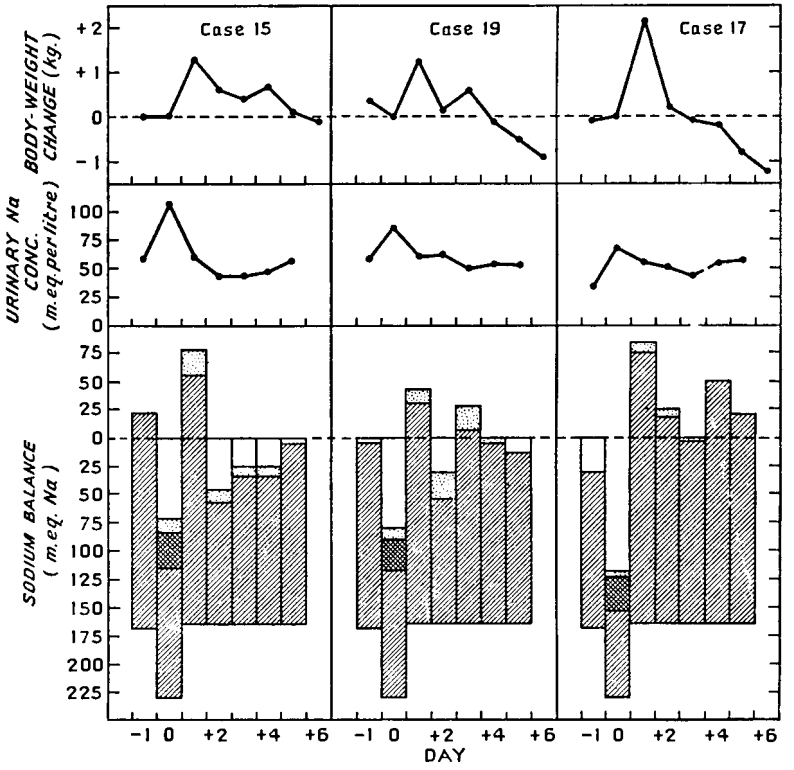


FIG. 11. Data from three cases receiving 100 m.equiv. potassium throughout the period of study, in addition to the intake of sodium chloride. Primary water retention and early sodium retention are still seen, but late sodium retention is less marked than in those cases receiving no potassium. (Le Quesne and Lewis, 1953.)

from the first by a period of negative balance. In the second the two phenomena are a little closer together, without a clear-cut negative sodium balance the day after operation. In the third the two phases have completely coalesced, and there is continuous sodium and water retention for three days

after the operation, with the weight going up and not coming down again below its previous level in spite of the very low intake of only 600 calories from intravenous glucose. At the end of four days the weight is still equal to the pre-operative weight. Notice the pattern of sodium concentration in the urine. Fig. 11 shows the effect of including 100 mEq. potassium in the intake. The effects are much less marked than in the previous cases—still there, but very much less obvious. We may say that the primary water retention was of the same order in the potassium cases as in the non-potassium cases. The difference was just significant, between the potassium and the non-potassium cases, as far as the primary water retention was concerned. As far as early sodium retention was concerned, there was no significant difference whether potassium was given or not. But the late sodium retention is very considerably reduced by giving 100 mEq. potassium a day. The weight changes are very much less striking than they were in the cases which were given no potassium at all.

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DISCUSSION

HELLER: I should like to say that as far as animal experiments go, I agree with Dr. Lewis. All kinds of stimuli do produce inhibition of urine flow. We (Ginsburg and Heller, 1953, *J. Endocrin.* **9**, 274) have recently analysed the jugular plasma of animals subjected to various "noxious stimuli" and have found increased concentrations of an antidiuretic substance in the blood. This, I think, bears out some of his observation in human beings. Secondly, it seems to me that there is no essential contradiction between the observations on the diuresis and the anti-diuresis group. I should not be surprised, for instance, if it could be shown that an outpouring of adrenaline, or perhaps noradrenaline, produced a diuresis and increased sodium excretion, and that then perhaps with a change in the nature or intensity of the emotional stimulus an increase in the output of the antidiuretic hormone supervenes. From the nature of an adrenaline diuresis, I should expect that it would be inhibited by the antidiuretic hormone.

DE WARDENER: May I make this position clear about emotion? There are three types of emotional disturbance, that is, three types of effect which emotion has on urine flow. One is that it may reduce it through the mechanism about which we have just heard, an outpouring of antidiuretic hormone; another is to increase it by an osmotic diuresis, and the third is an inhibition of the pituitary, giving a water diuresis. So you can have an excess production of ADH, an inhibition of ADH production or an osmolar diuresis. These are the three patterns which are well known, all produced by emotion—I don't say the same emotion.

LEWIS: We are not denying that. It is a question of the relative frequency. We have never seen a case like the one you published in the *American Journal of Medicine*,* where there was evidently an emotional inhibition of ADH. Marx showed in 1926 that you can inhibit ADH by suggestion under hypnosis and the patient will have a water diuresis. But we do not think that it happens very often.

DE WARDENER: I am sorry, I do not agree with you.

HELLER: Isn't one of the difficulties that when we say "emotion," we may not mean the same thing? The nature of the stimulus may be different in quality and in quantity and we may not realize that it is so.

PLATT: Dr. de Wardener, have you had the opportunity of producing an emotional state in the same person several times and do they always show the same pattern of response, or does one person at a different time or faced with a different emotion show a different response?

DE WARDENER: Yes, we have done it in the same person several times and have got more or less the same answer. In fact, the first case drew our attention to this by always producing a urine flow of up to 18 ml./min. That was our record, we never equalled it again. But we haven't tried enough to see what the effect would be of catheterizing *normal people during a water diuresis.* Our attention has been mainly focused on why this happens in hypertensives. In normals who were acting as controls we didn't do very much.

*12, 659, 1952.

BRADLEY: We failed to find a definite diuresis in man during infusions of *l*-noradrenaline and *l*-adrenaline. However, urine flow did decrease sharply on withdrawal of the drugs. In every instance both drugs caused a definite retention of sodium, despite maintenance of the urine flow.

I should like to comment on the effect Dr. Lewis has observed during the action of morphine. We have been interested in the possibility that the administration of morphine following operation might play a rôle in bringing about water and sodium retention. We followed one patient through a full day on repeated doses of morphine and failed to find evidence of an overall retention of water during the course of the day. On the other hand, a number of years ago, we made a study of the hæmodynamic effects of morphine, using the ballistocardiograph to measure cardiac output and the Hamilton manometer to measure intra-arterial pressure. We failed to find any definite hæmodynamic effect as a result of large doses of morphine. Robert Dripps in Philadelphia confirmed these findings a few years later but he found, in addition, that morphine markedly increased the susceptibility to orthostatic syncope. Thus there was, indeed, an important hæmodynamic action that was not apparent in the recumbent patient. It seems very likely that this response might play a rôle in producing antidiuresis.

LEWIS: We have studied that. Actually, the hypotensive effect of morphine is not, I think, very generally appreciated by clinicians. It may be one of the reasons why it is so successful in cardiac asthma. There is no doubt at all that if you take elderly people and give them morphine and stand them up, some of them will just go down flat. We have been studying younger patients and we took the blood pressure very carefully throughout the experiment; they showed no change from morphine at all, except the normal reaction to standing up. So it is not due to that, unless it is due to the circulatory adjustments that occur.

RAASCHOU: Is not the functional pattern of the kidney in heart failure identical with that in normal persons when they are tilted into an upright position, i.e. a slight reduction in the glomerular filtration, a reduction in the diodrast clearance, an elevation in the filtration fraction, a fall in the urine volume and in the excretion of sodium chloride in the urine? As Asmussen, Marius Nielsen and Howii-Christensen have emphasized, the circulation in the upright position of the human must be regarded as latently insufficient.

BLACK: I can briefly confirm what Dr. Lewis has said about the effect of potassium in modifying the post-operative response. Giving potassium in that sort of dosage also prevents you from getting a significant negative potassium balance. Whether that has anything to do with modifying the sodium relationships, I do not know.

SANDERSON: I should like to mention to Dr. Lewis some work which I think he would be interested in—it has not been published yet, but Dr. Victor Wynn has been doing it at St. Mary's (and I think he would give me permission to mention it). It is that post-operative water retention of the sort you observed presumably occurs almost constantly

in ordinary patients, but there are some patients in whom it goes on much longer, up to 10 days of so after operation.

LEWIS: We have never seen that.

SANDERSON: Of course, if those people are treated, as is still the fashion in some places, with liberal rectal water, they run into trouble.

BERLINER: Dr. Lewis, did the sodium diuresis occur at the time of the peak water retention?

LEWIS: No. It occurred when the water was coming out. The day after operation, you have a very large excretion of water, and the sodium is coming out too. Whether they could be entirely dissociated, I would not like to say.

BERLINER: There have been several recent studies of the effect of *large and continuous* doses of pitressin in normal individuals. When these subjects have retained considerable amounts of water, reducing plasma sodium to around 120 mEq./l. and expanding fluid volume, they suddenly have a considerable diuresis of sodium. I thought your observations might be related.

THE KIDNEY
G. E. W. WOLSTENHOLME

ELECTROLYTE EXCRETION IN STATES OF POTASSIUM DEPLETION IN MAN

M. D. MILNE, N. C. HUGHES JONES and B. M. EVANS

POTASSIUM depletion is seen in a number of clinical syndromes, including fluid loss from the gastro-intestinal tract, some cases of renal disease, diabetic coma, and after prolonged treatment with ACTH or cortisone. The biochemical situation is often complicated by other abnormalities, and therefore a study has been made of the metabolic and urinary changes associated with experimental dietary potassium depletion of moderate degree in healthy adult subjects. Any other abnormality or deficiency has been avoided as far as possible, but some observations have been made in states of combined sodium and potassium deficiency and others with unusually high sodium intake.

Three dietary regimes have been used to produce potassium deficiency:—

(a) A diet of three litres of milk which has been passed through a column of ion exchange resin charged with sodium ion.

(b) A low electrolyte diet similar to that described by Borst (1948) together with cation exchange resin charged with sodium taken by mouth.

(c) The electrolyte free diet of Bull, Joekes and Lowe (1949).

Details of these diets are given in Table I.

In the normal subject it is impossible to produce a severe potassium depletion by dietary means alone unless the experiment is prolonged. The amount of potassium lost in the urine rapidly falls (Fig. 1) and, unless ion exchange

Table I

Daily intake	*Daily K. (m.Eq.)*	*Daily Na. (m.Eq.)*
Diet I. Potassium depleted milk—3 litres. Glucose—125 g.	9	333
Diet II. Sucrose—150 g. Custard powder—100 g. Butter—50 g. Casilan (calcium caseinate)—50 g. Water—2 litres. Sodium ion exchange resin—40 g.	minus 12	80
Diet III. Glucose—400 g. Pea-nut oil—100 ml. Water—2 litres.	nil.	nil.

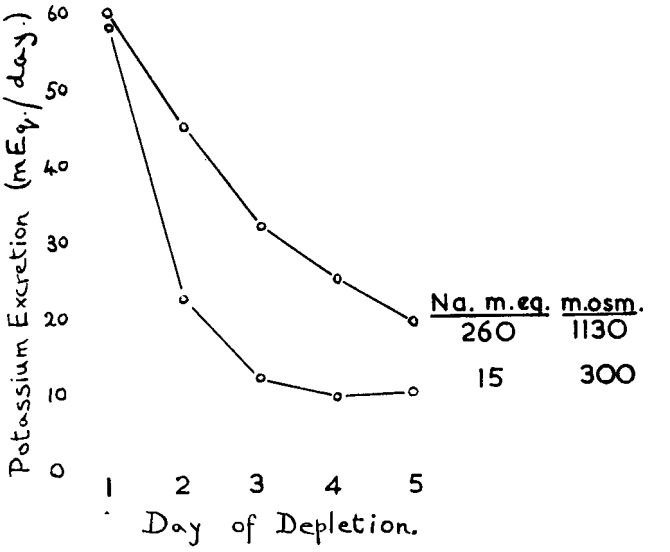

FIG. 1. Daily excretion of potassium during the first five days of potassium depletion. Above—milk diet. Below—protein and electrolyte-free diet. The sodium content and the osmolarity of the urines on the fifth day are compared.

resins are being taken, the fæcal loss becomes almost negligible. The potassium uptake of a sulphonic ion exchange resin taken with a low electrolyte diet averages 0·5 mEq. potassium per gram of resin.

Serum potassium rapidly falls to levels of about 3·5 mEq./l. during the first two or three days of depletion, but after that falls very slowly despite progressive potassium loss. A low serum potassium concentration is therefore a useful guide that potassium depletion exists, but is of no value in assessment of its magnitude. A metabolic alkalosis with increase of serum bicarbonate was seen in all cases, but was most obvious when the sodium intake was high. Bicarbonate levels up to 36 mEq./l. were obtained with a high sodium intake, but 31 mEq./l. was the maximum value with coincident sodium depletion. Evidence has been given (Black and Milne, 1952; Cooke *et al.*, 1952) that the cause of the alkalosis is an exchange of sodium and hydrogen ion for intracellular potassium with the production of an intracellular acidosis. The urine remains slightly acid in reaction, but rapidly becomes alkaline if potassium salts are taken. The competition of potassium and hydrogen ions for the exchange with sodium in the distal tubules as described by Berliner, Kennedy and Orloff (1951) may be necessary for the maintenance of the alkalosis but not for its initiation. Similar conclusions have recently been made from observations on nephrectomized, potassium depleted rats, by Orloff, Kennedy and Berliner (1953).

Urinary potassium levels are closely related to the degree of depletion rather than to plasma potassium concentration. The most efficient renal conservation of potassium is found in conditions of minimum osmolarity of the urine. As shown in Fig. 1, reduction in the daily urinary potassium is greater on the diet of Bull and associates (1949), in which both electrolyte and urea excretion are reduced to very low values. It will be shown later that the rate of potassium excretion is increased by osmotic diuresis both in the normal and depleted states.

There is a close relationship between the degree of depletion and the amount of potassium excreted following an oral dose of a potassium salt (Fig. 2). Urinary potassium levels are shown for a period of six hours after an oral dose of 7·5 g. potassium chloride and 2·5 g. potassium bicarbonate at

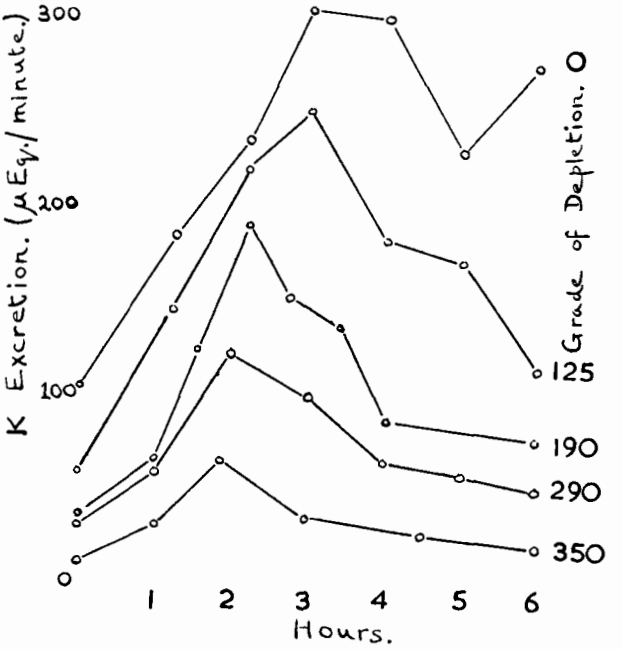

FIG. 2. Urinary potassium output following an oral dose of 125 m.Eq. of potassium salt at varying levels of potassium depletion from zero to 350 m.Eq. depletion.

varying degrees of depletion in a single normal subject. As potassium depletion increases, the urinary excretion becomes progressively less and the maximal excretion level occurs at a shorter interval from the time of potassium ingestion.

At the moderate degree of depletion obtained in these experiments (350 mEq. potassium or less) the urinary loss did not fall below 10 mEq. potassium per day. Considerably

lower levels of excretion have been obtained in œdematous patients treated with ion exchange resins over prolonged periods (Weston *et al.*, 1953). The ability of the kidney to excrete water is not impaired in uncomplicated potassium depletion. Conservation of potassium in the presence of maximal water diuresis is as efficient during depletion as in normal conditions and its excretion in unit time remains almost constant. It follows therefore that extremely low concentrations of urinary potassium are obtained by combining potassium depletion with maximal water diuresis. The lowest concentration recorded in this series of experiments was 1·2 mEq./l. Since the corresponding plasma potassium was 3·6 mEq./l., it is clear that the kidney is capable of excreting a urine of potassium concentration much lower than that of plasma. In contrast, osmotic diuresis produced by intravenous mannitol considerably increased the urinary potassium both in the depleted and normal subject (Fig. 3). The loss was much less in the former, showing that renal conservation is still partly effective.

There has recently been considerable interest in the mechanisms by which the kidney excretes an acid urine in potassium depletion despite an extracellular alkalosis. Berliner and his co-workers (1951) consider that there is competition between potassium and hydrogen ions for exchange with sodium in the distal tubule and therefore a greater exchange of hydrogen ion for sodium occurs in potassium depletion. It was considered that further information might be obtained by a study of the response to an increase in the existing alkalosis produced by sodium bicarbonate ingestion and by hyperventilation. The effects of both these stimuli were similar, and the response to a period of hyperventilation maintained at a steady level of 2·5 times the basal rate for a period of two hours is shown in Fig. 4. Alkalinization of the urine was found to be perfectly normal and the bicarbonate excretion was almost identical in the depleted and the normal subject, but potassium conservation was still effective in the former. Under these conditions *neither*

hydrogen ion *nor* potassium ion has exchanged for sodium, presumably due to lack of availability of both. This indicates that the intracellular reaction which governs the availability of hydrogen ion for exchange may play an important rôle in modifying the *p*H of the urine.

Potassium ingestion causes an increase of serum potassium with decrease of serum bicarbonate, together with an in-

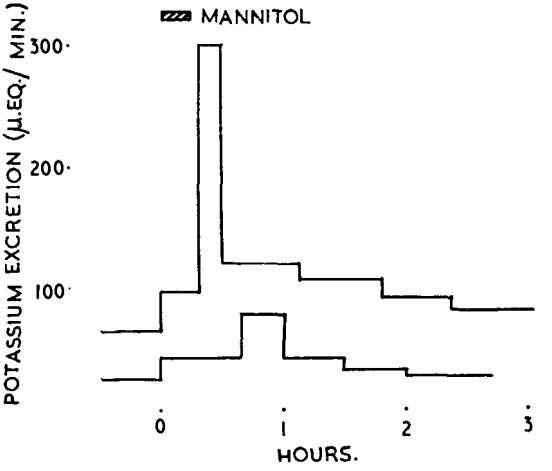

FIG. 3. Urinary potassium after intravenous injection of 300 ml. 25 per cent mannitol solution. Normal subject above. After potassium depletion below.

creased urinary excretion of potassium, sodium, chloride and bicarbonate. A special study was made of the relationship between potassium and bicarbonate excretion after oral potassium loading both in the normal and the depleted subject. As long as hypokalæmic alkalosis persists the bicarbonate output tends to be equivalent to that of potassium (Fig. 5). In contrast, in the normal state potassium excretion is much greater than bicarbonate. Close correlation between potassium and bicarbonate excretion is only seen during the period that urinary output of both ions is increasing. On a falling curve, the bicarbonate output decreases more rapidly than does

potassium. Similar observations were made after continuous oral loading at fifteen minute intervals with potassium chloride at a dosage of from two to six grams per hour (Fig. 6). Again, there is close correlation between potassium and bicarbonate excretion when the urinary output of each is

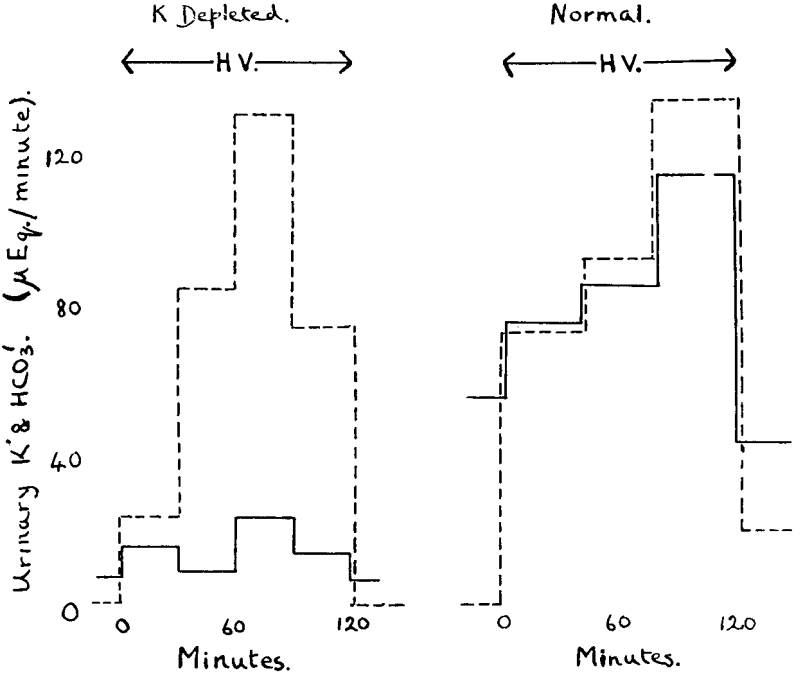

FIG. 4. Urinary potassium and bicarbonate excretion during a two-hour period of hyperventilation at 2·5 times the basal rate in a potassium depleted and a normal subject. Potassium excretion continuous line. Bicarbonate excretion—broken line.

rising, but as soon as potassium excretion reaches a constant equilibrium, the bicarbonate excretion falls. These facts suggest that bicarbonate is excreted when potassium is actually entering cells rather than when an equilibrium has been reached, and that a temporary intracellular alkalosis is produced by addition of base.

Fig. 7 illustrates the regression of bicarbonate and potassium in urine following oral potassium intake. Only values obtained when excretion levels are rising have been included since it is

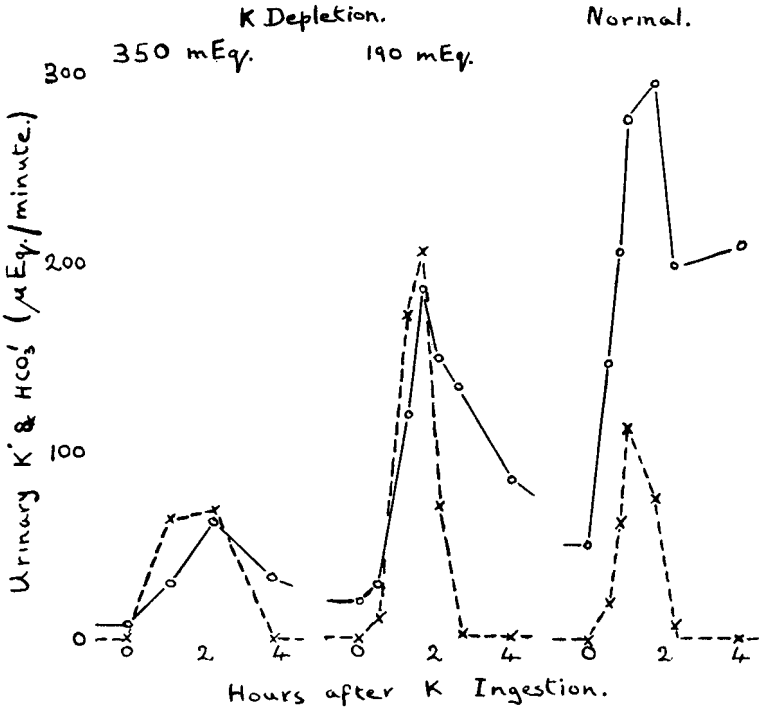

FIG. 5. Urinary potassium and bicarbonate excretion following 125 m.Eq. of potassium salt by mouth at 350, 190 and zero m.Eq. potassium depletion. Potassium excretion—continuous line. Bicarbonate excretion—broken line.

only under such conditions that a close correlation is obtained. The following regression equations were obtained:—

Potassium depletion:- $y = 1 \cdot 19x - 12$ (correlation cofficient $= + \cdot 86$).

Normal :- $y = 0 \cdot 38x - 27$ (correlation coefficient $= + \cdot 87$),

where "x" is the potassium excretion and "y" the bicarbonate excretion expressed in micro-equivalents per minute.

There was still a significant correlation between potassium

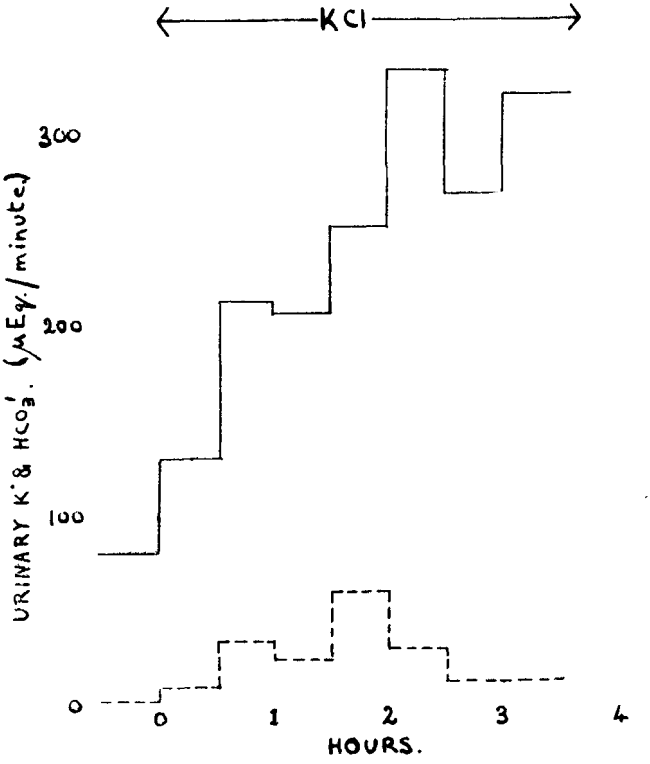

FIG. 6. Potassium and bicarbonate excretion during continuous oral ingestion of potassium chloride at a dosage of one gram every fifteen minutes. Potassium excretion—continuous line. Bicarbonate excretion—broken line.

and bicarbonate excretion when potassium excretion was steady or falling but it was much less striking:—

Potassium depletion:- corr. coef. $= +\cdot 44$; P less than $0\cdot 05$,
Normal :- corr. coef. $= +\cdot 58$; P less than $0\cdot 001$.

The results are in agreement with those of Roberts, Magida and Pitts (1953), who studied the relation between urinary potassium and bicarbonate during potassium infusions in normal dogs.

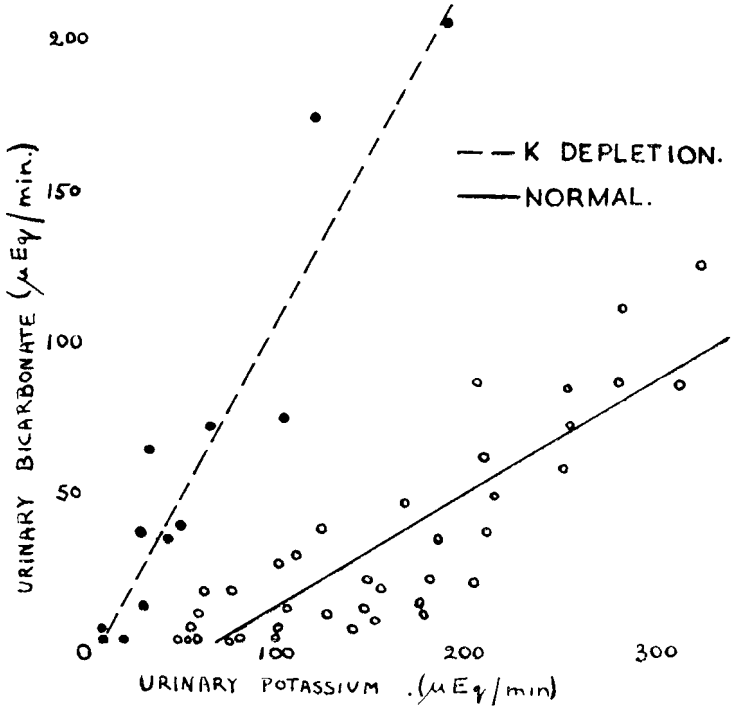

FIG. 7. Relationship between bicarbonate and potassium excretion after oral potassium intake in the normal and potassium depleted subject. Broken line—regression line during potassium depletion. Continuous line—regression in the normal subject.

Finally, preliminary observations have been made of the effect of potassium depletion on the diurnal variation in electrolyte excretion. It has previously been shown (Stanbury and Thomson, 1951) that this is unaffected by sodium depletion. In potassium deficiency, the diurnal rhythm of sodium, chloride, hydrogen ion and bicarbonate excretion was found

to be unaffected, but the diurnal variation in potassium excretion was greatly reduced. It is claimed that this observation is in support of a hypothesis that cyclical change of intracellular potassium and hydrogen ion concentration is the prime mover in the initiation of this diurnal rhythm. It has been demonstrated that change of potassium excretion during depletion causes a greater variation in the excretion of other electrolytes than a corresponding change in the normal state. It would therefore be expected that a cyclical change of potassium would be reduced during depletion if other electrolyte rhythms persisted unchanged.

Many arguments can now be brought against the earlier view that cyclical changes in ventilation directly related to sleep and wakefulness are the cause of this important diurnal rhythm. Attention is now drawn to complex metabolic changes within the cell with especial reference to intracellular acid-base balance. It is perhaps of significance that the most obvious electrolyte rhythm in the plasma and extracellular fluid, that of inorganic phosphate, must clearly be closely associated with variation of intracellular anion.

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DISCUSSION

BERLINER: There are many points on which I would like to comment, but I will have to sort them out. The one that should particularly be mentioned is the fallacy of equating hydrogen ion *excretion* with

hydrogen ion *secretion*. The hydrogen ion secreted is expended largely in reabsorbing the bicarbonate filtered; the bicarbonate excreted or the titratable acid represent only what is left over after this titration. In the falling phase of the bicarbonate excretion of the experiments which have been described, it is important to remember that we are dealing with a much decreased filtered load of bicarbonate, because at that time the plasma bicarbonate has usually fallen to a considerable extent. If we had some measure of the hydrogen ion actually secreted, we would probably find that the hydrogen ion secreted and potassium ion excreted are just as well correlated in that phase as they are during the rising limb of the curve. In the data of Roberts, Magida and Pitts, to which you referred, and in our data as well, we find that if potassium is given to a dog at a steady rate, the urine *p*H rises, stays up for a variable period of time and then goes down again. The latter fall we have associated with a marked reduction in plasma bicarbonate concentration.

PITTS: Dr. Berliner, how do Dr. Milne's experiments with mannitol in the depleted versus the normal state agree with your hypothesis that the urinary potassium is derived from a secreted moiety and not from a non-reabsorbed moiety?

BERLINER: I think they fit fairly well with that hypothesis. Since bicarbonate comes out in the urine, an amount of sodium at least equal to that bicarbonate must have reached the region in the tubule in which potassium and sodium are exchanged. I am more inclined to switch the emphasis from the total osmols excreted to the amount of sodium delivered to the exchange mechanism. I think the much greater and more rapid fall in potassium excretion in the individual with a low sodium intake is well in accord with this idea.

TREATMENT OF ELECTROLYTE-FLUID RETENTION BY ULTRAFILTRATION OF THE BLOOD *IN VIVO*

NILS ALWALL

THE essential pre-requisites for the studies to be briefly reported in this paper are partly an apparatus for ultra-

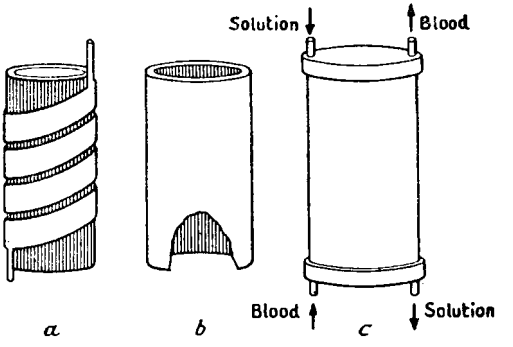


FIG. 1. Diagrams showing construction and assembly of Alwall's ultrafilter-dialyser (Alwall, 1947): (*a*) inner cylinder with cellophane tube wrapped round it; (*b*) outer cylinder, grooved on inner aspect; (*c*) cylinders assembled with top and bottom.

filtration of the blood *in vivo*, published in 1947, and partly the relatively high incidence of pulmonary changes of the type termed "uræmic lung" observed on chest X-rays among our renal patients, i.e. severe cases admitted to our clinic from the whole of Sweden.

The Ultrafilter-Dialyser

Fig. 1 shows the construction and assembly of Alwall's ultrafilter-dialyser (1947). The arterial pressure (or a pump)

drives the blood from an artery (or a vein) through the apparatus back to a vein. The cellophane tube through which the blood flows is wound round a cylinder of stainless steel with a longitudinally rifled outer surface. This is placed within another cylinder whose inner surface is similarly rifled. The apparatus is closed air-tight with bottom and lid. In this way the tube lies supported by rigid bars in a narrow space.

The cellophane tube of the apparatus used for patients is 12·6 metres long and its surface is about 8,500 sq. cm. A smaller model used for rabbit experiments has a tube 125 cm. long with a surface of 825 sq. cm. A suction pump or siphon brings about the negative pressure. At a filtration pressure of 700 mm. Hg the larger apparatus gives some 750–1,000 ml. filtrate per hour, and the smaller one some 100 ml. per hour. The body weight of the patient or animal is controlled continuously during the ultrafiltration treatment.

During dialysis, while the blood flows through the cellophane tube, a thin stream of electrolyte solution flows in the opposite direction along the grooves on the cylinders.

Uræmic Lung—Fluid Lung?

The following is a brief account of our 16 published cases of uræmia due to acute or chronic renal failure, in which chest X-rays revealed changes of the type indicated in the literature as "uræmic lung," "uræmic œdema," or "uræmic pneumonia," and so on (Fig. 2). We are reporting here only cases in which treatment described below could be carried out (Alwall, Lunderquist and Olsson, 1953). Thirty-four cases have been previously reported in the literature (*inter alia* Bass and Singer, 1950; Bass *et al.*, 1952).

We have started with the assumption that fluid retention constitutes a fundamental pathogenetic element. When radiological changes of this kind were noted at the routine X-ray examination of the newly admitted uræmic patient, which generally takes place on the first day, the following treatment was applied: induced thirst, or very reduced fluid

intake; and 200–400 ml. of a 25 to 50 per cent magnesium or sodium sulphate solution were given daily by mouth or administered by means of a stomach or duodenal tube. Should the patient vomit the solution, then 100 ml. of the solution were introduced rectally three to four times daily. Severe diarrhoea is induced especially by means of the former administration. Loss of up to 4 kg. of weight in one day can be registered.

It must be noted, however, that the patient loses relatively more water than electrolytes. The electrolyte surplus can be removed by subsequently dialysing the patient's blood against a hypotonic electrolyte solution.

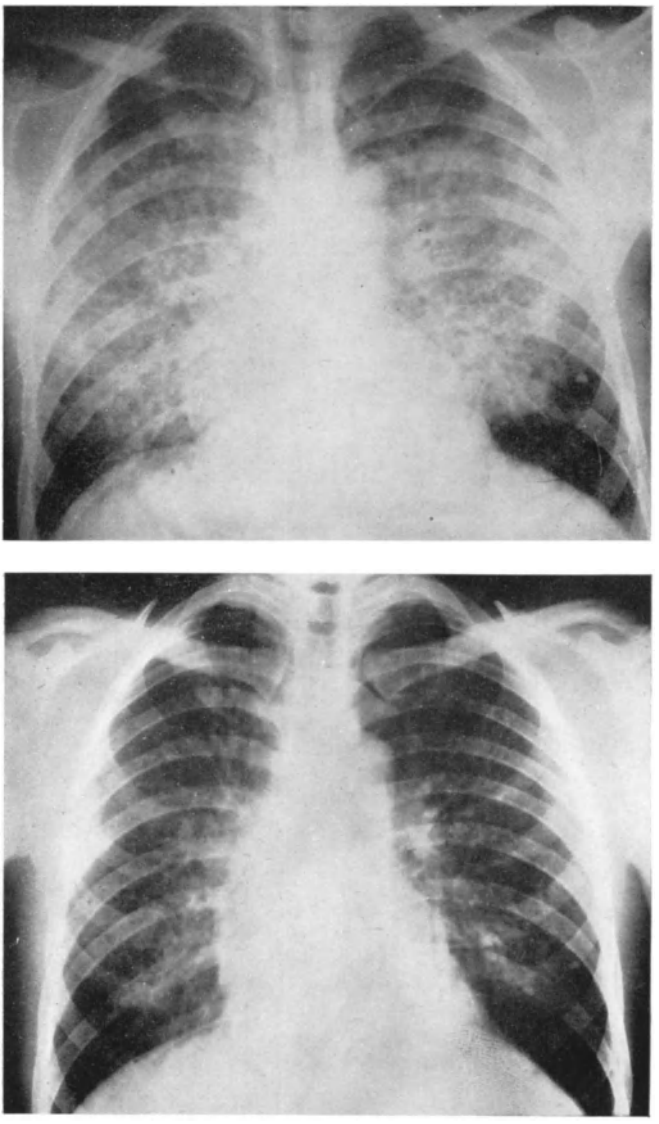
Figs. 2 and 3 reveal changes in the chest X-rays on the first day, which had practically disappeared on renewed X-ray examination two days later. During this time the patient's body weight was reduced from 67·3 to 62·8 kg. through loss of fluid. The concentration of N.P.N. in the blood had increased from 131 to 157 mg. per cent between the two examinations.

Fig. 6 shows a marked parallelism between the reduction in the body weight and the regression of the radiological changes in these cases of acute or chronic renal failure. The diagram indicates moreover the absence of any decisive relation between the changes in the chest X-rays and the values for N.P.N. and bicarbonate; we thus confirm the literature reports on these two points.

We have since treated ten more cases with similar result.

It seems justifiable to reckon fluid retention as a basic pathogenetic factor in the chest X-ray changes of the kind discussed here. For practical purposes it seems preferable to speak of "fluid lung" rather than of "uræmic lung." The former gives an indication as to appropriate therapy. This therapy, however, requires the condition of the patient to be not more serious than provides at least a couple of days' respite. The ultrafiltration treatment will be discussed later.

For more than five years X-ray examination has been performed as a routine procedure on all our newly admitted



FIGS. 2 AND 3 (Case 2 in Fig. 6). Chronic glomerulonephritis. Chest X-rays May 26th and 28th respectively. N.P.N. 131 mg. per cent and 157 mg. per cent, body weight 67·3 kg. and 62·8 kg. respectively. (Alwall, Lunderquist and Olsson, 1953.)

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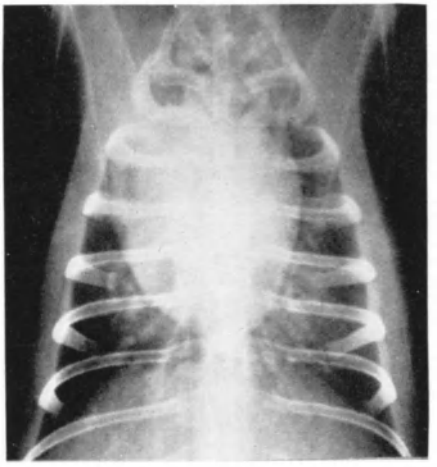

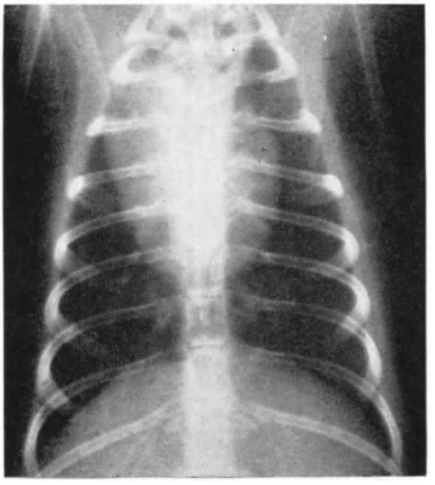

FIGS. 4 AND 5. Rabbit No. 1077. Body weight 1·97 kg. Fig. 4: Chest X-ray forty-four hours after ligation of the ureters and infusion of 300 ml. Ringer's solution. Forty-seven to fifty-two hours after the infusion 300 g. fluid was removed by means of ultrafiltration of the blood *in vivo*. Fig. 5: Chest X-ray seventy-two hours after the infusion; normal. Chest X-ray ninety-three hours after the infusion was still normal. The rabbit died seven hours later: N.P.N. 215 mg. per cent; no pleural or peritoneal effusion. (Alwall, Lunderquist and Olsson, 1953.)

patients with renal failure, generally on the first day, and at least once a week on the anuric-oliguric patient, as follows:

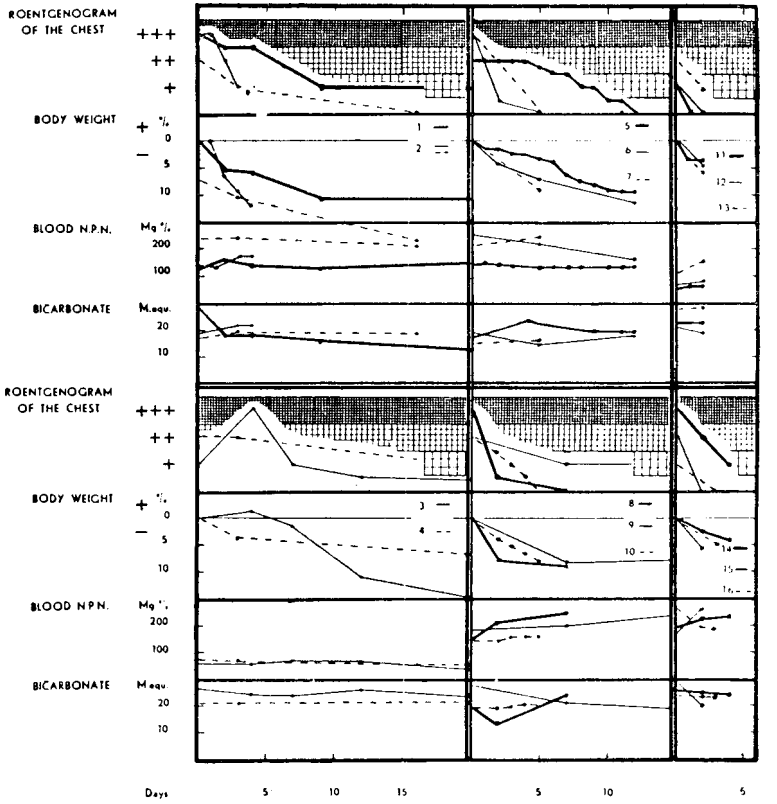

FIG. 6. The diagram illustrates (1) the degree of changes on the chest X-rays of our 16 patients, graded from +++ to 0; (2) the change in body weight expressed in percentage of weight on the first of the observation days as recorded in the diagram; (3) the concentration of N.P.N. in the blood; and (4) the bicarbonate value. (Alwall, Lunderquist and Olsson, 1953.)

(a) chest X-ray for "fluid lung," pleural effusion; (b) X-ray of the abdomen with a view to estimating the size of the kidneys, retroperitoneal œdema and peritoneal effusion. These

investigations give valuable information as to fluid retention. Our experiences in 25 anuric cases of acute tubular nephritis are being published (Alwall and Tornberg, 1953).

Treatment of Experimental Electrolyte-fluid Retention in Rabbits by Ultrafiltration of the Blood *in vivo*

The significance of fluid retention as a pathogenetic factor in this connection has also been elucidated by the results of animal experiments. Our first problem was to produce slowly supervening pulmonary œdema.

The ureters of rabbits were ligated during local anæsthesia or under "Narcotal"* narcosis. Subsequently, half the number of the animals in each group were given a quantity of Ringer's solution corresponding to 15 per cent of the body weight intravenously. The results are shown in Fig. 7 (Alwall, Lunderquist *et al.*, 1953). (1) Local anæsthesia: 19 rabbits, no positive radiological findings. (2) Narcosis: 18 rabbits, one positive X-ray (i.e. 5·5 per cent). (3) Local anæsthesia, fluid: 21 rabbits, five positive X-rays (i.e. 24 per cent). (4) Narcosis, fluid: 26 rabbits, 17 positive X-rays (i.e. 65 per cent); seven X-rays were positive already at the first examination, after some twenty hours. Several animals died relatively early from their pulmonary œdema.

Corresponding with the pulmonary changes there was, as a rule, increased lung weight, which is apparently not caused by

*"Narcotal" (Astra, Sweden)=Sodium isopropyl-β-bromallyl-N-methyl-malonylcarbamid.

FIG. 7 (*opposite*). The diagram illustrates chest X-ray findings and length of life of the rabbits. The ureters of the animals were ligated under local anæsthesia or under "Narcotal" narcosis. Subsequently half the number of the animals in each group were given a quantity of Ringer's solution corresponding to 15 per cent of the body weight intravenously. (Alwall, Lunderquist *et al.*, 1953.)

The animals have been arranged according to their length of life. The small dots indicate normal chest X-rays. The bigger dots indicate pulmonary changes of the type "fluid lung." Positive X-rays are further indicated by solid lines continuing until death takes place or the X-rays are negative. The lines are broken, narrow or broad, and indicate "probably significant," "significant" or "severe" changes in the chest X-rays respectively.

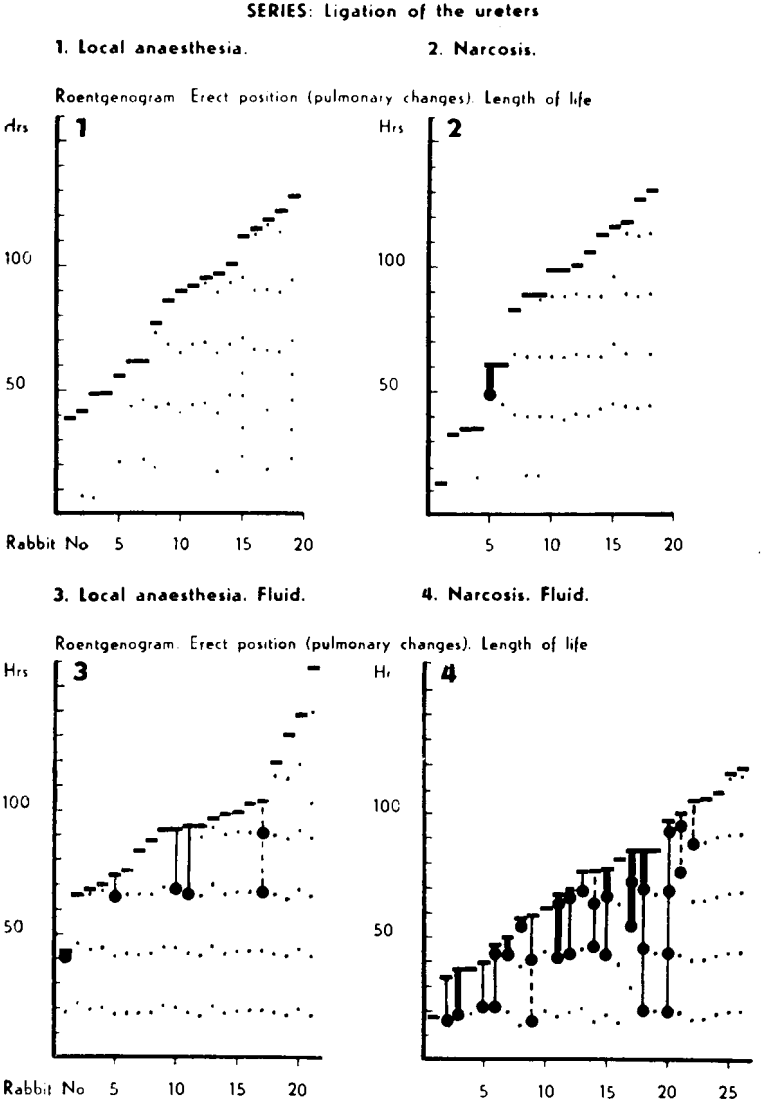
SERIES: Ligation of the ureters
1. Local anaesthesia.
2. Narcosis.
Roentgenogram. Erect position (pulmonary changes). Length of life
Hrs
1
2
100
50
Rabbit No 5 10 15 20
5 10 15 20
3. Local anaesthesia. Fluid.
4. Narcosis. Fluid.
Roentgenogram. Erect position (pulmonary changes). Length of life
Hrs
3
4
100
50
Rabbit No 5 10 15 20
5 10 15 20 25

FIG. 7.

inflammation. Mellgren and Bergman (to be published) performed the microscopic analysis of the lungs of our experimental animals.

Fig. 4 shows the chest X-ray of a rabbit with experimental fluid retention and "fluid lung." The technique is described below. Fig. 5 shows the chest X-ray of the same rabbit after removing the retained fluid by means of ultrafiltration of the blood *in vivo* (Alwall, Lunderquist and Olsson, 1953).

The following series (Lunderquist, 1953) shows the value of ultrafiltration treatment.

The ureters of the rabbits were ligated under "Narcotal" narcosis. Subsequently a quantity of Ringer's solution corresponding to 15 per cent of the body weight was infused intravenously. The animals were divided into two groups (Fig. 8). (1) Narcosis, fluid (i.e. the above-mentioned procedure): 26 rabbits. (2) Narcosis, fluid, ultrafiltration: 24 rabbits. After some twenty hours, an amount of fluid equivalent to that infused was withdrawn by ultrafiltration of the blood *in vivo* during some six hours' treatment.

In the first group (narcosis, fluid), 17 animals showed positive X-rays. With few exceptions the X-rays of the second group (narcosis, fluid, ultrafiltration) became normal or remained normal.

The same difference is obvious in the X-rays in a lateral position showing pleural effusion, and in the amount of pleural and peritoneal effusion found at death.

It should be stressed that the pleural effusion does not cause the changes in the chest X-ray here characterized as "fluid lung" (Alwall *et al.*, to be published).

Consequently, these experimental results confirm the above conclusion that fluid retention is a basic pathogenetic factor in changes of the type discussed here ("fluid lung").

FIG. 8 (*opposite*). The diagram illustrates the chest X-ray findings, in erect and lateral positions, length of life and the amount of pleural and peritoneal effusion found at death. The ureters of the rabbits were ligated under "Narcotal" narcosis. Subsequently a quantity of Ringer's solution corresponding to 15 per cent of the body weight was infused intravenously. The rectangles indicate ultrafiltration treatment. (Lunderquist, 1953.)

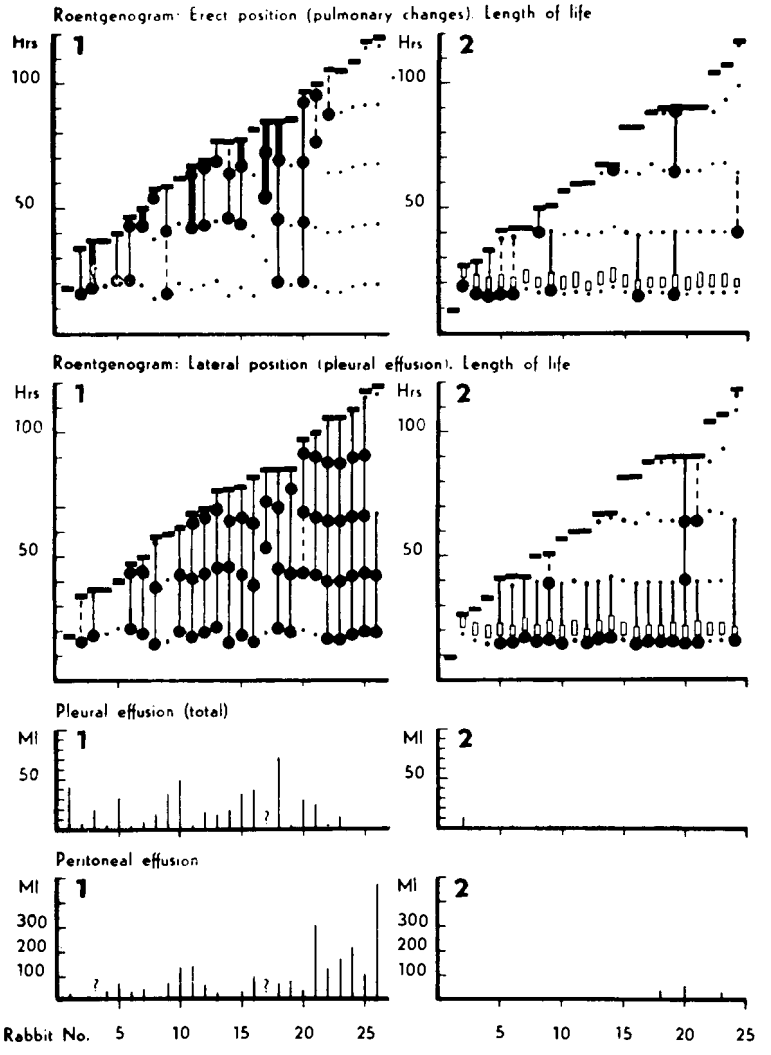
SERIES: Ligation of the ureters.
1. Narcosis. Fluid
2. Narcosis. Fluid. Ultrafiltration (Heparin).
Roentgenogram: Erect position (pulmonary changes). Length of life
Hrs
100
50
1
2
Roentgenogram: Lateral position (pleural effusion). Length of life
Pleural effusion (total)
Ml
50
Peritoneal effusion
300
200
100
Rabbit No.
5
10
15
20
25

FIG. 8.

Ultrafiltration (and Dialysis) Treatment of Patients

Figs. 9 and 10 show the effect of ultrafiltration treatment; furthermore, two cases had to be dialysed.

Case 2 has already been reported in brief by Lunderquist *et al.* (1952). Results of the ultrafiltration of the remaining three cases will be referred to by Tornberg (1953).

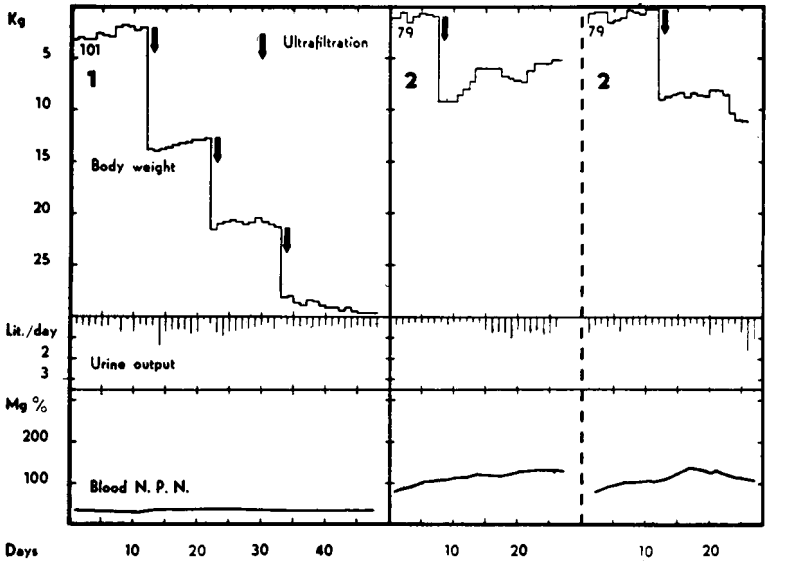

FIG. 9. Ultrafiltration treatment of two cases of chronic glomerulonephritis with nephrotic syndrome. Case 2 has already been reported in brief by Alwall, Lunderquist and Tornberg (1952). The two cases will be referred to by Tornberg (1953).

Fig. 9: Two men, aged fifty-eight and forty-five, suffered from chronic glomerulonephritis, and a nephrotic syndrome had quickly set in. Both were under observation one year and a half. With regard to the former, the urea clearance remained at about 30 per cent, and consequently there was no uræmia. The other patient had small kidneys, renal insufficiency and uræmia right from the beginning of the observation period.

Case 1*:* After three months' treatment with the prevalent nephrosis therapy, the œdema, of immense proportions, had not diminished, and the patient was totally immobilized. The initial weight was 101 kg. Through ultrafiltration of the blood *in vivo* about 30 kg. of œdematous fluid was removed in three treatments during one month. Although the nephrosis continued, the body weight did not increase, but on the contrary decreased still further. The patient has been able to leave his

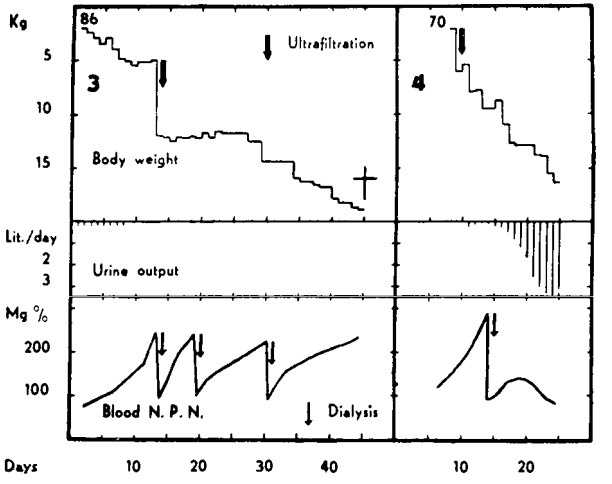

FIG. 10. Ultrafiltration and dialysis treatment of two cases of total anuria due to an acute attack of subchronic glomerulonephritis and probable acute tubular nephritis respectively. These two cases are being published by Tornberg (1953).

bed, and the general condition has been relatively good for the past six months.

Case 2*:* Life-menacing pulmonary œdema and then cerebral œdema and eclampsia were the indications for two ultrafiltrations, which were executed with a three months' interval. On both occasions his initial weight was 79 kg. A withdrawal of 7·4 and 8·6 kg. ultrafiltrate took place. It should be mentioned that the pulmonary œdema which necessitated the first treatment was induced by the administration of hypertonic

dextran solution intravenously, given with a view to combatting the œdema. One year after the first, undoubtedly life-saving treatment, the patient died of uræmia.

Fig. 10: Two men, forty years of age. Case 3 had an acute attack of glomerulonephritis and was totally anuric for six weeks. He was of course treated conservatively according to modern lines. The life-menacing uræmic intoxication was allayed three times by dialysis. During the days prior to the first treatment this man manifested increasing symptoms of "fluid lung." The intense agitation and air-hunger increased when the uræmic coma was treated by dialysis. Immediately after the dialytic treatment was finished, the patient was connected on to the ultrafiltration apparatus. When 2–3 kg. of fluid had been removed, the agitation and air-hunger ceased. Altogether about 5 kg. of fluid were eliminated. On the following day the chest X-ray was practically normal. The patient died later of uræmia and intestinal bleeding, the total anuria continuing to the end.

The last case had been admitted to a hospital in Copenhagen after a week at home with anuria, and he had drunk much fluid. A severe attack of lung-œdema with a still low non-protein nitrogen was the cause of his transference to our clinic. The chest X-ray revealed a severe "fluid lung." During the first day in hospital he was seized with a new attack of life-menacing pulmonary œdema. By means of morphia, inhalation of oxygen under excess pressure, alcohol inhalation and venesection, it was possible to prevent the gravest symptoms. Meanwhile, ultrafiltration treatment was started, but owing to the patient's agitation there were considerable difficulties to contend with. When 2 kg. of fluid had been removed, he was able to lie calmly in bed, and after a further weight-reduction of 2 kg. he slept peacefully. The pulmonary changes in the chest X-ray did not disappear. During the following days, dehydration with hypertonic sodium sulphate solution *per os* was continued. The weight of the patient decreased from 70 kg. by approximately 15 kg. during his three weeks stay at the hospital.

After the ultrafiltration the patient remained anuric for more than a week. The uræmic intoxication increased, and the patient became unconscious with a bad general condition. Dialytic treatment allayed the intoxication. Some days later the urine output began. The ultrafiltration and dialysis saved this patient's life. He recovered.

Consequently, ultrafiltration as well as dialysis are necessary complements to conservative treatment in rational renal therapy.

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DISCUSSION

BORST: I am greatly impressed by the results which Dr. Alwall has presented. I am more or less in the same position as he is, as patients with acute uræmia are also sent to the Amsterdam Clinic from other parts of the country. Our results are not as good as those of Dr. Alwall. We have no artificial kidney to reduce the volume of extracellular fluid. In the beginning we have often given too much fluid, and I am afraid that I killed several patients, especially as I was giving large amounts of sodium bicarbonate to correct for low bicarbonate figures. Dr. Bull warned me and convinced me that it was better to have an acidosis than an excess of extracellular fluid. At the moment we try to reduce the amount of circulating blood plasma when our patients are sent in with "wet lungs" (which is more or less the rule) by cuffs around the thighs. When the effect is insufficient we start a peritoneal dialysis with a high concentration of glucose. With that method it has been possible in several cases to reduce the fluid in the lungs and to save the patient. Our patients have always had a markedly increased blood urea and potassium level. With the peritoneal lavage we simultaneously removed the accumulated end products of protein metabolism and the

excess of extracellular fluid. It is hard to say which of the two contributed most to the striking improvement.

RUSSELL: Dr. Alwall, how many of your cases were grossly hypertensive? Because in our experience the fatal cases of uræmia and gross pulmonary œdema have very frequently been grossly hypertensive, often with malignant hypertension, and there has been a correlation between pulmonary changes, evidence of dilatation of the left ventricle, and degenerative changes in the myocardium. So that we have been tempted, in general, to interpret these pulmonary changes as more attributable to left ventricular failure than to any other one factor. Although I could very readily comprehend your excellent results in patients with the so-called nephrotic syndrome, in which there is great evidence of fluid retention in other tissues, in this hypertensive group of which I speak, there has not been that evidence. I wonder therefore whether it would be permissible to divide these cases of gross pulmonary œdema into two separate groups, which possibly might respond differently to that particular form of treatment?

ALWALL: Eleven of our first 16 cases published suffered from chronic glomerulo-nephritis. All except two had hypertonia. Two cases had had acute tubular nephritis with normal blood pressure.

We have later seen some 20 further cases with "fluid lung" in which the conservative treatment described above could be carried out. In all these cases the chest X-ray either changed in a normal direction or became quite normal after reduction of the electrolyte-fluid retention—uræmia still continuing. Also in this material we found several cases, especially acute cases, with normal blood pressure.

DE WARDENER: What was the venous pressure?

ALWALL: As a rule, I think the venous pressure is rather high.

BORST: I can confirm that there is always a high venous pressure in these cases.

PLATT: We meet this syndrome of "fluid lung" I should say most frequently in acute nephritis, in the fairly early stage of acute nephritis; that being a recoverable condition, we have used conservative therapy and I think always got away with it. I do not, at any rate, remember a recent death from acute nephritis. The syndrome is of course independent of the state of the non-protein nitrogen. Most of them have a moderate degree of hypertension. We have usually given antibiotics because we feel that there is sometimes an infective factor which may be secondary, the temperature goes up and so on, and we put them on the routine diet that one gives to such patients, with a very low protein, electrolyte and water intake.

BULL: I should like to support Dr. Alwall. Hypertension is not necessary, even in acute nephritis. The patients with acute nephritis who are anuric are quite often normotensive, and these people may get pulmonary œdema just as do those with hypertension. I do not think this is just an ordinary congestive cardiac failure. It is peculiar in a number of ways. The venous pressure is raised, the cardiac output and the circulation time may be normal, and the "failure" does not respond

to digitalis. This makes one think that it is not the ordinary type of congestive cardiac failure.

MERRILL: We have studied a number of uræmic patients under these circumstances, and a good many of them actually have an increased cardiac output. Dr. Goodale feels that there may be a syndrome of so-called high output failure in this type of condition which is not due to the degree of anæmia which they have. I think the key to the point about the "fluid lung" is given by Dr. Alwall's experiments with the rabbit. The best way to treat these patients probably is not to overhydrate them before they come in, because in our experience, too, the "uræmic lung," when reviewed in our radiology department, boils down to pretty good-looking pulmonary œdema of the sort that can occur not just with hypertension and not just with overhydration alone, but with a combination of any of these things.

DARMADY: Has Dr. Alwall tried treating any cases that have been given too much fluid after operation? One of my greatest difficulties in treating these cases is to combat the patient who arrives overhydrated. Has he any experience of that?

ALWALL: There is the same risk in Sweden. I have seen several cases that have had excess-fluid treatment after operation. Especially some years ago the patients were given too much fluid in order to *force* diuresis—it is a good method to *stop* diuresis!

BRADLEY: What is the general opinion regarding the use of digitalis? I must confess that I have always believed that these patients suffered from heart failure and I have treated them with digitalis. But I get the impression from the opinions that have been voiced here that it may have no value.

BULL: I give it but I do not think it does any good!

BORST: Observations in patients with chronic heart failure and normal kidneys suggest that the effect of digitalis is dependent on the condition of the patient's heart, that is defined by a point on the Starling curve (see Fig. 1, Borst). Digitalis improves the function of the heart and brings the Starling curve on to a higher level. The difference between the two curves is most marked near the top. In glomerulo-nephritis, in severe, acute and many cases of chronic renal failure, normal amounts of NaCl and water taken with the food are not excreted. Accordingly the venous pressure rises and initially the arterial pressure and pulse pressure rise too, probably reflecting an increased cardiac output. However, the rise in the renal output of water and sodium chloride which would ensue in the presence of normal kidneys is missing and the venous pressure continues to rise. Then the top of the curve is passed; the arterial pressure and especially the pulse pressure may fall to a subnormal level. Fig. 1 shows that if the patient is far beyond the top little benefit from digitalis is to be expected. It may do harm in promoting ectopic beats. But also when the patient is in the condition indicated in the figure by a point between "b" and "c," the effect of digitalis is not so impressive as it is if the kidneys are normal, since the diuresis resulting from an improvement of the circulation is lacking. Accordingly the venous pressure remains at a high level and there is no

appreciable return in the direction of point "b." Finally, one has to take into account the fact that the left and the right heart both have their individual Starling curves. If the improvement of the right heart exceeded that of the left side the net result could be negative. However, some improvement is likely to occur if the patient is not in a very serious condition.

PLATT: Dr. Borst, is this largely hypothetical or based on cardiac output measurements?

BORST: In the case of anuric patients it is hypothetical. The curve is based on observations made by Dr. Molhuysen on the relation between venous pressure and the output of sodium and chloride in patients with

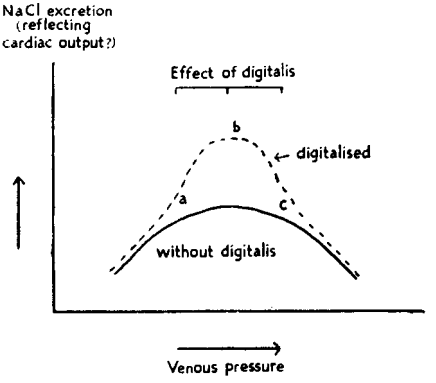

FIG. 1. (Borst.)

chronic heart failure and normal kidneys. There is, however, no reason to think that the heart in a patient with anuria would not obey Starling's law.

PLATT: I am not convinced that this is heart failure at all. I think it is hæmodilution secondary to salt retention.

BORST: I think it is the same thing. Excessive hæmodilution leads to heart failure.

ALWALL: I agree with you, Prof. Platt. None of our patients has been given digitalis. I have used dehydration as the only therapy.

PLATT: I think also that it is very difficult to judge the results of digitalis therapy in the presence of a normal rhythm, even in heart failure.

BULL: It does not alter anything that is measurable.

BORST: Fig. 2 (Borst) is one of Dr. Molhuysen's figures (Molhuysen, J. A., Thesis, Amsterdam, 1953). Every point (or circle) represents the results for one day. Determination of venous pressure were made two or three times a day with the method we have published in *The Lancet*.*

*BORST, J. G. G., and MOLHUYSEN, J. A. (1952). *Lancet*, ii, 304.

The average of the daily results is plotted against the amount of sodium chloride excreted on that day. In the beginning of the experiment, the patient, who had an aortic incompetence, was adhering to a salt-free diet; 3 g. of sodium chloride were excreted daily and his venous pressure fell from +2 to $-1\frac{1}{2}$ cm. Then he was digitalized. After five days 15 g. of salt were added to the food daily. Gradually the venous pressure rose and the salt output increased in parallel. However, after five days the NaCl excretion went down while the venous pressure continued to rise; the patient was over the top of the curve. Then digitalis was omitted and that brought the patient into a very bad condition. A salt-free diet was supplied and very slowly his venous pressure went

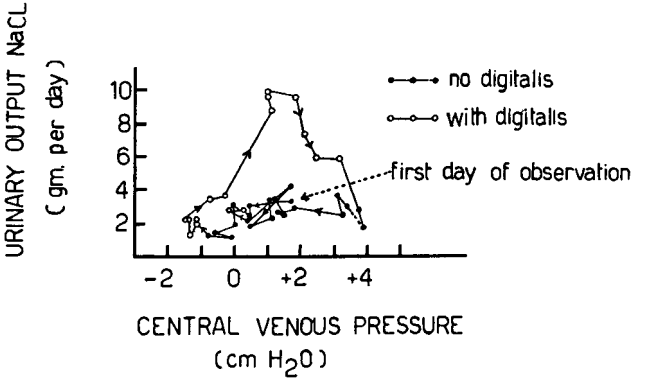

FIG. 2. (Borst.)

down. As in the first days of the observation, he excreted only small amounts of sodium and chloride in this period.

Similar curves or parts of curves have been recorded in 13 patients. Increments of venous pressure initially increase the output of sodium and chloride. Usually at a point representing a central venous pressure of 4–6 cm. above the level of the centre of the right auricle, the curve bends down, and salt excretion falls. When the rise in venous pressure continues, the patient is in a state of severe heart failure. In patients with predominantly left-sided failure, the top of the curve is at a low venous pressure and the experiment is not without danger, because of the possibility of the sudden development of lung œdema. Patients with manifest lung œdema are usually beyond the point "c" on the curve, where digitalis is of little benefit. Probably in a patient with salt retention due to renal disease, going beyond the top of the curve will have the same harmful effect; however, in this case the curve cannot be recorded in the same way since the kidney is blocked.

REUBI: I believe we are dealing with quite different things. I think some speakers are talking about acute nephritis, others about the

nephrotic syndrome in chronic nephritis and others about cardiac insufficiency in hypertensive patients. Now Dr. Borst says the kidney in these patients is unable to excrete anything. That might be true in chronic nephritis.

BORST: I meant anuria. I should have explained more clearly that I use the knowledge obtained in studying the salt excretion in patients with heart failure and normal kidneys in explaining phenomena found in patients with heart failure due to primary inability of the kidney to excrete salt.

REUBI: Yes. But in acute nephritis the mechanism is probably entirely different. I do not know whether Dr. Merrill has any explanation of the œdema in acute nephritis? We have had the opportunity of observing several œdematous and hypertensive cases with entirely normal renal function at the initial stage. Glomerular filtration rate is perfectly normal, PAH clearance is normal, the extraction ratio is also normal, there is no anoxia of the kidney because the oxygen extraction is also normal, and we have never found in such patients an increased cardiac output. I also believed formerly that the hypertension and œdema of acute nephritis could be due to high output, but I no longer think this is true.

MERRILL: Do not mistake me, in the pulmonary œdema of chronic nephritis or acute nephritis this high output is rather a rare finding, but it is well documented when it does occur. It is only in a small percentage of cases. I do not mean to imply that it is the sole cause of congestive heart failure in any of the other syndromes that have been mentioned.

REUBI: I wonder whether you have any explanation in such cases, where cardiac output and filtration rate are normal? Do you assume that a tissue factor plays a rôle, or the adrenal?

LEWIS: Do you remember when Doniach first described the pathology of one of these cases of uræmic lung, he did not think they were solely due to œdema. It is possible that you may be sharing his views but I do not know whether people still accept that. If they do, there is yet another group that would certainly have to be differentiated off from the rest.

MERRILL: It has, of course, been described a good number of times. I can only say that the X-ray picture as we have reviewed it in our people is not different enough for our radiologists to say it is a separate entity. I think the same thing is true of the pathological findings.

SHEEHAN: In acute renal failure, we were never able to find any definite correlation between pulmonary œdema and generalized œdema. I am talking now of the days when invariably, when we had a case of anuria, we loaded them with fluid—our criterion of an adequate "head of fluid" was that they should have slight œdema of the ankles. Now, at postmortem, these patients usually had some œdema of the posterior abdominal wall and sometimes a little ascites, but practically never did we see any fluid in the lungs. In patients dying early, we sometimes saw multiple areas of hæmorrhage, and we suspected that there was some inflammatory basis for it, because in the centre of the hæmorrhages

there were usually two or three alveoli full of polymorphs. But we never seemed to be able to find the "fluid lungs" that everyone talked about. Possibly this was because we were doing the autopsy early after death, before postmortem hypostatic œdema had developed in the back of the lungs.

ALWALL: According to my experience the signs of fluid retention appear as a rule in the following order: (1) Pleural effusion on the chest rœntgenogram in lateral position. (2) Fluid lung, sometimes in combination with cerebral œdema. (3) Marked general œdema, as a rule combined with retroperitoneal œdema on the abdominal rœntgenogram, sometimes combined with peritoneal effusion.

Thus, X-ray examination is a necessary method for detecting electrolyte-fluid retention and should be employed by way of routine continuously, especially in anuric cases of acute renal failure.

It is very interesting that with very big changes on the X-ray, the clinical symptoms are very insignificant. Have you had no clinical signs? No air hunger, nothing at all?

SHEEHAN: Our patients had sometimes been diagnosed as pulmonary œdema but we could not substantiate this at autopsy.

ALWALL: Overhydrated cases?

SHEEHAN: Yes, very definitely overhydrated. In those days we used to keep on giving the fluid to maintain the slight œdema of the ankles throughout the period of anuria.

THE KIDNEY
G. E. W. WOLSTENHOLME

SOME ASPECTS OF CALCIUM AND PHOSPHORUS EXCRETION

C. E. DENT

THE excretion of calcium and phosphorus by the kidney involves us in problems of great complexity. It is my intention today to stress the clinical importance of this aspect of renal function and to discuss some of the factors involved. I hope that the ensuing discussion may give us ideas for future work.

I know of no systematic work on the physiology of calcium excretion by the kidney. Clearly this must be largely because of the difficulties in determining ionized and diffusible non-ionized calcium levels in plasma. Another difficulty is the relatively small range of calcium levels that physiological considerations enforce upon us. Clinical studies however have shown that large, up to a hundredfold, changes in urine output can occur in various circumstances in the absence of significant changes in plasma concentration. A strong case can therefore be made out for the hypothesis that reabsorption of calcium by the renal tubule is the main factor determining urine output, and that there are many ways in which this calcium reabsorption may be affected.

I will mention first the influence of vitamin D. This is important from the clinical diagnostic point of view but it also reflects an interesting aspect of renal function. Fig. 1, taken from the paper by Chu and co-workers (1940), illustrates well the exceptionally low calcium outputs in a fully developed case of D deficiency and their slow rise to normal levels on the addition of only a small dose in the form of eggs, followed by the gradual fall again when the eggs were discontinued. Note the small changes in total serum calcium levels. The independence of urine output from blood level is shown even better in Fig. 2 (from Liu *et al.*, 1940) where the gradual

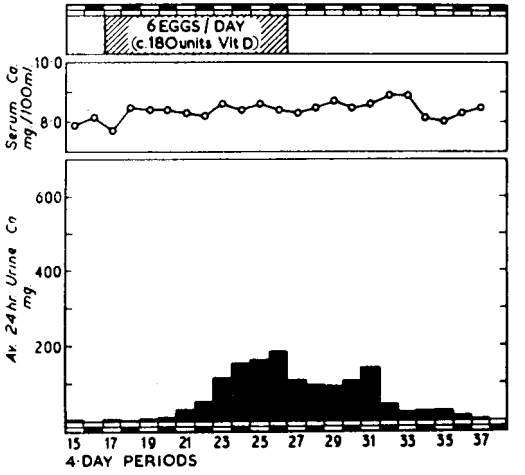

FIG. 1. Effect of a small dose of vitamin D in the form of eggs on the urine calcium output of a patient with osteomalacia (modified from Chu *et al.*, 1940).

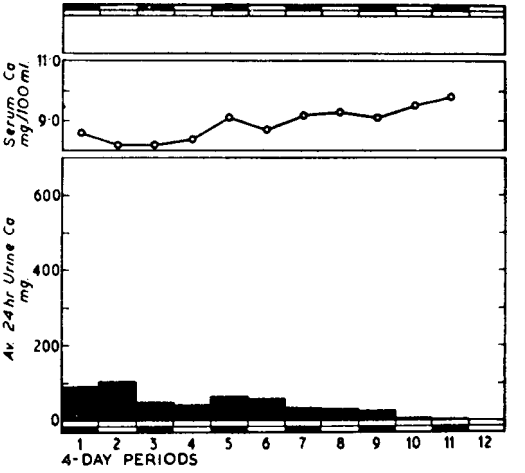

FIG. 2. Effect of vitamin D-free diet on urine calcium output. Note that the blood level happened to rise at the same time as the output fell (modified from Liu *et al.*, 1940).

depletion of vitamin D as shown by the fall in urine output happens to coincide with a rise in serum calcium. These rises and falls of urine calcium were accompanied by larger falls and rises respectively in fæcal calcium content, confirming that they were due to a true vitamin D action. Fig. 3 shows the similar responses shown by one of our osteomalacia patients. To do this, however, he required doses about 2,000 times larger than the others. I know of no explanation for

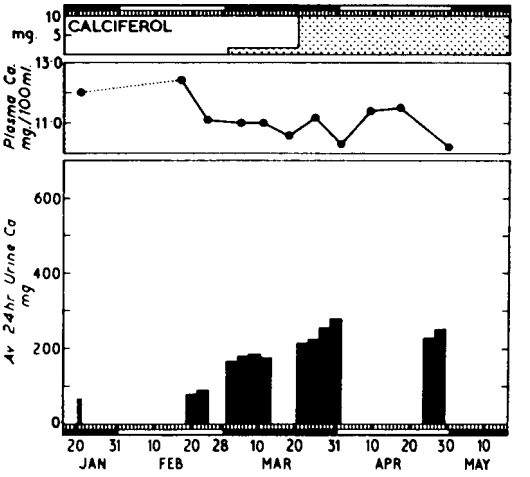

FIG. 3. Effect of large doses of calciferol (1 mg.=40,000 units vitamin D) on urine calcium of a patient with "vitamin D resistant" osteomalacia.

this difference in sensitivity to vitamin D except to add that this rare condition of extreme resistance is often hereditary.

Another factor which appears to affect kidney tubular calcium reabsorption is the thyroid hormone. The effect of addition and removal of the hormone is shown in Figs. 4 and 5. These changes likewise seem independent of total plasma or serum calcium levels (Robertson, 1942). A similar change is well known to occur in acidosis. An especially good illustration of this is shown in Fig. 6 which has been taken from the paper by Chu and co-workers (1940). Here the osteomalacic patient

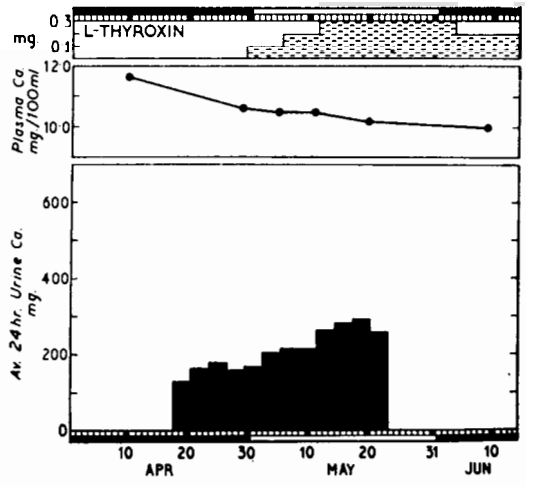

FIG. 4. Effect of thyroxine on urine calcium output of a cretin. Note that the blood calcium level fell when the urine calcium rose.

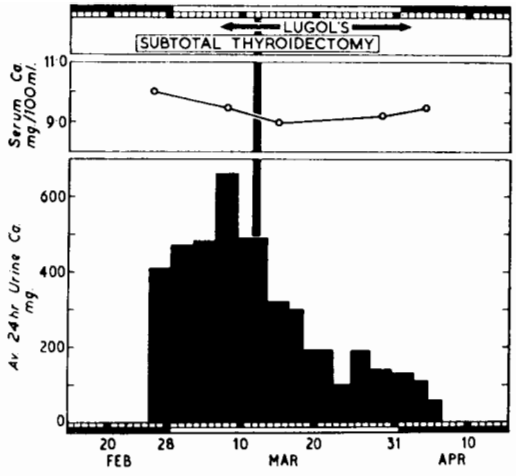

FIG. 5. Effect of sub-total thyroidectomy and Lugol's iodine on urine calcium of a thyrotoxic patient (modified from Aub *et al.*, 1929).

had a serum calcium of only about 6 mg. per cent and practically no urine calcium. The prompt increase in the latter on giving a small dose of ammonium chloride by mouth took place without any rise in serum calcium. A very high calcium excretion with a normal blood level occurs constantly in some people for no certain reason with the result that they are subject to recurrent urinary stones (Flocks, 1940). Many

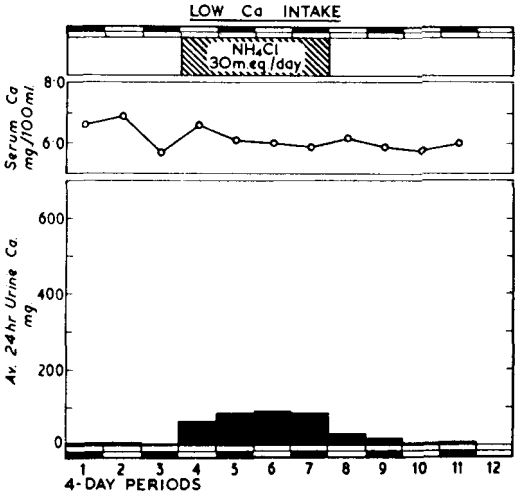

FIG. 6. Effect of ammonium chloride acidosis on urine calcium output of a patient with osteomalacia (modified from Chu *et al.*, 1940).

other factors such as diet, parathyroid activity and immobilization are known to affect calcium excretion. I will not stress these here as they need not be caused only by changes in tubular function since they are usually accompanied by changes in blood calcium levels.

The problems of phosphate excretion are in some ways simpler than those posed by calcium. All the ion is ultrafilterable and plasma levels can be greatly raised and lowered experimentally, thus enabling proper renal studies to be performed. These have shown that under suitable conditions

a definite phosphate Tm exists in man (Schiess, Ayer, Lotspeich and Pitts, 1948) and dog (Pitts and Alexander, 1944), which in dogs is fully working when the filtered load/Tm ratio is above 1·5 (Pitts and Alexander, 1944). It is interesting that all the factors mentioned above as having an effect on calcium excretion also affect phosphate excretion, though not always in the same direction. However the problem of phosphate excretion is more complex in one respect since other substances such as glucose and certain amino-acids seem to lower the Tm, and moreover the uncertain factors involved in diurnal rhythms operate conspicuously, so that large changes in phosphate output without apparent relation to the plasma level may occur spontaneously during short term experiments in the fasting subject.

I wish to report now some work on the action of parathyroid hormone carried out with my collaborators Dr. A. D. Kenny and Mr. G. Philpot. It has been known for a long time that a high phosphate clearance, roughly calculated from a blood level and timed urine collection, usually occurred in hyperparathyroidism. When, therefore, various workers (Ellsworth and Howard, 1934; Goadby and Stacey, 1934; Albright, Burnett, Smith and Parson, 1942; Kleeman and Cooke, 1951) found that an increased phosphate output, usually associated with a fall in plasma phosphate, occurred in the three hours after an intravenous injection of parathormone (200 U.S.P. units), it was natural to ascribe this to the normal physiological action of the hormone. On the basis of this rapid action Albright and Reifenstein (1948) have argued strongly that this indeed represents the most important action of parathormone and that its other known (but slower) actions, e.g. the dissolution of bone, the hypercalcæmia, etc., all follow inevitably from its renal action on phosphate excretion. I will not deal with the further details, but it must be clear by now that the problem has important clinical and theoretical repercussions. We therefore decided to reinvestigate the matter.

We have carried out the parathormone test as near as possible to the conditions of Ellsworth and Howard. The

fasting subject provided six hourly collections of urine, three before and three after the intravenous injection of 200 units of hormone. Plasma phosphate and calcium levels were

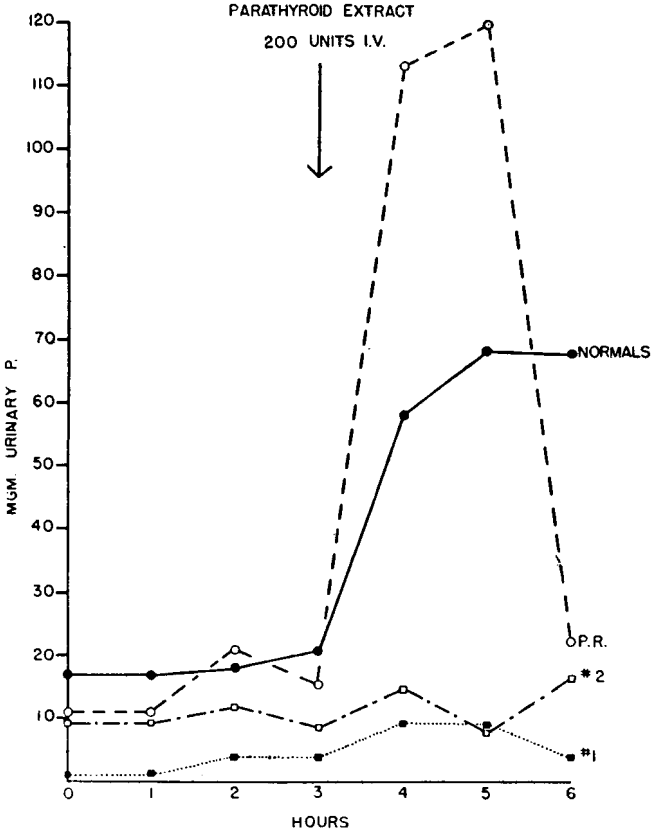

FIG. 7. Effect of parathyroid hormone on phosphate excretion (Albright and Reifenstein, 1948). P.R., Idiopathic hypoparathyroidism. Nos. 1 and 2. Pseudo-hypoparathyroidism.

determined before the injection and three hours after. The tests were done eight times on four normal subjects, once each on three hypoparathyroid patients, on seven patients with various other diseases not involving the renal tract and on one

patient in renal failure. Fig. 7 taken from Albright and Reifenstein (1948) illustrates the type of result obtained by earlier workers. Our results in brief were very different from these. Only two of the three hypoparathyroid patients showed a phosphate diuresis, and this was only about a two-fold increase as against the six-fold shown in Fig. 7. These latter were post-operative cases and had high plasma phosphate levels at the time, which fell about 1 mg. in the course of the test; the third, a typical "idiopathic" case, had a plasma phosphate of 4·8 mg./100 ml. The parathormone neither lowered this nor increased the phosphate output significantly. The remaining cases gave variable results. There were no significant falls in plasma phosphate levels except in two tests on one child with marble bones disease who for some curious reason always had a high level of the order of 7–8 mg./100 ml. (Fig. 9). The urine phosphate outputs likewise changed variably and insignificantly except for a good increase in the child with marble bones disease. (It is interesting, in passing, to point out that this is the one disease in which bone dissolution cannot be obtained under the action of parathormone.) These results are illustrated in Figs. 8 and 9. The averaged results in terms of hourly P output are: hypoparathyroid patients, before 18 mg., after 43 mg.; other patients and normals, before 24 mg., after 32 mg. We cannot analyse these figures properly without more values from control experiments. As far as they go the changes are clearly much smaller than those shown in Fig. 7 on the basis of the earlier work.

It occurred to us quite early on in this work that our failure to repeat the Ellsworth-Howard test might be due to differences in the hormone preparations used. All these preparations are crude extracts assayed on the basis of their serum calcium raising power. It could either be that the phosphate excreting power of the extract was due to a different hormone than that responsible for calcium raising or else it could be a pure artifact. The latter possibility is strongly suggested by the recent work of Stewart and Bowen (1952) who were able to obtain a definite but weakened phosphate diuresis from a

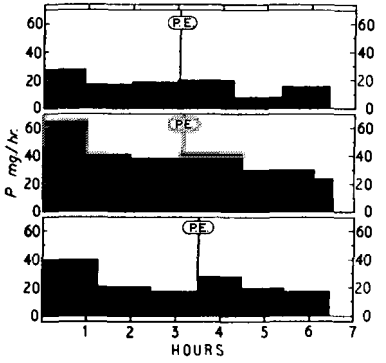

FIG. 8. Urine phosphorus output before and after parathyroid extract (200 units U.S.P. i/v). In the three examples shown here three different batches of extract from two manufacturers were given on different occasions to the same normal volunteer. These results are typical of those encountered in other normals.

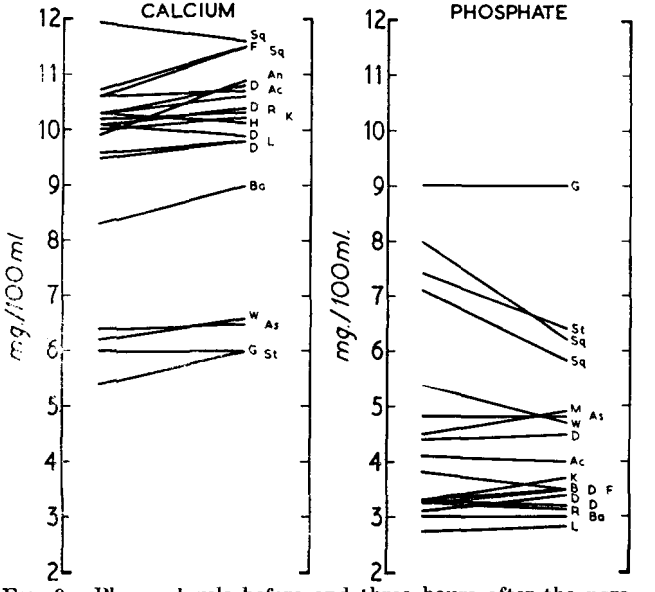

FIG. 9. Plasma levels before and three hours after the parathyroid hormone injection. Case G, chronic renal failure; Sq., marble bones disease; St and W, post operative hypoparathyroidism; As, idiopathic hypoparathyroidism; the remaining cases had normal renal and parathyroid function.

parathyroid extract whose calcium raising power had been destroyed by formaldehyde treatment. They were also able to obtain an extract from spleen which increased phosphate excretion without having any effect on serum calcium levels. It begins to look as if we are wasting our time experimenting with an extract of such a crude nature. A more fundamental objection can also be made, namely that we are perhaps asking too much of our method of investigation. I will end with a

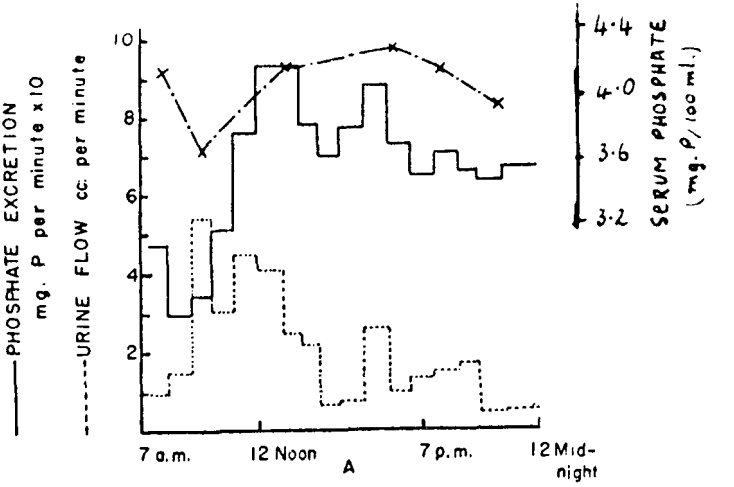

FIG. 10. Diurnal variations in urine phosphate output in a fasting normal human (modified from Ollayos and Winkler, 1943).

reference to the chart (Fig. 10) taken from the work of Ollayos and Winkler (1943). This shows the spontaneous changes in phosphate output in a fasting patient observed during a whole day. Note that had we done a parathormone test on that person on that day we would have injected the hormone after the first three hours' collections and then continued to collect the next three hours' urines. The result, had the hormone had no effect, would have been very similar to the "response" of the normal subject to parathormone shown in Fig. 7.

I hope I have shown you some features of interest concerning calcium and phosphorus excretion by the kidney. I think there is considerable scope for clinical research along the lines indicated. There is probably more scope still for studies on animals. However, too much of the animal work involves experimental conditions far removed from those met in the development of disease, as for instance when Tm's are being measured. This was well illustrated by the work of Schiess and co-workers (1948) on the effect of acidosis. It is well known clinically that acidosis increases phosphate excretion and lowers the blood level and this was confirmed by Schiess and his collaborators on the dog when normal blood levels were concerned. When however they raised the blood level for the purpose of determining the Tm for phosphate, this Tm was found to be the same as in normal non-acidotic dogs. Because of this I am in considerable doubt as to the exact procedures to be followed in investigating the various disorders of calcium and phosphorus metabolism in the human, and I should therefore appreciate your help and advice.

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DISCUSSION

MILNE: I think, Dr. Dent, that one extension of the Albright theory which you did not mention is the fact that they claimed that reduction of plasma phosphate from parathyroid action was the actual cause of the increased blood calcium from solubility product effects. I think you will agree that this does not occur by reduction of serum phosphate by other means, e.g. glucose and insulin together, causing transfer of phosphate into cells, or reduction of plasma phosphate by going on a low phosphate diet, will not cause any rise of blood calcium. Do you agree with that?

DENT: Entirely. We have studied carefully three cases in which we reduced the blood phosphate level with aluminium hydroxide by mouth, which is the same as going on a low phosphate intake. It was interesting to note that the plasma calcium did not change, with the patients on a constant diet, while in two of them the phosphate output practically disappeared as soon as Algelox (aluminium hydroxide) was given. The plasma phosphate fell about a milligram too. That was continued for over a week. We also used glucose-insulin; I thought this might have been too acute to show anything. That is why we continued the aluminium hydroxide experiments for a longer time. The third patient on the aluminium hydroxide continued to put out phosphorus in the urine even when the plasma phosphate was lowered to 2·5, when normally urine phosphate disappears. This patient had had a renal stone in her only remaining kidney and presumably had defective capacity for reabsorbing phosphate. I agree entirely that you can pick holes in the Albright scheme in various places.

MILNE: A further very striking discrepancy is the fact that it was claimed that the reduction in plasma phosphate, which must be distributed through the plasma and the extracellular fluid, was due to the loss in the urine. If you apply this principle to their data and our data, the loss is about one-third of that expected from the fall of plasma level —phosphate must go into the cells.

DENT: I think we are wasting our time with parathyroid hormone which is still obtainable only in very impure form. We have either got to work only on patients with parathyroid diseases for the time being or wait till the chemists turn out a pure hormone. When one is determining something so sensitive to change as phosphate output in the urine, one can easily imagine any toxic substance damaging the kidney tubules in such a way that they will reabsorb phosphorus differently. In the changing values of phosphate output we have got a sensitive measure of a particular cell function. The kidney tubule is accessible to investigation in a manner unlike that of any other organ in the body, and I think we shall probably find that a lot of objectionable substances given intravenously might affect the kidney tubule cells as well as other

body cells. The effect on the tubule would at once show up if one were happening to do a renal study on that patient.

STANBURY: Dr. Dent, when you were showing us a slide of the effect of parathormone on serum calcium, there was a vague hint that you found diurnal changes in plasma calcium. Could you elaborate on that?

DENT: Mr. G. Philpot and I have made some attempts to follow spontaneous changes in plasma calcium levels in the same subject repeatedly venesected from early morning to late evening of the same day. We could not make much sense of the results which varied seemingly haphazardly and quite differently in different people. The values varied as much as 1·5 mg./100 ml. on the same day. The practical issue which arises is the importance of standardizing the time of day for taking blood for routine analyses. We like to be fussy about it and have chosen to do it at 12 noon before the midday meal when the patient's stomach is fairly empty and, we hope, other factors are fairly constant.

STANBURY: Have you taken into account the possible effects of change of posture on phosphate excretion? Some time ago Mills and I (*J. Physiol.*, 1952, **117**, 22). did a series of experiments in which for forty-eight hours we tried to repeat a twelve hour cycle of activity. We slept three hours in each period of twelve hours, or at least became recumbent, and some slept while others did not succeed. Under these circumstances there was in each of four subjects a double peak in phosphate excretion, whereas there was the normal twenty-four hour rhythmicity in the rate of excretion of the major electrolytes. We thought that it might be postural changes that disrupted the diurnal rhythm of phosphate excretion. I wonder whether some obvious change of posture was responsible for the fact that you found the peak of phosphorus excretion at midday rather than at the more usual time of about 4 p.m.

DENT: It seems that the spontaneous changes in serum phosphate level can vary up to 2 mg./100 ml. and I wish Dr. Pitts would tell us what to do if we are to try and superimpose renal studies on this inconstant base line. Do we have to give up acute experiments? Shall we make them very much more acute, so that we do everything in an hour, or should we just do twenty-four hour experiments?

STANBURY: Don't you really have to establish your norm for the individual subject? It is fairly easy because they tend to reproduce.

DENT: We have not done enough normals in the same person at different times to decide really how reproducible it is, but you say it would be sufficient?

STANBURY: Yes, I think so.

THE KIDNEY
G. E. W. WOLSTENHOLME

Part V—Renal Share in Volume Control of Body Fluid

THE CHARACTERISTIC RENAL EXCRETION PATTERNS ASSOCIATED WITH EXCESSIVE OR INADEQUATE CIRCULATION

J. G. G. BORST

FLUCTUATIONS in water and electrolyte excretion are largely the resultant of three diuresis mechanisms. This can be demonstrated in subjects who are kept in bed and who receive food, fluid and electrolytes in equal quantities at three hourly intervals during the twenty-four hours (Borst and de Vries, 1950; Borst *et al.*, 1952). The bladder is emptied completely exactly at midnight, 3 a.m., 6 a.m. and so on. Under these conditions the renal excretion of water, sodium chloride and potassium shows no other fluctuations than the regular tides of the diurnal rhythm (*first type of diuresis*).* The output of creatinine is almost constant, though there is usually a slight depression in the beginning of the night (Fig. 1). Probably this is at least partly due to the effect of the "dead space" (de Vries *et al.*, 1952).

The drinking of an extra amount of water elicits a *second type of diuresis* strictly limited to water. The brisk diuretic response starts with a delay of twenty minutes and within four hours all the water is eliminated. During the night the delay time is prolonged often up to an hour and only 70 per cent of the water is excreted in four hours (Blomhert, 1951). The excretion pattern of the electrolytes and creatinine is essentially unaltered. The little peaks that often occur during the steep rise in urinary output are probably due to the washing out of the concentrated urine in the collecting kidney tubuli and pelvis ("dead space"). Similarly the injection of antidiuretic hormone is without influence on the excretion

*The order has been changed; in previous papers the diurnal excretory rhythm was indicated as the *third type of diuresis*.

of electrolytes; only the elimination of water is restricted (Fig. 1). These facts confirm the view expressed by Chalmers, Lewis and Pawan (1950) and are at variance with the opinion of other workers that the antidiuretic hormone in physiological concentrations increases the output of electrolytes.

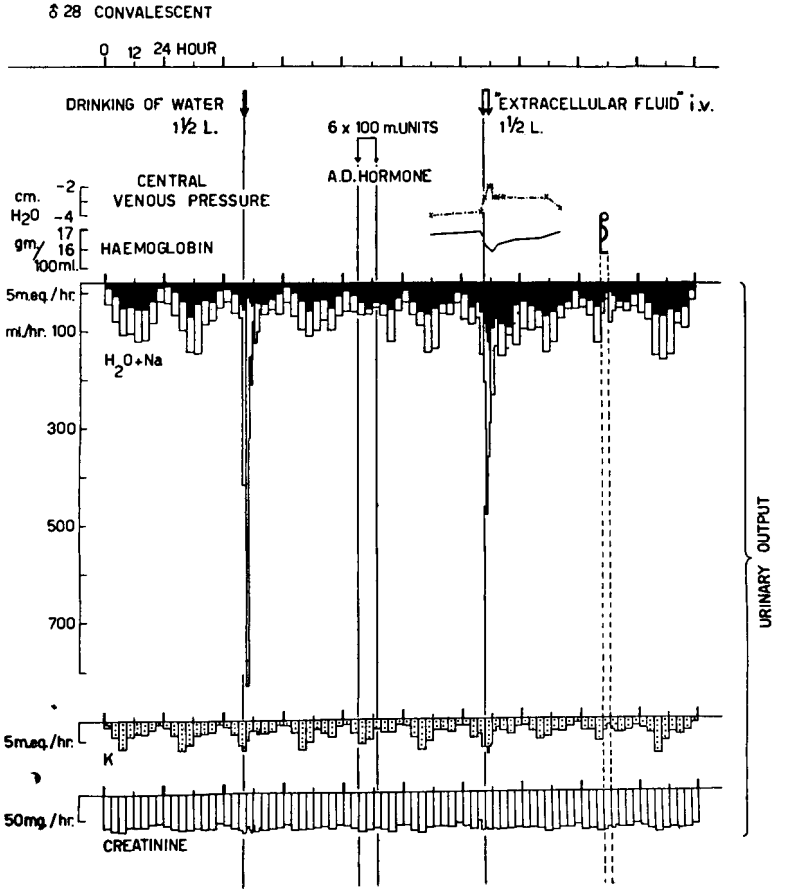


FIG. 1. The three types of "natural" diuresis and their corresponding oliguria. Composition of injected "extracellular fluid": NaCl 112 m.eq./L., $NaHCO_3$ 30 m.eq./L., KCl 3 m.eq./L.

The *third type of diuresis* is seen following the drinking or intravenous injection of a salt solution of the same composition as extracellular fluid. The first response is as if plain water were taken (Blomhert, 1951; Blomhert *et al.*, 1951). After a delay of twenty minutes the output of water rises steeply, while the excretion of the electrolytes is unaltered. After three-quarters to two hours the output of sodium and chloride rises moderately and sometimes there is also a slight increase in the potassium excretion. In normal subjects it usually takes two days before the excess of extracellular fluid is excreted. During this time the excretion of sodium, and with the exception of the first three hours also that of water, is only slightly increased, while the diurnal rhythm is fully maintained. Sometimes the greater part of the extra sodium is excreted during the second day. With the exception of the initial water diuresis the change in the excretion pattern is not very conspicuous unless the diet is poor in salt.

There are slight variations in this diphasic response. In some cases the first phase of the diuresis is very pronounced and may continue for several hours in spite of a marked rise in the blood plasma sodium level (Fig. 2). If the observations are limited to the first few hours and if the subject is not under strictly standardized conditions it may even be impossible to distinguish between the effects of the intake of water and of saline. In other cases the initial water diuresis is not impressive and can be overlooked if the urine collections are not made at short intervals. During the late evening hours the response to the administration of "extra-cellular fluid" is limited to a water diuresis and may even be completely absent. Then a largely increased matutinal rise in the output of water and sodium chloride is the first reaction on the part of the kidneys to the excess of extracellular fluid existing since the preceding evening.

The physiological counterpart of the diphasic diuresis can be studied in convalescent patients, when they are for the first or second time out of bed (ten Holt *et al.*, 1952). The excretion of water and sodium falls sharply. The potassium

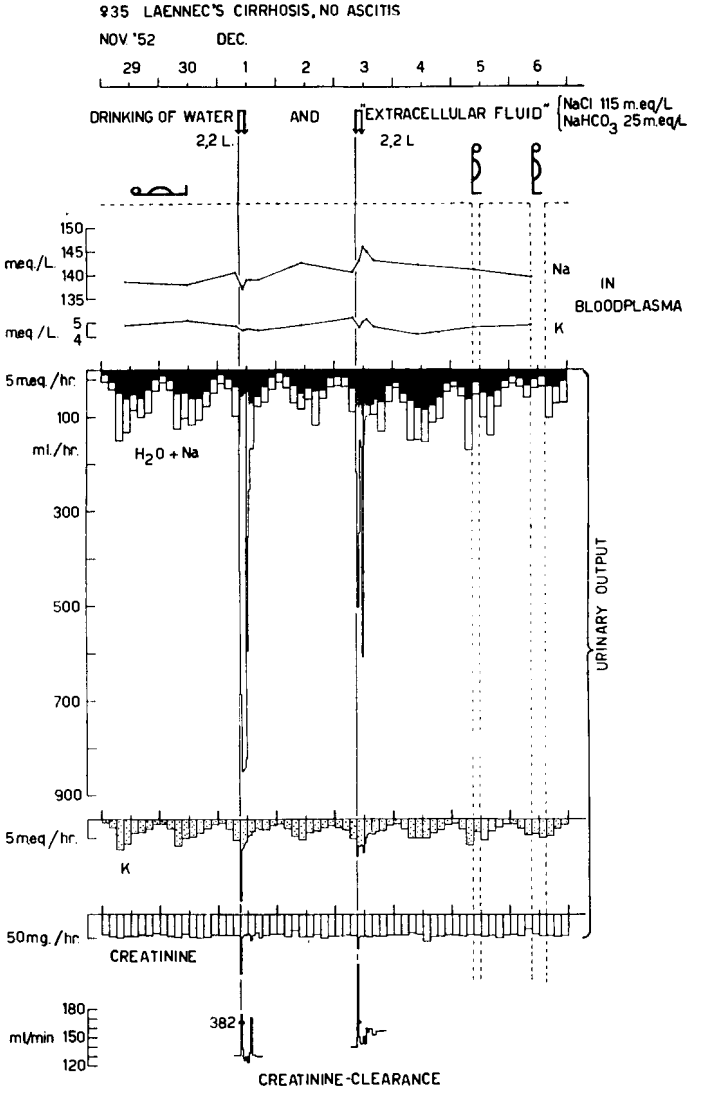


FIG. 2. Long-lasting "water diuresis" following the drinking of "extracellular fluid." Sharp rise in the sodium level of the blood plasma. Maximum output of sodium on the second day.

output is also reduced but to a lesser extent and it soon returns to the control level. If we allow for the effect of the "dead space," the output of creatinine is hardly affected. Resuming recumbency gives rise to a gradual increase in the sodium excretion often preceded by a water diuresis (Fig. 1

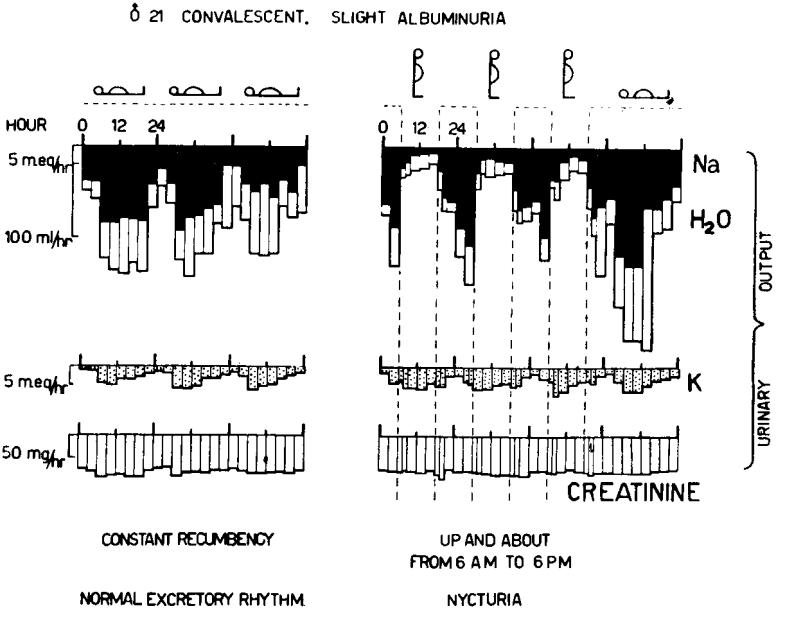


FIG. 3. Effect of posture dominates over the diurnal rhythm in the output of sodium and water but hardly affects potassium excretion. Commencement of standardized diet on the first day of observation; balance in intake and output not established before third day.

and 18). The response is in every respect similar to that produced by the administration of saline. The twenty-four hour sodium output remains below the control level. If the patient is kept in bed during the next day the retained sodium is eliminated (Fig. 3). In normal subjects the influence of posture and activity on the output of water and electrolytes is of minor importance. The excretion is dominated by the

diurnal rhythm. If this were not so sleep would be disturbed by the need to empty the bladder several times during the night. In convalescents the diurnal rhythm in the output of extracellular fluid may be completely reversed, whereas the potassium excretion continues in the normal way with the

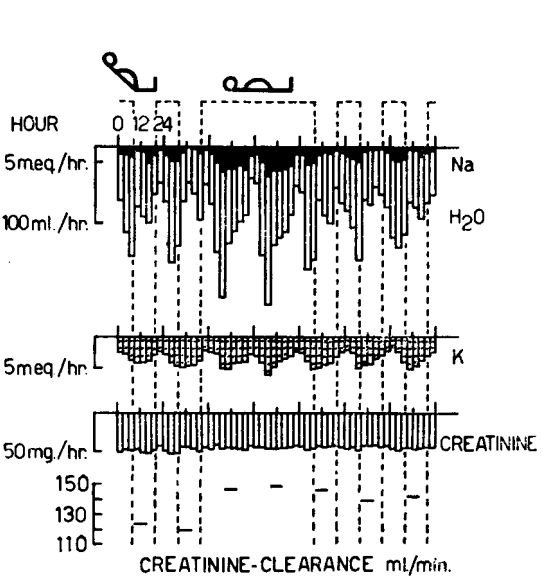


FIG. 4. "Unaccountable" fluctuations of sodium and water output in spite of continuous bed-rest disappear on lying flat during sixty hours. Potassium output is unaffected. No significant variations in creatinine clearance during the last five days.

maximum during the day (Fig. 3). In patients with œdema a slightly higher position on the cushions and light activities in bed during the day may suffice to reduce the output of extracellular fluid without affecting the potassium excretion, so that nocturia may develop in spite of continuous bed-rest (Smits, 1948; Borst and de Vries, 1950). Complete recumbency and inactivity during the twenty-four hours restores the normal rhythm (Fig. 4).

The fall in the sodium output on changing from recumbency to a more upright position is prompt, by contrast with the slow and gradual rise on resuming recumbency or following the drinking of saline. But also the negative response is progressive, till eventually the accumulation of ingested extracellular fluid leads to a blood dilution and counteracts the effect of the erect posture and the excretion of sodium and water rises (Figs. 3 and 18). Apparently not only the intensity but also the duration of the stimulus plays a part in the magnitude of both the positive and the negative response.

This third diuresis mechanism by which the body regulates the excretion of extracellular fluid is the main subject of this paper.

The Common Factors in Conditions leading to Alterations in the Excretion of Extracellular Fluid

The characteristic diphasic diuresis and its corresponding oliguria are found in a great many physiological and pathological states. It is highly probable that the identical responses on the part of the kidney in various conditions are the result of (positive and negative) stimuli mediated through the same pathway. If we accept this as a postulate the number of possibilities is restricted, so that we can infer from the available knowledge how the mechanism operates.

Hæmodilution, hæmoconcentration and blood volume. The intake of saline and the resumption of recumbency in convalescents are accompanied by a fall in the level of hæmoglobin and serum proteins, the change from the recumbent to the upright position by a rise in both levels (Figs. 1 and 17). Hence it might be conceived that the hæmodilution provided the stimulus for the diphasic diuresis and the hæmoconcentration for the corresponding oliguria. This would be in keeping with the fact that the drinking of saline in patients with the nephrotic syndrome hardly raises the output of extracellular fluid and does not lower the hæmoglobin and total protein levels significantly, while the injection of a plasma expander produces both hæmodilution and diuresis

(Fig. 5). However, a massive and often more prolonged diuresis is obtained by a blood transfusion in spite of a rise in the levels of hæmoglobin (Fig. 6) and of all the fractions of

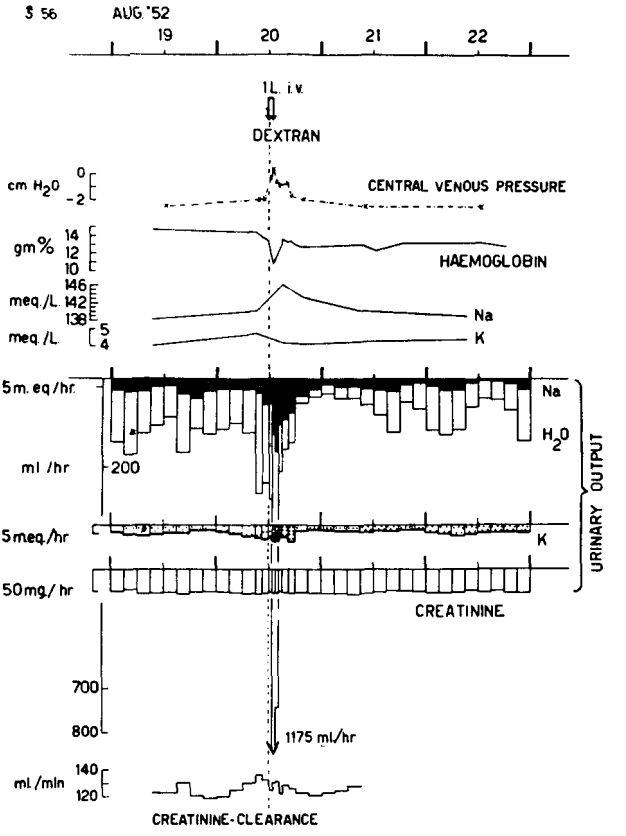


FIG. 5.

the serum protein (Borst, 1948). In patients with chronic anæmia a transfusion produces a similar but less pronounced diuretic response (Fig. 7) while the removal of a large volume of blood leads to a hæmodilution accompanied by a retention

of extracellular fluid (Fig. 8). These facts warrant the conclusion that blood dilution, unaccompanied by an increase in blood volume, provides no stimulus for a diphasic diuresis.

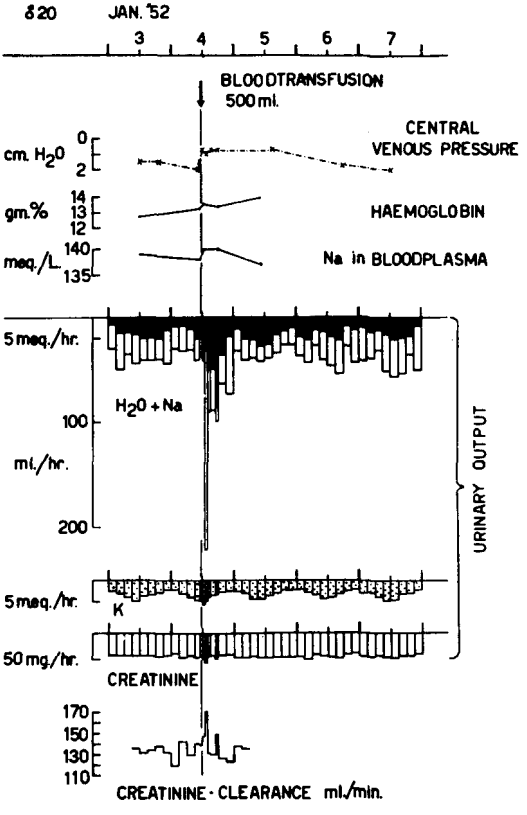


FIG. 6.

Blood volume and central venous pressure. In most instances the increase in blood volume that initiates a diphasic diuresis is accompanied by a measurable rise in the central venous pressure (Figs. 1, 5, 6 and 9). Removal of a large quantity of blood lowers the central venous pressure and reduces the

output of water and sodium. A fall in both central venous pressure and excretion of extracellular fluid can also be recorded following paracentesis in Laennec's cirrhosis; the

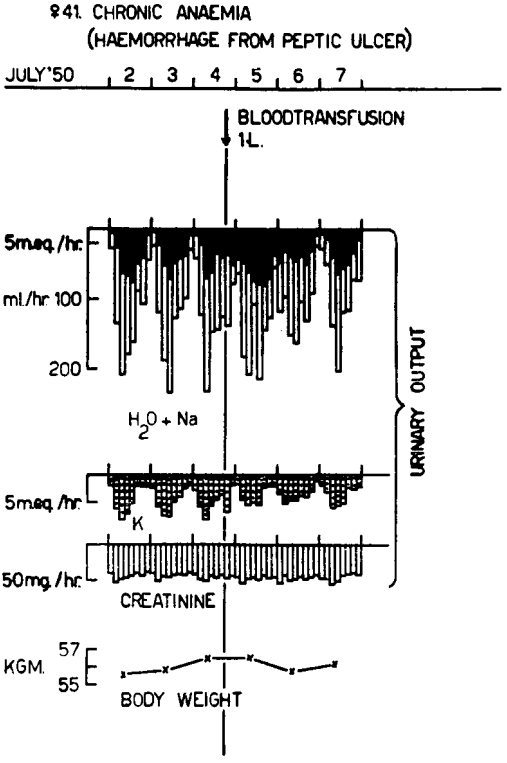


FIG. 7. Diuresis following blood transfusion in the evening is greatly modified by diurnal rhythm. Essentially all characteristics of a (not very pronounced) diphasic diuresis are present. Potassium output shows short-lasting unusually large initial peak.

hæmoglobin level may remain constant, indicating that the blood volume did not decrease (Fig. 9). Re-injecting a large amount of the removed fluid intravenously results in an almost parallel rise in central venous pressure and urinary output

of sodium chloride and water. In patients with hunger œdema a blood transfusion may produce a marked increase in blood volume unaccompanied by a rise in venous pressure. Then the diuretic response also fails (Borst, 1949).

These facts might suggest that changes in the venous pressure would directly stimulate the kidneys to parallel changes in the excretion of extracellular fluid. This, however, can be ruled out on the basis of the following observations.

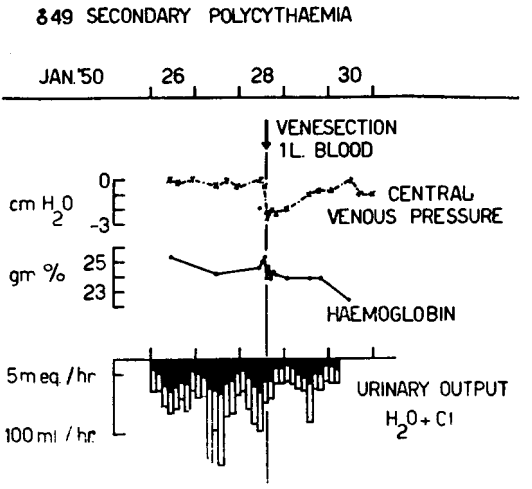


FIG. 8.

Central venous pressure and cardiac function. The massive diuresis following the injection of digitalis in moderate heart failure is preceded by a steep fall in central venous pressure (Fig. 10). In patients with œdema not resulting from heart failure, digitalis usually produces a slight but definite increase in the output of extracellular fluid, which may be preceded by a short-lasting fall in central venous pressure. Fig. 11 shows that the diuretic response was prompt if the injection was given in the morning but that there was a delay till the next morning if the digitalis was injected in the

♀ 64 LAENNEC'S CIRRHOSIS, ASCITES

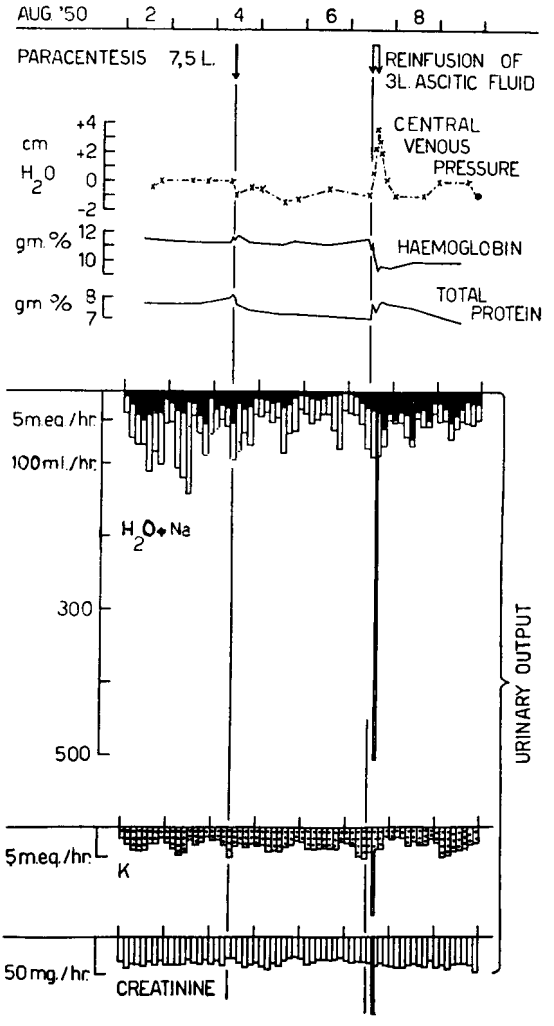


FIG. 9.

evening hours. Also in this respect the similarity to the effect of a blood transfusion in anæmia (Fig. 7) and to the drinking of saline is striking.

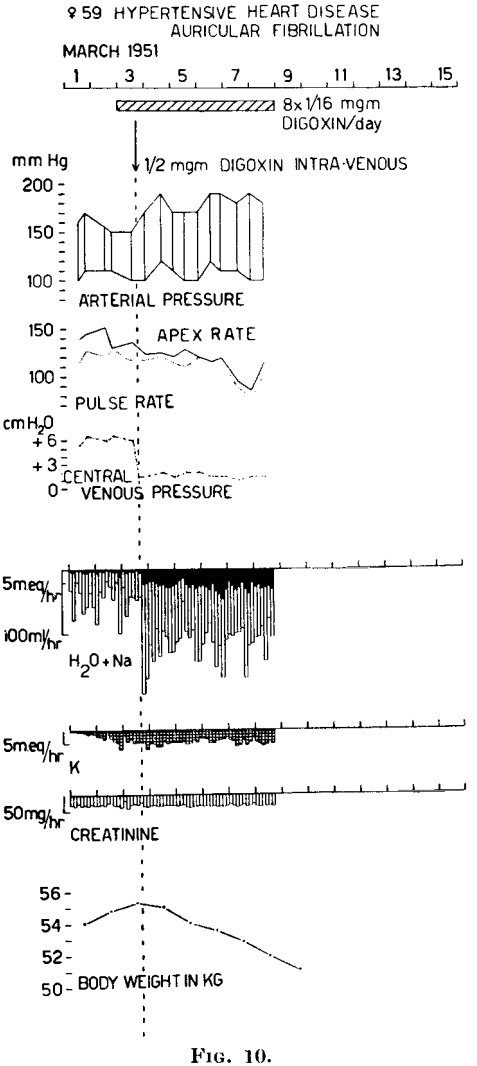


FIG. 10.

A good opportunity to study the effect of a primary excessive function of the heart is provided by patients who have attacks of paroxysmal tachycardia unaccompanied by heart failure (Borst, 1948; Borst *et al.*, 1952). If the heart rate does not exceed 170 per minute and the heart is otherwise normal these attacks give little discomfort to the patients. They

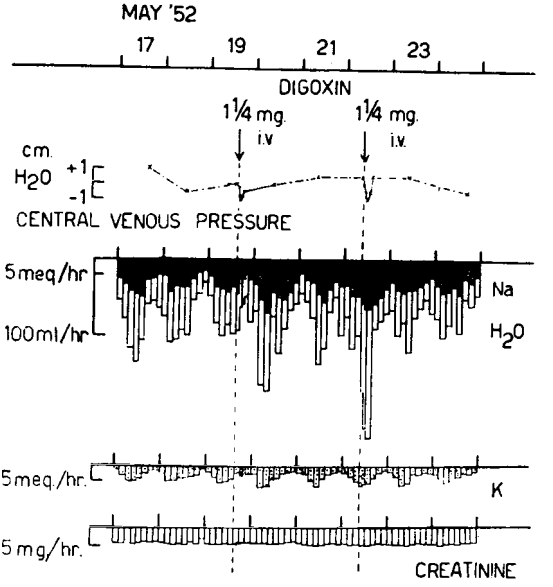


FIG. 11. Slight but definite effect of digitalis in patient with cachectic œdema (no heart failure). Effect of injection in evening hours is postponed till next morning (compare with Fig. 7).

are nearly always accompanied by a copious diuresis which cannot be distinguished from that following the injection of a large amount of saline. The loss of fluid may be so excessive that dehydration develops in a few hours. In some cases we found a fall in venous pressure and a shortening of the circulation time indicating an excessive cardiac output.

Fig. 12 presents the findings during an attack that developed in a period during which the patient was on the standardized

diet. It is noteworthy that the diuresis only affects the extracellular fluid. Potassium and creatinine excretion are almost constant. The initial water diuresis, which may be very

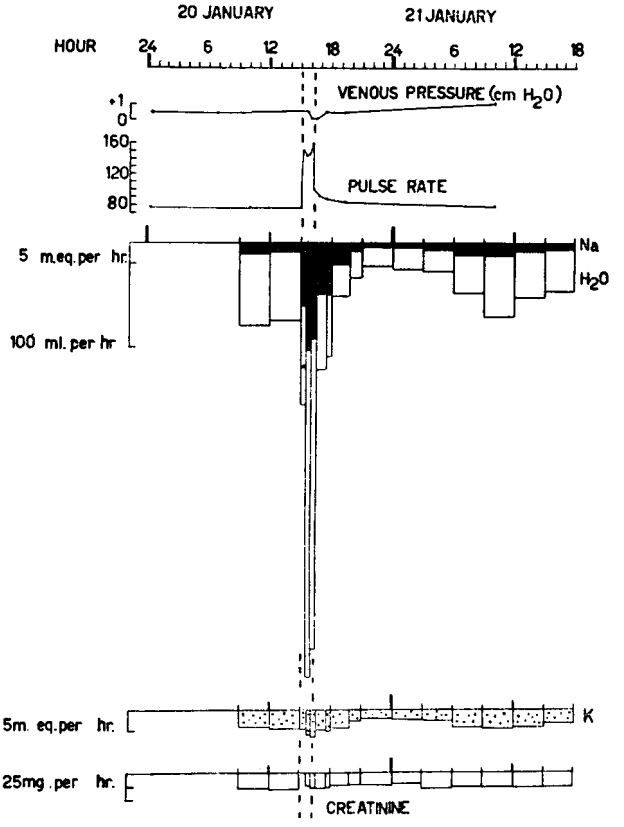


FIG. 12. Attack of paroxysmal tachycardia accompanied by a diuresis similar to that following injection of saline. No initial water diuresis in this case (with permission from *Nederl. Tijdschr. voor Geneesk.*, 1952, III, 2235).

pronounced, is lacking in this case. The sodium output gradually decreases in the four hours following the restoration of a normal pulse rate, showing that the effect of the attack continues long after the circulation has become normal.

A primary depression of the function of the heart, such as that precipitated by coronary occlusion or other acute heart disease, always leads to a fall in the output of extracellular fluid and a rise in central venous pressure. The same type of oliguria can be produced by the administration of drugs which have a depressive action on the heart (e.g. quinidine) (Fig.

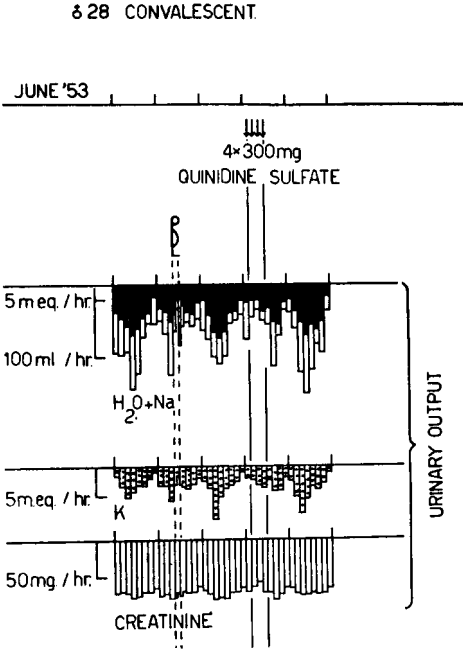


FIG. 13. Similarity in response to the upright position and to a high dosage of quinidine.

13). These facts lead to the conclusion that alterations in cardiac function, either primary or secondary to alterations in the central venous pressure, are the stimulus to parallel changes in the output of extracellular fluid. Though probably essentially correct this concept appears to be too simple.

The effect of alterations in the demand. The characteristic retention of extracellular fluid is also found in conditions in which the metabolic requirements increase while there is no

fall in the central venous pressure and an increase rather than a depression in the function of the heart, e.g. during muscular exercise (Kattus *et al.*, 1949) and during fever (Borst, 1948). The retention of sodium during fever is accompanied by a

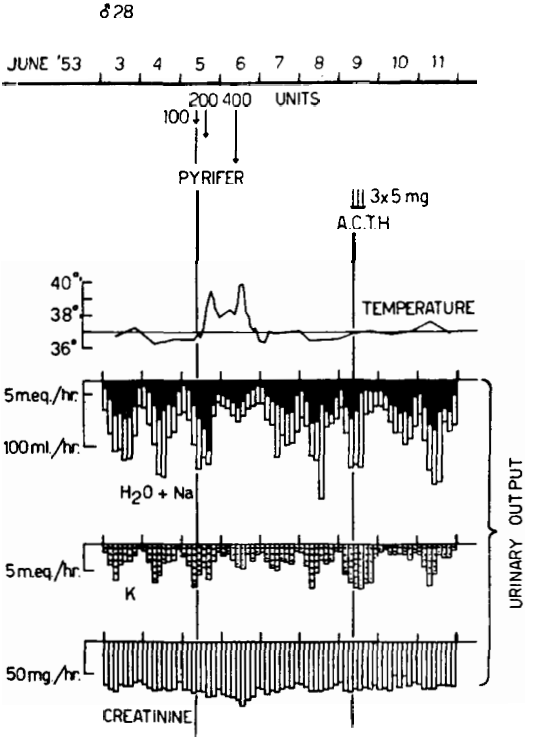


FIG. 14. Response to fever with retention of water, sodium and potassium, preceded by a nine-hour's diuresis (due to hyper-circulation?). Fundamental difference with the response to ACTH: increased potassium output during Na retention.

slight reduction in the output of potassium. It is the same pattern that is found in conditions leading to a depression of the circulation. There is a striking difference in the retention of water and sodium chloride produced by ACTH. This hormone provokes an increase in the excretion of potassium (Fig. 14). Hence the stimulus to the retention of extracellular

fluid resulting from fever is not mediated by the adrenal cortex and cannot be regarded as a part of the stress syndrome.

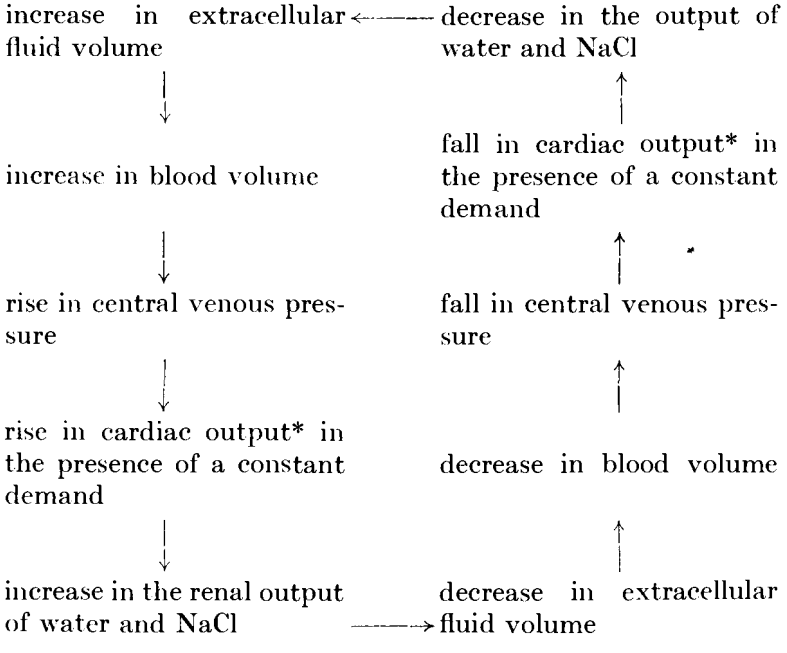
The similarity in response either to a depression of the circulation or to an increase in metabolic demands suggests that the excretion of extracellular fluid might be under the control of a substance that is formed in excess or insufficiently washed out if the metabolic requirements are not met by the circulation.

The following table summarizes the conditions in which the characteristic diphasic diuresis and the corresponding oliguria were found:—

DIURESIS	OLIGURIA
infusion of	acute blood loss
isotonic saline	
blood	
blood plasma	
plasma expanders	
reinfusion of ascitic fluid (in liver cirrhosis)	
change from the upright position to recumbency in convalescents	change from the recumbent to the upright position in convalescents
	abdominal tapping (ascites)
paroxysmal tachycardia without heart failure	acute heart failure
administration of digitalis in heart failure	administration of drugs depressing the function of the heart
	fever, muscular exercise

Several workers postulated that the excretion of extracellular fluid is directly related to the cardiac output and is largely independent of the level of the central venous pressure. They came to this conclusion independently and along different lines (Borst, 1938, 1941; Starr *et al.*, 1943; Warren

and Stead, 1944). The concept was extended by several authors (Dock 1947, 1949; Borst, 1948; Leiter, 1948; Starr, 1949; Youmans and Huckins, 1951). It is essentially the same hypothesis that Starling advanced in 1896 in an article in *The Lancet* and maintained in his monograph on the fluids of the body (1909). In modernized form it is as follows:—



Actually the two sequences of events are continuous.

During a constant intake and bed-rest there is a steady state but as soon as any change in volume, pressure or cardiac output intervenes a chain reaction comes into play and continues until the cardiac output is again adapted to the demands. The mechanism prevents a long-lasting excessive circulation by reducing the volume of the extracellular fluid and consequently of the circulating blood. It also counters

*Probably "cardiac energy output" would be more correct, see Discussion, p. 286.

even the slightest insufficiency of the circulation by retaining extracellular fluid provided the salt intake is not restricted. In the presence of a hypo-albuminæmia, a portal hypertension or a severely damaged heart this leads to œdema. If no salt is taken an (often latent) circulatory insufficiency reduces the sodium output to a trace within a few days.

Homeostatic Effect of the Inter-relationship between the Circulation and the Excretion of Extracellular Fluid

The homeostatic effect greatly impedes the study of the sequence of events, since deviations from the normal are self-limiting and never become very marked in normal people. We have to depend on pathological cases to discover all the steps in the sequence of events. Verney, who disclosed the chain of events in the post-pituitary diuretic mechanism met with the same difficulty. The fall in osmolarity in the blood plasma that initiates the chain of reactions is hardly measurable. A marked fall is only obtained if the last reaction is prevented by injection of antidiuretic hormone. Similarly the rise in venous pressure following the intake of saline is slight and can only be recorded beyond doubt in 25 per cent of normal subjects. If the intake continues, and the output is suppressed by anuria, or reduced by adreno-cortical hormones, a measurable rise in venous pressure never fails to develop (Fig. 17).

The slowness of the response of the "circulatory" diuretic mechanism largely contributes to the difficulties. Especially in healthy people other *more rapidly acting compensating mechanisms* interfere. The effect of posture on renal excretion can therefore better be studied in convalescents or in patients with orthostatic hypotension, and the effect of blood transfusion in patients with chronic anæmia and especially with hypo-albuminæmia. Here the rapidly acting compensating mechanisms which in normals prevent excessive circulation are less alert. For the same reason paroxysmal tachycardia, in which the heart rate has become independent of compensating nervous regulation, affords a unique opportunity

to study the effect of excessive circulation on the excretion of extracellular fluid.

Emotional diuresis. Even in patients who are kept in bed and who receive their food and fluids at equal intervals, seemingly unaccountable fluctuations in the output of water

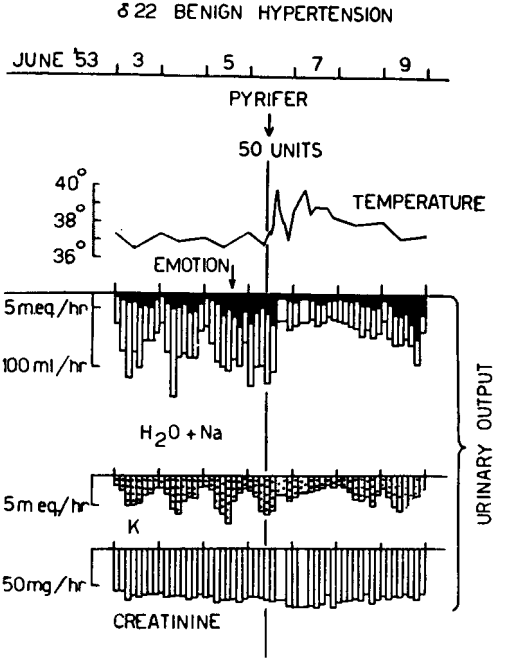


FIG. 15. Emotional diuresis obviating the normal nightly decrease in water and sodium excretion precedes pyrifer injection. Potassium output is unaffected.

and electrolytes may occur. Strong emotions may produce a diuresis of the "circulatory" type: the output of water and sodium chloride increases; the excretion of potassium and creatinine is unaffected. This often precedes the experiments, when the patient is aware of the arrangements that are being made. An example of a long-lasting diuretic effect is presented in Fig. 15. We had not informed the patient that treatment

with pyrogens would be started the next morning. When the results of urine analysis were charted we discovered a highly diuretic reaction restricted to water and sodium chloride, and learned from the patient that he had heard part of a discussion between two students about a new and hazardous treatment that was to be tried the next morning. This happened at 4 p.m. on the day preceding the injection.

It is perhaps noteworthy that we found the strongest emotional diuresis in a patient with hypertension; in our other cases the blood pressure was normal. Yesterday Dr. de Wardener reported on several patients with hypertension who reacted in the same way.

Alterations in the Renal Function that affect the Retention of Extracellular fluid

Since the discovery of the retention of extracellular fluid in circulatory insufficiency the following possibilities have been considered.

Renal insufficiency. Observations on salt retention during recovery from hæmorrhage from peptic ulcer have shown that a retention of sodium and chloride, in the presence of a high level of both electrolytes in the blood plasma, may continue when the urea clearance has returned to normal (Fig. 16). Potassium, phosphates and creatinine also could be normally excreted in the presence of a complete suppression of the excretion of NaCl and a minimal excretion of water. This led to the conclusion that the retention of extracellular fluid during circulatory insufficiency was due to a regulating mechanism and not to an impaired kidney function (Borst, 1938).

In severe heart failure, however, the output of all the urinary constituents, including creatinine, is markedly reduced. The same holds true for severe peripheral circulatory failure. Obviously this complete depression of excretion is not an appropriate reaction to an insufficient circulation but the result of renal insufficiency, and is fundamentally different from the selective retention of water and NaCl. Probably

most of the patients examined by Black (1942), Merrill (1946) and by Lauson, Bradley and Cournand (1944) belonged to this category. Their observations do not disprove that the organism is able to regulate the output of extracellular

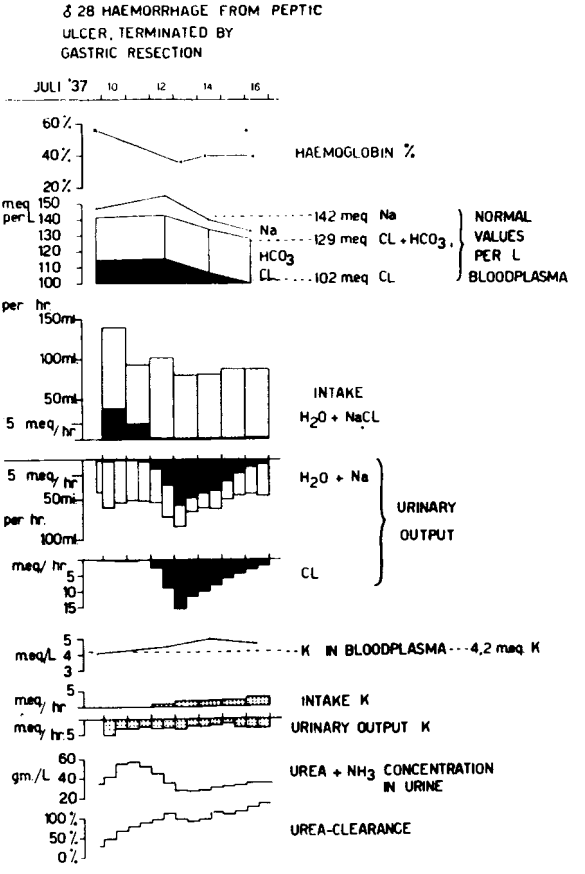


FIG. 16. Gastric resection terminating blood loss was performed on July 9th. Rapid improvement is accompanied by a fall in hæmoglobin. Excretion of retained sodium and chloride starts when post-hæmorrhagic blood dilution is completed. Restoration of urea clearance precedes salt excretion. (With permission *Acta Medica Scandinavica*, 1938, **97**, 68).

fluid independently from the elimination of waste products. In young people suffering from serious diseases causing high fever a complete suppression of sodium excretion and a high sodium level in the blood may be found in the presence of a urea clearance that is far above normal (Borst, 1948).

Regulation of the sodium output by slight alterations in the filtered sodium load. On the basis of the observations of Merrill (1946), who found that a markedly reduced cardiac output was always associated with a decreased glomerular filtration rate and a still more decreased renal plasma flow, Wesson, Anslow and Homer Smith advanced the hypothesis that the filtered sodium load was the determining factor in the excretion of sodium chloride. Actually, in severe circulatory insufficiency, the retention of extracellular fluid is always accompanied by a subnormal glomerular filtration rate, and even in the absence of an obvious circulatory failure a retention of NaCl is rarely accompanied by a high glomerular filtration rate. The clearances of substances that have to be administered at a constant rate are, however, not suitable for following the glomerular filtration rate during long periods. In the recovery phase of circulatory insufficiencies, during fever, during exercise and following changes in posture the parallelism between urea clearance and endogenous creatinine clearance on the one hand and the excretion of extracellular fluid on the other may be completely lacking. During recovery the clearances nearly always rise more rapidly to the control level than the excretion of sodium chloride. This fact has been confirmed by several workers. The observations shown in our figures also provide examples of a lack of correlation between the excretion of extracellular fluid and creatinine clearance during excessive circulation (Figs. 5, 6 and 18).

Hormonal factors. The objections raised by many workers against the concept of the dominating rôle of the filtered sodium load drew attention to the factors affecting tubular reabsorption of water and sodium chloride. On *a priori* grounds it is highly improbable that the retention of water and sodium chloride rests mainly on an increased level of

antidiuretic hormone in the blood. Nevertheless we must accept the possibility that in circulatory failure the retention of water, in the presence of low serum sodium, is at least partly due to an increased release of antidiuretic hormone. The initial water diuresis accompanying all conditions that tend to increase the circulation also provides strong evidence that the secretion of the post-pituitary is under the control of the circulation (Blomhert *et al.*, 1951).

The hypothesis that the kidney during circulatory failure is stimulated to sodium retention by an excess of adrenocortical hormones is inconsistent with many well-established facts. Till now no adrenocortical hormones or related substances have been detected that suppress the output of sodium chloride and water without increasing at least temporarily the output of potassium. ACTH has the same effect (Figs. 14 and 17). By contrast, the retention of extracellular fluid in patients with circulatory failure is usually accompanied by a moderate potassium retention; only if the sodium level in the blood is high (as during dehydration) may potassium excretion be increased. In patients with Addison's disease who do not react to ACTH the output of extracellular fluid is sharply reduced during standing (ten Holt *et al.*, 1952) and during fever. The response is in every respect similar to that in convalescents with intact adrenals (Figs. 18 and 19).

Sustained administration of ACTH, adrenocortical hormones or related substances to both patients with Addison's disease and in normal subjects leads to a retention of extracellular fluid, a blood dilution, a rise in central venous pressure and a rise in arterial pressure and in pulse pressure. Then the excretion of extracellular fluid rises too and a new equilibrium is established in spite of continued therapy (Fig. 17). Apparently the diuresis connected with the excessive circulation opposes the hormonal retention of extracellular fluid (Molhuysen *et al.*, 1950).

Conclusions. The retention of extracellular fluid resulting from failure of the circulation is characterized by a typical excretion pattern which can be distinguished from that

associated with a marked reduction in glomerular filtration and also from that induced by post-pituitary and adrenocortical hormones.

At the onset of the diuresis produced by an excessive

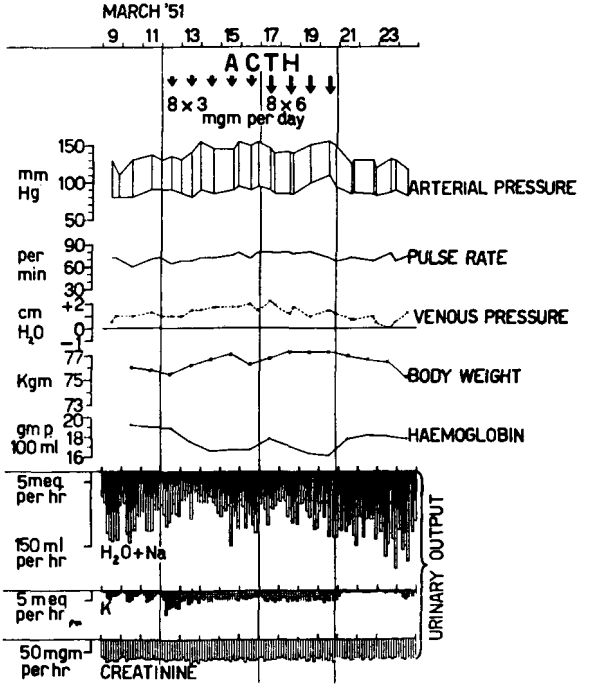


FIG. 17. ACTH injections produce a retention of extracellular fluid that terminates when venous pressure and arterial pressure rise. Potassium excretion shows an initial rise and the rhythm in excretion disappears (with permission from F. H. Wolthuis and L. A. de Vries; *Nederl. Tijdschr. voor Geneesk.*, 1951, IV, p. 3175).

circulation a suppression of the release of antidiuretic hormone probably plays a part.

The circulatory diuresis mechanism must be regarded as an independent type of response which is able to regulate the excretion of extracellular fluid without the mediation of

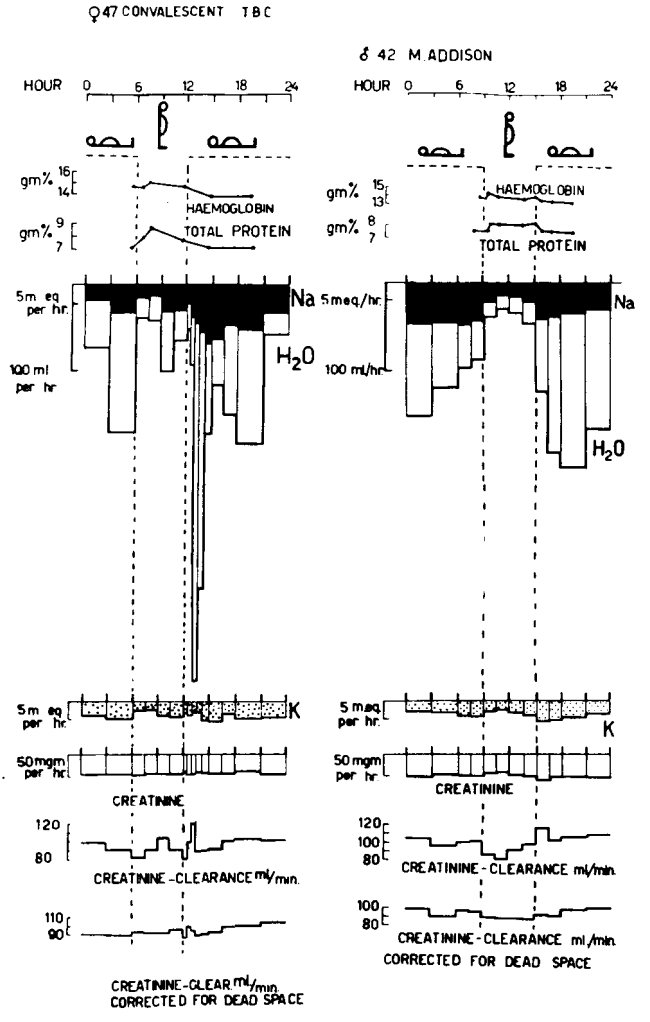


FIG. 18. Identical responses to the change from lying to standing position and *vice versa* in a convalescent with intact adrenals and in a patient with Addison's disease. No effect on the creatinine clearance, corrected with the assumption that the dead space contained 12 cc. (With permission from L. A. de Vries *et al.*, *Nederl. Tijdschr. voor Geneesk.*, 1952, III, p. 2251).

alterations in glomerular filtration and without the mediation of changing blood levels of adrenocortical hormones.

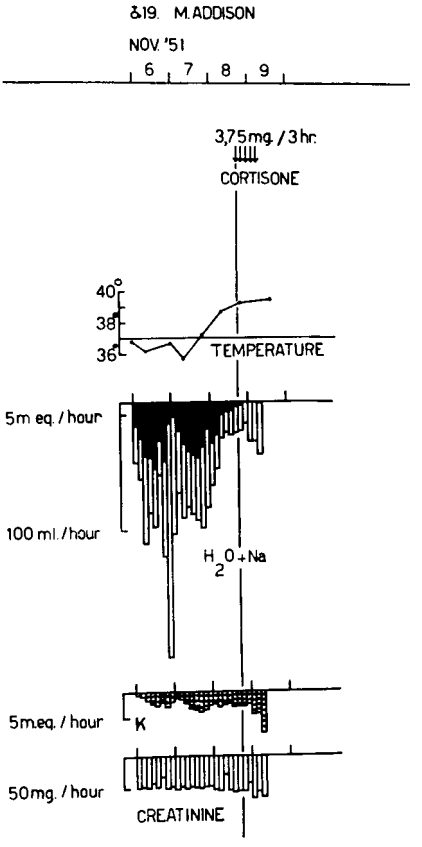


FIG. 19. Fever reduces extracellular fluid output in a patient with Addison's disease as it does in normal subjects (compare Fig. 14). Potassium is retained to a lesser extent. Small amounts of cortisone promptly increase potassium output.

Summary

The spontaneous fluctuations in urinary output are mainly the resultant of three diuretic mechanisms:—

(a) that associated with the diurnal rhythm;
(b) that associated with alterations in the osmolarity of the blood;
(c) that related to alterations in the circulation.

The latter is especially considered in this paper.

Factors favouring a decrease in the cardiac output (decrease in blood volume and central venous pressure, damage of the heart) or increasing the metabolic demands (fever, exercise) are accompanied by a retention of water, sodium and chloride and to a lesser extent of potassium, whereas the excretion of creatinine may remain constant. The reversed type of excretion is seen when in the presence of a constant metabolic demand factors are active that tend to increase the cardiac output. Often the lengthy saline diuresis is preceded by a brisk water diuresis (diphasic diuresis). With this exception, the renal responses are slow and last many hours after the stimulus has ceased to exist; the magnitude of the response is dependent on the intensity as well as on the duration of the stimulus.

Both negative and positive renal responses are sharply characterized and are essentially different from the response to the administration of the adrenocortical hormones and to adrenal insufficiency.

Patients with Addison's disease have a normal renal response to alterations in the circulation.

The "circulatory" diuresis and its corresponding oliguria are often accompanied by an almost constant endogenous creatinine clearance.

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DISCUSSION

MCCANCE: Prof. Borst's paper certainly provides us with a good example of what can be done by patient observation in the wards. I have had the privilege of being in Amsterdam and seeing the man in action, so to speak. He has no great facilities or variety of apparatus. Indeed he is working in a very old hospital and he has few modern aids.

WIRZ: It is in a way very comforting to think of that direct link between cardiac output and excretion of sodium especially. Of course there must be a way in which the kidney is informed, and apparently it is neither the filtration rate nor, seemingly, the adrenal cortex. I should like to know what Prof. Borst suggests could be the way by which the kidney is informed what it should do.

BORST: I have insufficient evidence for any mechanism. However, we have to consider the following points. Salt retention is induced by

a reduction in the general circulation, and in spite of the fact that compensating mechanisms in the kidney itself may keep glomerular filtration at a normal level, it is probably a pure renal mechanism. The kidney "has a memory"; it continues to retain sodium chloride for many hours after the circulation has become normal and it may react to a short-lasting excessive circulation with a prolonged saline diuresis. An increased metabolic demand has an effect that is probably in all respects similar to that of a reduction in the circulation. All facts are in keeping with the tentative hypothesis that a substance (a hormone?) with a sodium-retaining effect is continuously produced in the kidney; excessive circulation suppresses its production or promotes its washing out; insufficient circulation, absolute or relative to an increased demand, leads to an increase in its production. This, however, is mere speculation, there are other possibilities.

MERRILL: There are two things I should like to ask Dr. Borst about. There have been some experiments in which, in the upright position, the increase in blood volume that occurs following albumin does not result in any increase in sodium and chloride excretion. That distresses me a little bit. The other thing is that you showed, I believe, a slide of a patient without any heart failure, with a normal heart, who following the administration of digitalis had an increased diuresis. Now in the normal heart with normal rhythm, the cardiac glycosides typically, I think, cause a decrease in cardiac output. I wondered how you could fit those observations into this scheme.

BORST: You say that sometimes an injection of plasma or an injection of blood is not accompanied by a typical diuresis but even by oliguria?

MERRILL: Yes, in the upright position, an infusion of albumin may have no effect on sodium excretion, although plasma volume is increased.

BORST: In normal people the effect of measures tending to produce an excessive circulation is obviated by compensating mechanisms. In patients who have insufficient circulation for a long time the compensatory mechanisms that would act in the presence of an excess are, so to speak, sleeping. When in a normal person the blood volume is suddenly increased by a blood or plasma infusion the reaction on the part of the kidneys is poor even in the recumbent position. When the same person has lost blood during a long period and is in latent circulatory failure, the infusion of an equal amount of blood can produce an impressive diuresis. This explains, I think, why carefully planned experiments in normal volunteers are often less illustrative than routine observations in patients.

In a normal person, on changing from the recumbent to the standing position a marked fall in the circulation is prevented by powerful compensatory mechanisms. If the volume of extracellular fluid is then suddenly increased, a relaxation of the compensatory effort is sufficient to prevent an excessive circulation and there is no stimulus for a diuresis.

With regard to the second question, I do not think that the results of the cardiac output determinations are convincing. Great fluctuations are possible in normal subjects especially when they are apprehensive. Moreover, technical difficulties make it difficult to get recordings that

are exact to 10 per cent. I assume that longer-lasting changes in the average cardiac output of less than 10 per cent may be responsible for variations in the renal excretion of water and sodium chloride. We have no methods for very exact and continuous recording of cardiac output during long periods. Therefore I have more confidence in well established indirect evidence. When a patient with moderate heart-failure is treated with digitalis he shows a marked rise in arterial pressure and in pulse pressure, sometimes without decrease in pulse rate. His kidneys respond with a characteristic type of diuresis that is also found in patients with an insufficient blood volume following a blood transfusion. In a case of non-cardiac œdema, digitalis gives rise to a less pronounced diuresis of the same type. A normal subject has only a very slight diuretic reaction to digitalis and to a blood transfusion. I infer from this that the normal heart reacts to digitalis, but that compensatory mechanisms neutralize its effect. Dr. Molhuysen's curves, one of which was presented yesterday (Fig. 2, Borst, p. 239), show that digitalis has its maximum effect on salt excretion if the heart is near to the top of the Starling curve. A negative effect was never found.

DE WARDENER: I do not know whether you are right or wrong, but could you not cut out the cardiac part of your scheme and then you would not be quite so vulnerable? You have measured the venous pressure, and you do not know that pressure changes might not be causing it?

BORST: I am sure it is not the venous pressure itself. In a patient with heart failure who is over the top of the Starling curve a further rise in venous pressure produces a decrease and not an increase in the renal output of sodium chloride and water. This can be seen on the curves of Dr. Molhuysen (*see* pp. 238 and 239). A rise in venous pressure only leads to a diuresis if the heart is able to respond with an increased cardiac output. Moreover the diuresis following digitalis injection in heart failure is preceded by a fall in central venous pressure. The massive polyuria precipitated by paroxysmal tachycardia provides another example of the independence of the diphasic diuresis from the venous pressure; during the attack the venous pressure may fall. Therefore we cannot escape the conclusion that the increase in the output of extracellular fluid must be mediated by an increased function of the heart. I cannot miss that link. I must confess, however, that according to Starling's law the cardiac output may remain constant if the arterial resistance is raised concomitantly with the central venous pressure. However, a rise in central venous pressure must result in a rise in "cardiac energy output" (product of cardiac output and mean arterial pressure). There is no absolute proof that our patients who responded to a rise in venous pressure with an increase in arterial pressure and a massive diuresis also had an increased cardiac output. There is another reason why it is with hesitation that I speak of an increased cardiac output. The excretion of extracellular fluid is also affected by the metabolic requirements.

WINTON: Do you imagine that the metabolism of the heart liberates a hormone that affects the kidney?

BORST: No.

PITTS: Did not Farber describe a direct diuretic action of certain digitalis glycosides on the kidney, independent of change in venous pressure or of cardiac output?

MERRILL: Yes, to digoxin.

BORST: I cannot exclude a direct effect on the kidney, but the evidence is against it. In normal subjects there is hardly any effect of digitalis on the renal output of extracellular fluid. In a person with latent heart failure there is a definite effect. We can more or less say that the more serious the cardiac insufficiency, the larger the diuresis produced by digitalis. The excretion pattern has the same characteristics as that following a blood transfusion in a patient with anæmia. This suggests a circulatory factor.

MILNE: What evidence is there that the diuresis in the diurnal rhythm—stressing the word diuresis, i.e. an increase in water output—is fundamentally different from an osmotic diuresis? It seems to me they are both conditioned by and are secondary to electrolyte output. I agree the temporal relation is different, but as a renal mechanism is there any fundamental difference in the diuresis?

BORST: The reaction to the administration of saline is diphasic. It starts with a brisk water diuresis and continues after one and a half hours with a moderately increased output of both water and sodium chloride. But also in the diurnal rhythm there is no parallelism between water and electrolyte output. For instance, if a patient takes 80 g. of urea daily, the diurnal rhythm in the excretion of electrolytes continues in exactly the same way, but the rhythm in the output of water nearly disappears.

MILNE: More strictly, can we replace the word electrolyte by total osmotic load?

BORST: I do not think that relatively slight changes in osmolarity have that effect on the water. We had observations on the diurnal rhythm in patients who had a salt-poor diet and who excreted only small amounts of sodium chloride. Nevertheless, the elimination of water during the day was nearly double the amount during the night. We have also studied patients who had a diet poor in all electrolytes. They still had the rhythm in water excretion. In patients with circulatory insufficiency who completely retain sodium, a normal rhythm in the potassium excretion and a reversed rhythm in the water output may be found.

LEWIS: In answer to a question of Dr. Milne's I studied a case of panhypopituitarism with regard to electrolyte and water output on the Borst method, giving a fixed intake every four hours, and the electrolyte and water rhythms were quite dissociated. The patient had a diuresis at night, not an osmotic diuresis, the specific gravity came right down to 1002–1003, but she showed retention of water during the day. The electrolyte rhythm was normal. So I do not think they are really anything to do with each other. When we gave her ACTH the electrolyte rhythm remained the same, but she was able to respond to a water load during the day.

FLUID BALANCE IN ANURIA

JEAN HAMBURGER and GEORGES MATHÉ

WHEN an anuric subject is given as much water as the insensible loss, the proportion of water in the body rises. If electrolyte losses are not replaced, the increase of body water only affects the intracellular space; the so-called extracellular fluid actually seems to diminish.

The purpose of the present paper is to give the evidence for this, to suggest the reasons and to discuss the clinical implications.

Material

Twenty-nine dogs and 64 human subjects were studied. In the former, both ureters were tied under pentothal or chloralose anæsthesia. Their only food was a negligible quantity of sucrose.

Twelve animals (Group 1) were given this without water or electrolytes. By deducting alimentary losses from the weight loss, we estimated that the insensible loss was more than 20 ml./kg. per day.

Eight animals (Group 2) were given 20 to 50 ml./kg. water, intraperitoneally, every day, to replace the estimated insensible loss plus the alimentary losses.

Nine animals (Group 3) were given 100 mg. of sodium chloride and 50 mg. of sodium bicarbonate per kg. daily, intravenously, with a negligible amount of water, while no water was given by mouth.

The following investigations were carried out, either daily, every other day, or at the beginning and end of the experiment: body weight, heavy water space,* thiocyanate space, plasma specific gravity, proteins, chloride, bicarbonate, sulphate, phosphate, sodium, potassium, magnesium, calcium, freezing

*Determinations kindly made by Prof. A. Coursaget, Laboratoire des Isotopes, Hôpital Necker. Paris.

point, resistivity and *p*H, blood sugar, urea and cholesterol, mean corpuscular volume and estimation of the body water content of muscle by desiccation.

Sixty-four cases of anuria were also investigated at the Hôpital Necker between 1951 and 1953. In these, the data are more difficult to interpret, as anuria was often complicated by shock, septicæmia, etc., and the need for treatment often restricted the number of investigations that could be performed.

Body Water

In both the dogs and the clinical cases the body weight and water balance were followed. The muscle water and heavy water space were studied in the dogs.

In the dogs of Group 2, in which the insensible loss was replaced, the muscle water rose by about 5 per cent. This was less in animals on a dry diet. An increase in muscle water was also found in anuric sheep by Harris and co-workers (1952).

Complete figures for heavy water space are available only for six dogs. In these, the pre-operative total body water was 46 to 74 per cent of body-weight (apparently varying according to the fat content). After ureteric ligation, the plasma heavy water level fell steadily (in both the animals on a dry diet and the others), and heavy water space increased, by over 10 per cent of the body weight. Even allowing for the errors of this estimation, these results indicate an increase in total body water, even in dogs on a dry diet. Table I shows the results in one dog. The heavy water increased from 74 per cent to 86 per cent.

Table I

Dog No. 31	*Before ligature*	*3rd day*
D_2O Space (ml.)	4·500	5·300
(per cent)	74	86
Muscle Water (per cent)	73	74
Thiocyanate Space (ml.)	991	866
(per cent)	16	14·5
Effective Osmotic Pressure of Plasma (mOsm.)	319	315
Mean Corpuscular Volume	70	73

In the clinical cases, the same phenomenon can be observed (Hamburger and Richet, 1952). All were given 400 g. of glucose daily. The amount of water given varied according to the indications to be discussed later. These are the results in a case of mercurial anuria: nine days before the onset of

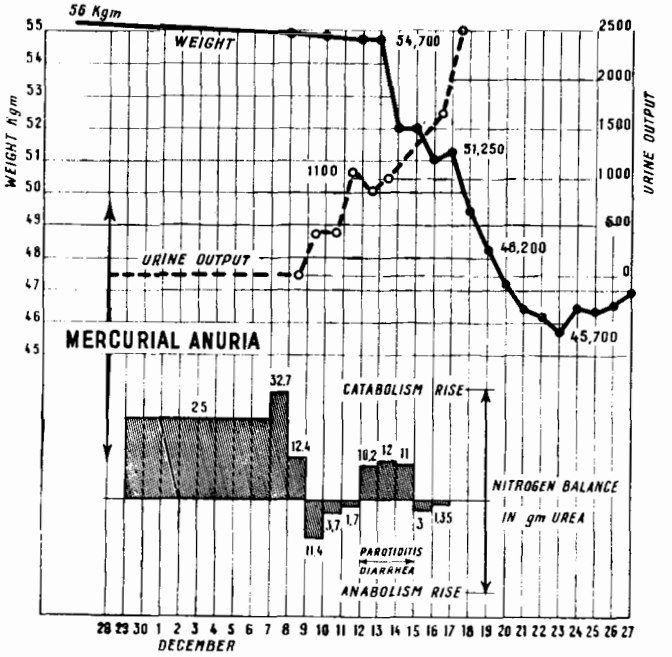


FIG. 1. Weight curve, daily urine output and nitrogen balance in a case of mercurial anuria (see text).

diuresis, 25 g. of urea were being formed a day (equivalent to 75 g. of protein), so that several hundred grams of tissue were being broken down daily, while the body weight stayed between 54 and 55 kg. With the onset of diuresis, the patient rapidly lost 9 kg., at a time when the nitrogen balance became positive and over two thousand calories a day were being ingested. Fig. 1 shows the weight, urine output, and nitrogen balance in this case.

The same sequence of events was observed in all ten patients. The average loss of body weight with the onset of diuresis was 10 kg., which must be regarded as a loss of the excess water accumulated during the period of anuria. Swann and Merrill

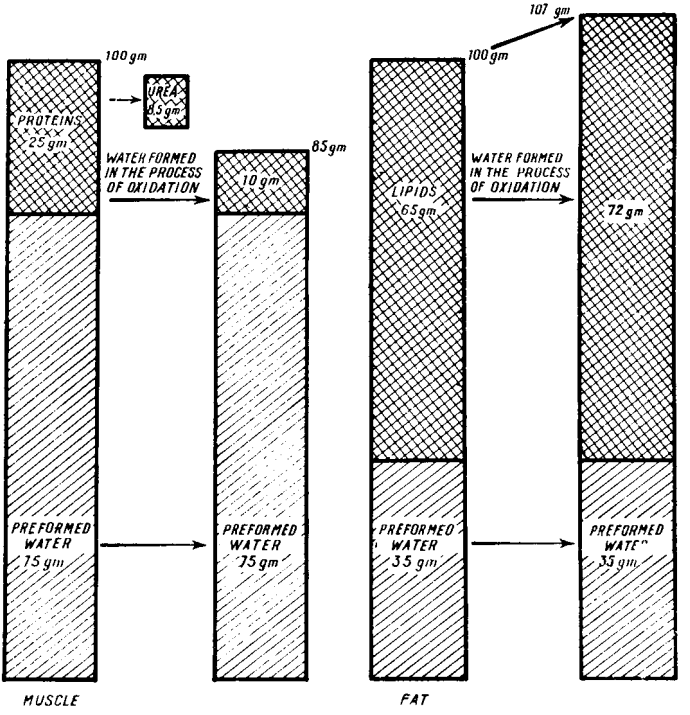


FIG. 2. Preformed water and water of oxidation derived from 100 gm. of muscle and fat respectively.

(1953) have reported that the heavy water space was increased in the four cases in which it was measured.

This water must be derived from the following sources (the calculation is illustrated in Fig. 2):

From protein catabolism. The endogenous urea production averaged 240 g. The water of combustion would be 280 ml., and the preformed water liberated 2100 ml., making an average total of 2400 ml. of water from this source.

From glucose catabolism. Our patients were given 400 g. of glucose a day (an average of 2800 during the period of anuria); 1500 ml. of water would derive from this.

From fat catabolism. Deducting the weight loss accounted for by protein catabolism, it appears that 6·8 kg. of fat were burnt. The water of combustion would be 4900 ml., and the preformed water liberated 2400 ml., giving a total of 7300 ml. from this source.

From these three sources the subjects derived on the average 11 litres of water. This figure agrees well with the weight loss at the onset of diuresis. Part is made up of water liberated from broken-down tissue (4300 ml.), but the greater part (6700 ml.) is water formed in the organism as a result of the increased catabolism during anuria.

A similar explanation can be given for the increased heavy water space in the dog, but the preformed water is included in this space both before and after tissue destruction. Here, water derived from protein and carbohydrate is found to be small, so that a very large amount of fat must have been burnt. This averaged two-thirds of the total body fat. The low calorie intake must be partly responsible for this, which is perhaps evidenced by the hyperlipæmia and increase of blood cholesterol: in the animal previously shown (Table I), weighing 6 kg., 600 g. of fat were burnt.

Water Distribution

The increase of body water affects the intracellular fluid alone: this is increased not only by the new water formed, but probably also by a shift of water from the extracellular space.

This is suggested by the changes in: (*a*) plasma specific gravity, plasma proteins, and hæmatocrit; (*b*) mean corpuscular volume; (*c*) volumes of distribution of test substances.

(*a*) *Plasma specific gravity, plasma proteins, and hæmatocrit.* Plasma specific gravity and proteins rose in the dogs. An increase in plasma proteins was also found in anuric sheep by Harris and co-workers (1952). In one dog, from which no blood was removed, the hæmatocrit rose slightly. In the

human subjects, there was often evidence of the same phenomenon on arrival in the ward, before any correction had been made. In eleven cases, the plasma proteins exceeded 8·5 g. per cent, rapidly returning to normal when saline was given.

Plasma specific gravity and protein, and, in the absence of any complicating disorder of the blood itself, the hæmatocrit, give an indication of any change in extracellular fluid volume; but it cannot be assumed that this will still hold in renal insufficiency or that changes in plasma volume are proportional to changes in extracellular fluid volume.

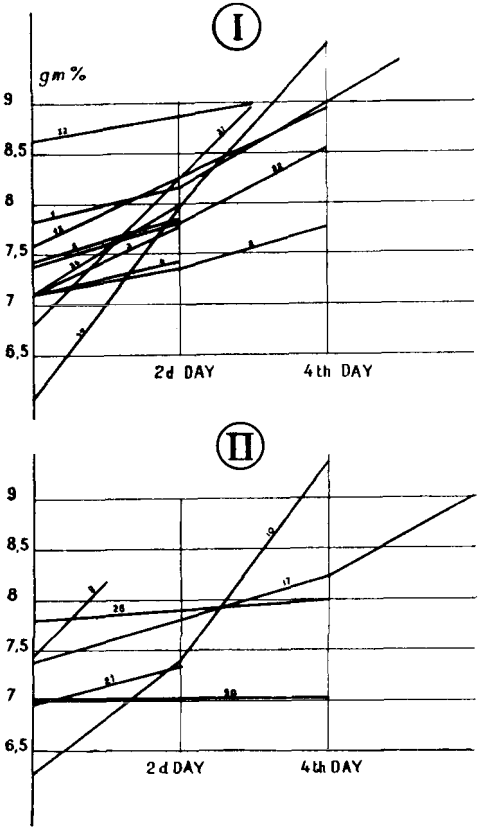
(*b*) *Mean corpuscular volume.* The mean corpuscular volume constantly rose in the dogs of Group 2, in which the insensible losses were replaced. It frequently rose in those given sucrose alone. It rose in most of the clinical cases. This suggests that water moved into the cells, although it could be debated whether the response of red cells to extracellular osmotic changes is the same as the response of other body cells.

(*c*) *Volume of distribution of test substances.* The volume of distribution of inulin and thiocyanate form the accepted methods for estimating the extracellular fluid volume, but, unfortunately, it is likely that the cell membrane is greatly modified in renal insufficiency. Merrill and his co-workers (Finkenstaedt, O'Meara and Merrill, 1953) found a progressive fall in the level of plasma inulin after its injection into 13 anuric patients, so that its exclusively extracellular position is evidently not maintained. The same phenomenon occurred in nephrectomized dogs, and these authors question the validity of this method in renal insufficiency.

In both species we have studied, only the thiocyanate space was estimated. This substance is normally eliminated by the alimentary canal, as well as by the kidney, so that after ureteric ligation in dogs the plasma level, plotted semilogarithmically against time, should fall slowly along a straight line. This has been the usual finding, but in several animals the plasma level has risen slightly, evidently indicating a contraction of the extracellular fluid volume; we have found

in other animals that the plasma blanks do not vary enough to account for this.

It may be concluded that in the case of anuric animals,



PLASMA PROTEINS

FIG. 3 (*a*)

thiocyanate is better than inulin for the study of extracellular changes. In one dog, repeated estimations of the thiocyanate space were made, and the results suggested that water moved into the cells. This occurred in all seven dogs of Group 2 in which estimations were made; it occurred in five out of nine

dogs studied in Group 1. The difference between the two groups is probably due to the frequency of vomiting and

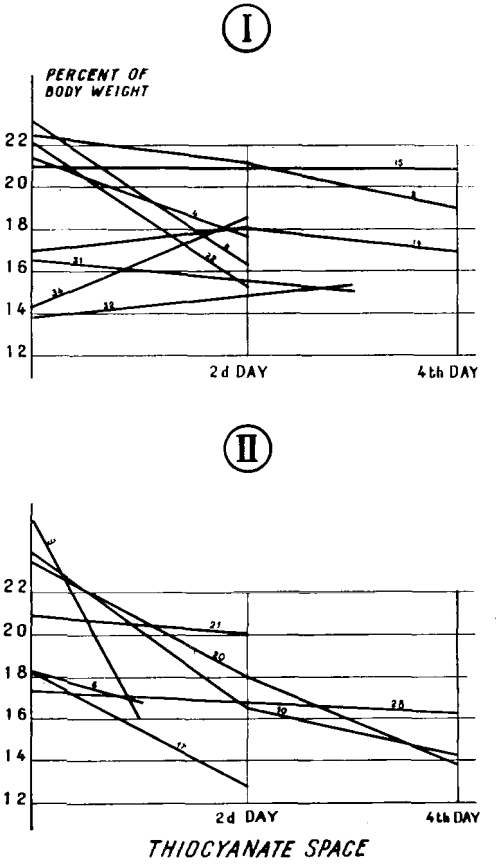


FIG. 3 (*b*)

FIG. 3. Plasma proteins and thiocyanate space in two groups of dogs after ureteric ligation (Group 1: dry diet; Group 2: insensible water loss replaced).

diarrhœa in Group 2, for reasons to be given later. Fig. 3 shows the rise in the level of plasma protein and changes in the thiocyanate space in the first two groups of dogs.

The plasma volume was found to decrease in anuric sheep by Harris and co-workers (1952) and in rabbits by Muirhead and co-workers (1952).

In the clinical cases, it was seldom possible to measure the thiocyanate space before treatment began. Fig. 4 shows the

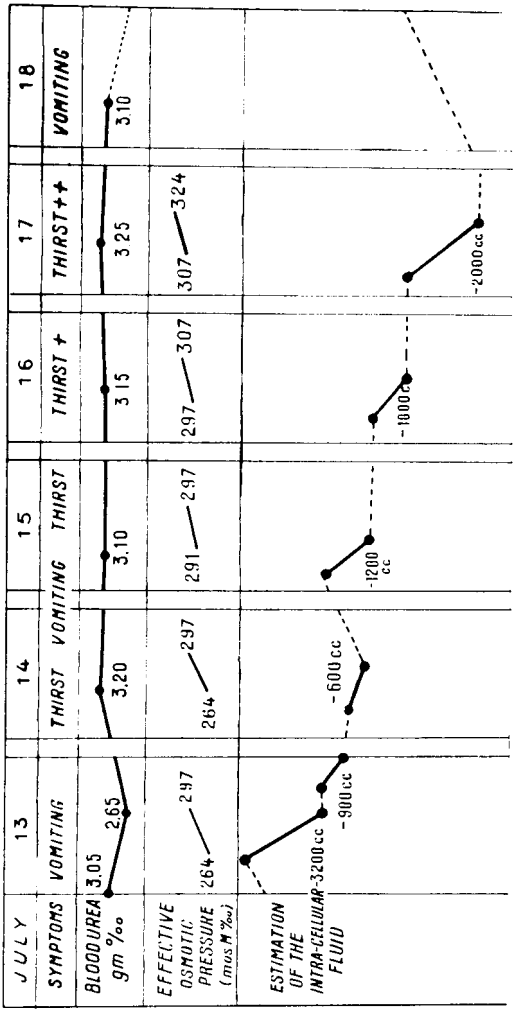


FIG. 4 (*a*)

extent to which intestinal dialysis increased the thiocyanate space at the expense of the cells in a case of mercurial anuria. This strikingly illustrates the extent of cellular over-hydration in anuria, 9 litres being removed.

These abnormal shifts of water appear to depend on either

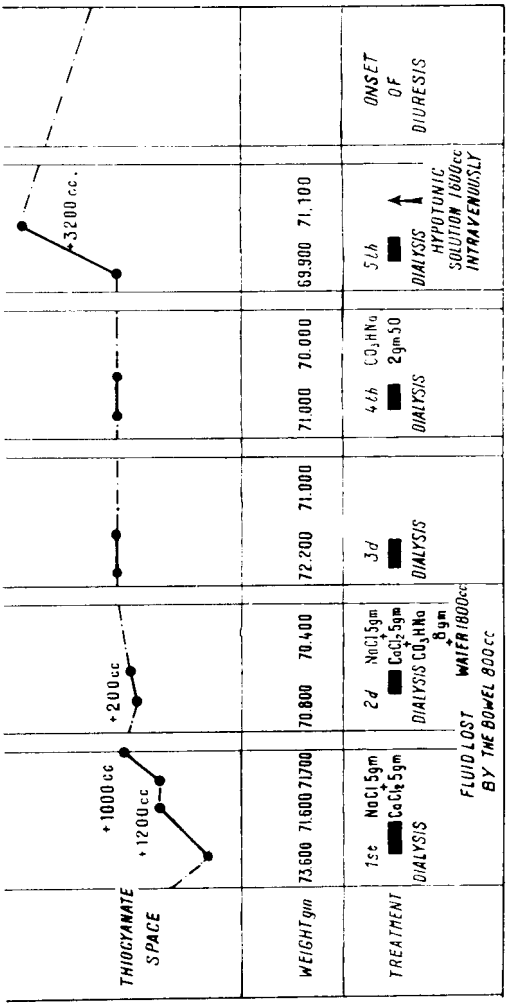


FIG. 4 (*b*)

FIG. 4. Details from a case of mercurial anuria showing changes in extracellular and intracellular fluid volumes. The former is estimated from the thiocyanate space and the latter from the changes in this and in the body weight.

Repeated intestinal dialysis removed nearly 9 l. of I.C.F. and increased E.C.F. by 5·6 l. (Average normal effective osmotic pressure is 302 m.Osm./l.).

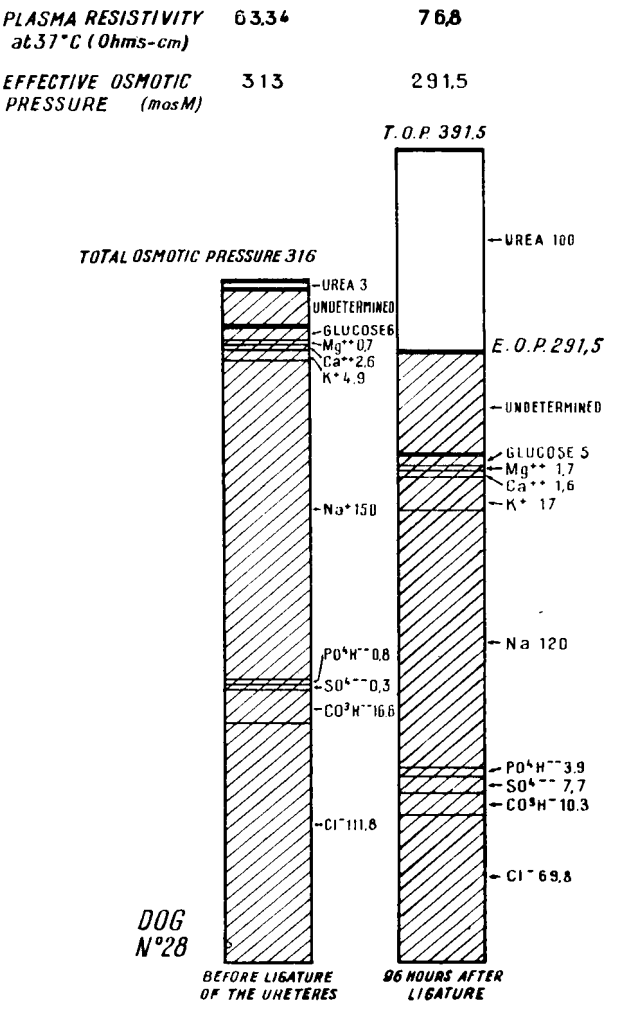


FIG. 5 (*a*)

changes in the tonicity of the extracellular fluid or on intracellular changes of an unknown nature.

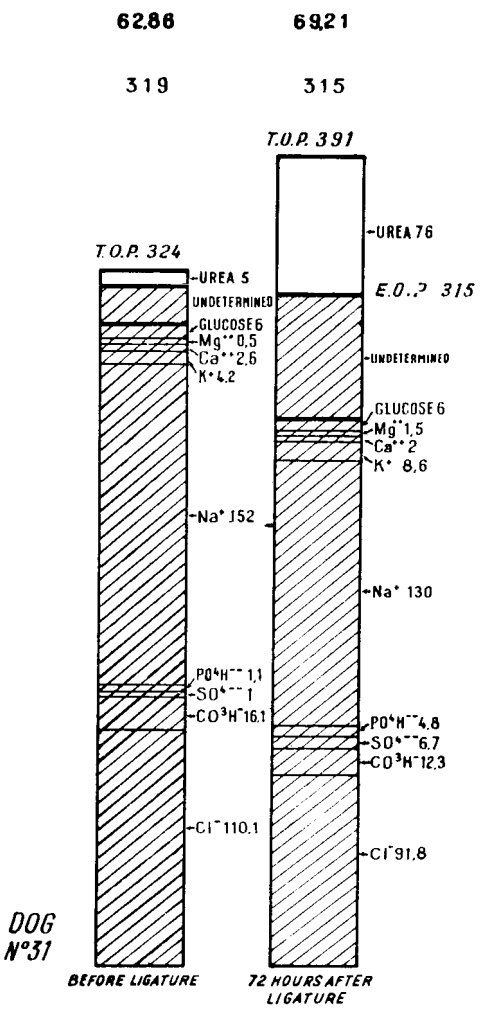


FIG. 5 (*b*)

FIG. 5. Electrolyte structure of plasma in two dogs before and after ureteric ligation, expressed in mOsm./l. "Effective osmotic pressure" is derived from the plasma freezing point (see text). In dog 28 (given 20 cc. water/kg. daily) there is severe hypotonicity. In dog 31 (dry diet) this is less marked.

Rôle of variations in extracellular tonicity

It is known that cells immersed in a hypotonic medium absorb water; thus a decrease in the extracellular fluid volume can occur. In renal insufficiency, the osmotic effect of urea must be discounted for this is distributed throughout the body water. The "effective osmotic pressure" of the plasma is therefore calculated by deducting that exerted by the urea from the figure derived from the estimation of the freezing point (Hamburger and Mathé, 1952). This value can also be derived from the estimation of the electrical conductivity or from the sum total of the plasma electrolytes themselves. Fig. 5 illustrates these methods.

In anuria, plasma hypotonicity is very commonly observed. This is constant in dogs of Group 2. Those in Group 1 usually remain isotonic (some become slightly hypotonic).

Extracellular hypotonicity was found in 39 of 64 patients on arrival in the ward.

This might possibly be accounted for by: (a) electrolyte losses from diarrhœa and vomiting; (b) shifts of electrolytes into cells. In the dogs, even those without diarrhoea, the total extracellular sodium declined.

Rôle of intracellular disturbances

Even if we know the tonicity of the extracellular fluid, we know nothing of that of the intracellular. Indeed, the latter is so heterogeneous that we prefer to use the purposely vague term "force of cellular hydration." There is evidence that this depends on the cellular metabolism. Not only has this been demonstrated for cells and tissues by Robinson (1950), but we have shown it for the whole organism (1951–1952); we showed that anoxia and acidosis produced a shift of water from the extracellular space into the cells. This perhaps partly explains the facts observed.

Clinical Implications

Extracellular dehydration. The symptoms and signs in man consist of severe asthenia, rapid weak pulse, and dry inelastic skin. They are found in most cases before treatment.

This dehydration may itself impair renal function and delay recovery.

Cellular over-hydration. In man and animals, the signs are the same.

Vomiting and diarrhoea were much commoner in the dogs

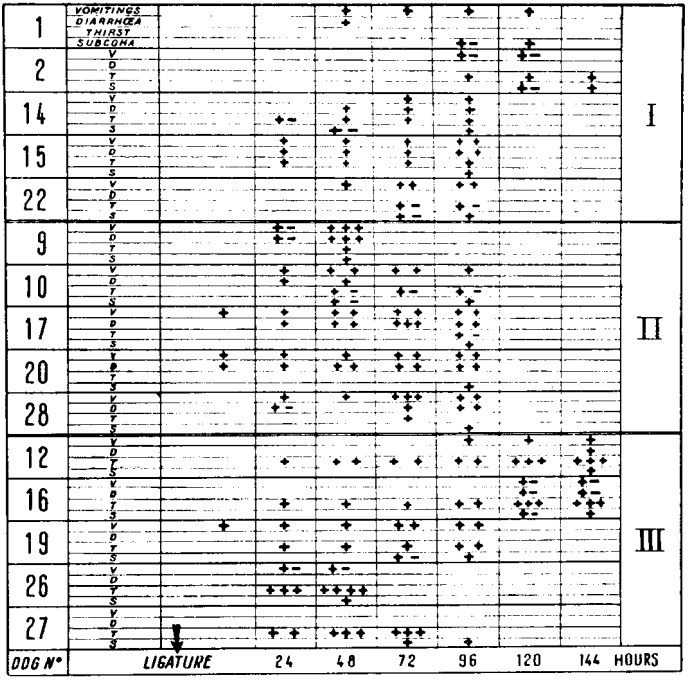


FIG. 6. Comparison of the symptoms in the three groups of dogs after ureteric ligation. (Group 1: dry diet. Group 2: insensible water loss replaced. Group 3: given hypertonic NaCl and $NaHCO_3$). Vomiting and diarrhœa were commoner in Group 2, thirst was evident in Group 3.

of Group 2, in which the insensible loss was replaced, than in those on dry food or those given sodium chloride and bicarbonate. Fig. 6 compares the symptoms in five dogs of each group.

In man, asthenia, hypothermia, anorexia, nausea, vomiting, cramps, headache, depression, psychosis, and even convulsions

and coma occur. Electroencephalographic tracings in a few cases show generalized symmetrical slow waves. All these symptoms disappear after treatment with hypertonic saline.

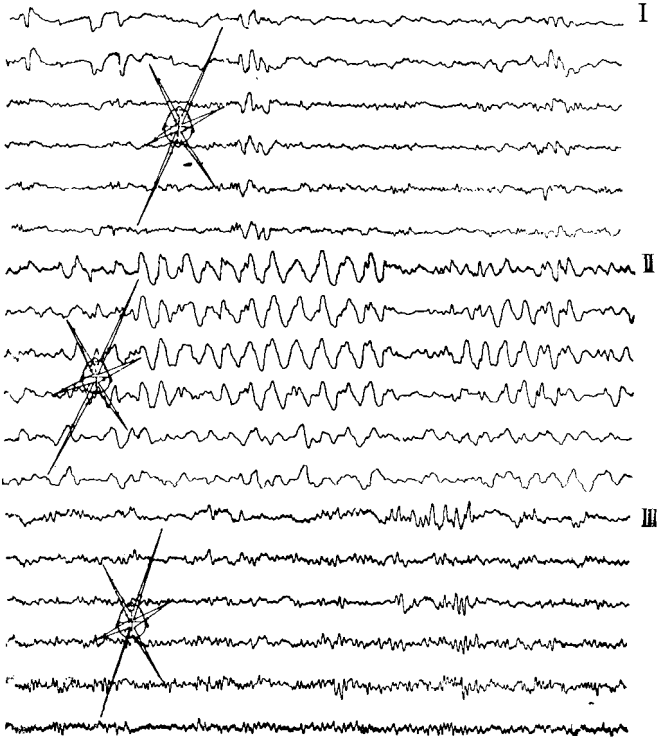


FIG. 7. Electroencephalograms from a case of acute renal insufficiency.
(I) Early in the course.
(II) After large quantities of water had been given, when the symptoms of over-hydration were present.
(III) After intestinal dialysis and administration of salt.

Fig. 7 shows the tracings from a case of acute renal insufficiency.

Many observations suggest that the onset of diuresis may be delayed by cellular over-hydration (Funck-Brentano, 1953).

Effect on nitrogen catabolism. Comparing the blood urea levels of the three groups of dogs, those of Group 2 were the highest and those of Group 3 (given salt and bicarbonate) the lowest. The significant difference only appears on the fourth day, so we have only considered dogs that have survived longer than this. This is seen in Fig. 8. These results

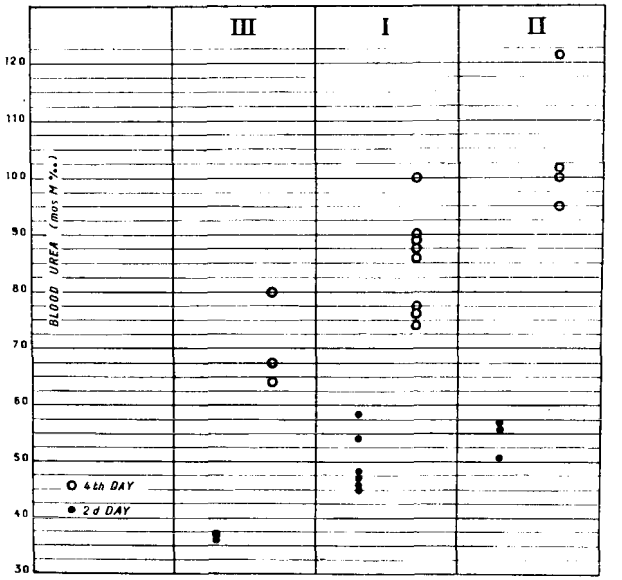


FIG. 8. Blood urea in the three groups of dogs. By the fourth day this was highest in those in which the insensible water loss was replaced.

may have a bearing on the "Azotémie par manque de sel" described by Pasteur Vallery-Radot (1914).

Therapeutic Implications

We have outlined the general tendencies in anuria, but each case must be treated on its own merits. Many cases have already had some treatment before arriving in the ward, and,

in many of these, both intra- and extracellular over-hydration is already established.

In most of the untreated cases, the tendency to cellular over-hydration and extracellular dehydration must be corrected.

The administration of water. If the metabolism of the patients with anuria were normal, the amount of water required would be 300 ml. less than the insensible loss, which itself is often increased. Our experience shows that patients do best on an intake of about 700 ml. At any time, an accurate indication of the need for water is given by the symptoms and the osmotic pressure of the plasma; low osmotic pressure with nausea, vomiting and nervous disturbances are indications for prohibiting water; thirst and high osmotic pressure the reverse.

The administration of electrolytes. Although the diet in anuria should be salt free, it must be remembered that the usual cause of cellular over-hydration is extracellular hypotonicity. In this case, besides fluid restriction, intravenous hypertonic saline may be indicated. The amount should not exceed 100 milli-osmols at a time, because of the danger of pulmonary œdema, and the blood pressure must be checked as a control. If the symptoms persist, as may occur when there are large extra-renal losses, the infusion should be repeated.

The choice of the sodium salt to be administered depends on the electrolyte balance. Sodium chloride may be combined with citrate, lactate or bicarbonate if there is severe acidosis.

Extra-renal dialysis. In certain cases, especially after mistakes in therapy, there may be gross over-hydration. In such cases, it may be dangerous to try and correct the tonicity by giving salt. Extra-renal dialysis (for example, intestinal dialysis—Hamburger, Mathé and Crosnier, 1950) is then indicated to remove water.

Summary

Sixty-four cases of anuria in man and 29 cases of experimental anuria in the dog have been studied.

In anuria there is a tendency to an increase in the total body water, which appears to result from increased production of water, mainly derived from increase of catabolism.

This over-hydration only affects the intracellular fluid while the extracellular fluid volume seems to diminish. This is partly due to a fall in extracellular fluid tonicity, but also to changes in the cells themselves.

The clinical implications are discussed.

The authors are indebted to Dr. A. A. G. Lewis for the translation and help in the preparation of this paper.

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DISCUSSION

MERRILL: I have talked about this problem with Dr. Mathé before. We have obtained quite surprising results in measuring heavy-water spaces in people, with what we thought was adequate restriction of fluid to replacement of the insensible losses. The catabolic response to the trauma that one almost invariably sees as a precipitating factor in acute renal failure, certainly, in my opinion, results in a good bit more catabolic water than we had realized, and satisfactorily accounts for the water diuresis that may occur in patients in whom we have thought the fluid restriction was adequate. We thought it might account for the hyponatræmia that we had previously attributed to intracellular shifts

of sodium. I do not know quite how to reconcile that with Dr. Mathé's data on extracellular fluid contraction, because although we found total expansion of body water we found equal expansion of extracellular fluid. With regard to inulin, although our observations made us certain that inulin did not just stay in the extracellular space in the anuric patient, nevertheless the rate of disappearance was slow enough so that over a period which we thought was adequate for rough equilibration, one could say that in general our patients had an expanded extracellular fluid volume—or a better term, I think, would be an expanded volume of distribution of inulin, whatever that measures. I think that has an important therapeutic connotation, both in terms of our concepts of how much water should be administered, and also from the standpoint of the caloric intake. Dr. Mathé and our group have found that a good bit of this water comes from the catabolism of fat. Dr. Moore in his surgical patients pointed out that as much as 3,000–3,500 fat calories may be utilized per day by the patient who has had major surgical procedures. If that is true, one can easily see where the endogenous water comes from, but above and beyond that it makes me wonder a little about the concept—this may stimulate some discussion—of giving exogenous fat to these people. I think we know, in the patient or the animal with acute trauma, that the provision of large amounts of calories does not conclusively influence the initial catabolic phase. Ingle has gone one step further and shown, so far as the relationship is catalysed by the adrenal, that fat and protein will not affect it but glucose will. If we all agree that acute renal failure is a self-limited disease and that people have adequate fat stores to begin with, I wonder if the provision of excess fat calories in these patients is really going to help us much. I would sharply delineate what I have said about this situation from that of the chronic patient in whom the fat stores have been depleted, in whom the liver glycogen is low and in whom there is no acutely catalysed catabolic response.

RAASCHOU: A problem, which can be solved by performing aspiration biopsies in acute renal failure, is whether or not there is interstitial œdema in the kidney, and if so, whether it forms part of the pathological picture or may be due to overhydration. In fact, in our patients who were examined in the oliguric phase, we have found an interstitial œdema; in these patients we considered the fluid restriction to have been adequate.

MATHÉ: Answering Dr. Raaschou, I wish to point out that, besides the œdema between the tubules, there may be, in some cases of anuria, a cellular overhydration. This hypothesis was suggested to us by several clinical cases, in which the correction of the hypotonicity alone was sufficient to provoke the diuresis. This view is also supported by various experiments of Funck Brentano: in these, he has compared the effects of isotonic saline and glucose solutions in large amounts. The former, which are known to produce essentially an extracellular overhydration, resulted in an abundant diuresis; while the later, known as responsible for overhydration both extra and intracellular, proved, after an initial polyuric phase, to be anuric in their final action.

LEWIS: What about renal decapsulation?

RUSSELL: Has it ever worked, that is what I would like to know? I have seen the kidney decapsulated in these circumstances but I have yet to learn that it is effective.

MERRILL: The classical observation, always used as an argument against decapsulation, has been made by Talbot—I think it was—who catheterized both ureters and decapsulated one kidney and found that the diuresis occurred in both kidneys at the same time. So that the causal relationship has been in some doubt. I must say, I know of no real evidence that it has done any good. There is, however, an increasing amount of evidence that perhaps there is interstitial œdema and perhaps increased intrarenal pressure may have something to do with this, but I certainly cannot evaluate it now and I personally will not treat my patients by decapsulating at present.

WINTON: Judging from dog's kidneys, I do not think decapsulation would necessarily overcome the implied obstruction, because it is interstitial. The renal tissue is very rigid indeed, and in the dog's kidney decapsulation does not reduce the tension very much.

MERRILL: I think that may be the answer.

DE WARDENER: We have been doing that for the last six months. We have been taking the intrarenal pressure bilaterally and simultaneously with continuous recordings via capacitance manometers. On one side the kidney has been decapsulated and on the other left intact. We find that at normal intrarenal pressures there is no difference between the two sides. If we raise the intrarenal pressure by mannitol diuresis, there is no difference between the two sides. The rate at which it goes up or the extent to which it goes up makes no difference. If we tie a noose around the inferior vena cava and get a rise of intrarenal pressure to 50 mm. Hg, again it is the same on the two sides. Finally we have perfused the two kidneys with a pump and got a rise of intrarenal pressure of up to 100 mm. Hg, and again found the same on both sides. I do not think the capsule, if it has any effect at all, has a very appreciable one.

ALWALL: The size of the kidneys is, as a rule, always increased on the X-ray plate in acute anuria. In our rabbit material, there is no difference between the increase of NPN after ligation of the ureters in the following two series: (1) control animals and (2) rabbits that had been given an amount of fluid corresponding to 15–25 per cent of the body weight after the ligation of the ureters.

If the fluid supply during anuria-oliguria is limited to an amount corresponding to the real need, there should be no increase in the extracellular fluid, i.e. the body weight is to be continuously reduced by a weight corresponding to the breaking down of the tissues (liberation of tissue fluid). If the body weight is kept constant, there will be a continuously increasing fluid retention (increase in the extracellular fluid) during the anuric-oliguric stage. This fluid retention may explain the frequent appearance of pleural effusion and fluid lung in cases with constant or too slowly reduced body weight during anuria-oliguria and the later rapid loss of body weight during the polyuric stage. The

increase of the extracellular fluid (fluid retention) may be avoided by adequate therapy, i.e. *sufficient* limitation of the fluid supply during the anuric-oliguric stage.

MATHÉ: What is your solution?

ALWALL: Ringer's.

DARMADY: Going back to the question of the extracellular volume in acute tubular necrosis, we have found that, allowing 1000 ml. as insensible loss, the extracellular volume is increased and tends to expand. That is one of the reasons why we have now taken the fat out of our mixture and use glucose almost entirely. Unfortunately, some of these cases come with an apparently already raised extracellular volume, they often have a raised effective osmotic pressure which theoretically would seem to prevent diuresis and one is worried about giving extra fluid in order to produce a diuresis, although I have been able to do this. I have actually two cases which show the effect of giving salt to patients whose plasma was hypotonic and have produced a diuresis in that way, which does confirm Dr. Mathé's evidence. I do think that the volume of water to be given for insensible loss should be reduced, but 1000 ml. is satisfactory when glucose alone is given.

ALWALL: Formerly I gave sodium chloride in order to normalize the value of serum chloride. Several times œdema followed and the diuresis stopped. Don't you think there must be a cellular shift? Nowadays I correct hypo-electrolytæmia in uræmic cases only if absolutely necessary.

MERRILL: There is the point which Dr. Mathé makes on which we have no information, that there is a marked difference between the sodium and chloride levels, plus urea, and the total freezing point depression—I had not realized that and am interested to hear it.

MATHÉ: Indeed, in the course of acute renal insufficiency, we have, both experimentally and clinically, noticed a difference between the osmotic pressure as measured by the freezing point and the one obtained by summing chemically each of the plasma electrolytes.

This difference, which often amounts to several dozen of milliosmoles, leads us to accept with some reservation the osmotic pressure estimation based upon the level of sodium or chloride.

BULL: That effective osmotic pressure is measured by electrical conductivity, is it not?

MATHÉ: We obtain the "effective osmotic pressure" by measuring the freezing point. From the value thus obtained, we subtract the part which is due to urea. However, electrical resistivity is also of great value in the estimation of effective osmotic pressure.

The formula is as follows: $E = \frac{1 \cdot 8 \times 10^6}{\rho\,(100 - 0 \cdot 25P)}$

where E = total number of milliequivalents per litre.
ρ = resistivity at 37°, in $\omega/cm^2/cm$.
P = concentration of proteins in grams per litre.

RENAL FACTORS IN VOLUME CONTROL

D. A. K. BLACK

In considering any regulatory process, we should have knowledge of the stimulus which sets it in motion, the steps by which the "message" is transmitted to the end-organ, and the precise manner in which the end-organ responds to it. When these matters have been defined for one regulatory mechanism, we are then in a position to consider its integration with other regulatory mechanisms, similarly defined, and so to build up a comprehensive picture of one sector of biological homeostasis. Let us make a drastic simplification of the problem of volume control, by reducing it to the control of water, sodium, and potassium; in doing this we relegate anions generally to a "mendicant position," to use Gamble's expression. This may not be altogether a safe procedure, for some renal operations could be primarily on anions—for example mercurial diuresis may be basically a chloruresis; but in the time available I think we must limit ourselves to water, sodium, and potassium. Even so, it will be apparent to all of you that I cannot go into any detail, and water, sodium, and potassium excretion have each been the subject of separate papers in our symposium. My purpose is rather to examine in respect of these substances how much—or how little—we know, in broad outline, of the stimulus, transmission, and renal mechanism of their control. By this method of analysis, I think I can expose some rather surprising gaps in our knowledge, in spite of the mushroom literature in this field, much of which seems to be strangely repetitive.

Let us take water, sodium, and potassium, in that ascending order of ignorance. With water, thanks largely to the work of Verney, we really get tolerably close to the objective which I have just defined—a knowledge of stimulus, transmission,

and end-organ effect. The effective stimulus to water conservation is an increase in the osmolarity of the plasma perfusing the internal carotid artery. This stimulates secretion of posterior pituitary antidiuretic hormone (ADH), probably not directly, but through the mediation of hypothalamic "osmoreceptors." ADH is then transmitted humorally to the kidney. The orthodox view is that in the kidney the proximal tubules carry out an essentially iso-osmotic reabsorption, so that we must place active water conservation distal to them, most probably in the distal convoluted tubule. We have, however heard Wirz advocate a different view of hypertonic urine formation, and it is also possible that ADH could inhibit a process of water secretion in the distal tubule. While there is therefore some uncertainty as to the precise renal mechanism of water conservation, I think we possess first approximations to a knowledge of the stimulus and mediation which induce the kidneys to conserve or discharge water. It is important to note, however, that the established stimulus for the ADH mechanism is a change in osmolarity, and not a change in flow-rate or volume. Most recent studies suggest that ADH has no direct effect on sodium or potassium excretion. We can, therefore, invoke the ADH mechanism to explain the regulation of body fluid tonicity, which may be well maintained in spite of considerable changes in the volume of body fluids; but for an explanation of these volume changes we must look elsewhere, to the mechanisms which control sodium and potassium output. We may also observe that "companionship of water and electrolytes" (Gamble, 1951) is not entirely produced by the ADH mechanism, but that changes in solute excretion are accompanied by similar changes in water excretion through the intrinsically renal mechanism of osmotic diuresis.

When we come to sodium excretion, we have not so clear a picture as in the case of water. This may be partly related to the comparative difficulty of estimating sodium in blood and urine, which has only quite recently been obviated by flame photometry; and the past few years have produced many

papers on this subject. With regard to stimulus, the concentration of sodium in extracellular fluid (ECF) is clearly an adequate stimulus to either sodium conservation or to natriuresis. There are, however, many situations—including diurnal variation—in which the rate of sodium excretion shows great changes in the absence of any detectable change in the plasma sodium concentration; and many workers have sought for a stimulus to sodium conservation independent of the plasma sodium level. One possibility is a mechanism sensitive to changes in ECF volume, and comparison of sodium output in recumbent and sitting subjects has suggested that there may be an intracranial apparatus of this type. Since the assumption of a sitting posture leads to a considerable fall in cardiac output, the fall in sodium output could be in part at least related to this, as Borst has just claimed. An alternative approach lies in the infusion of an isotonic electrolyte solution, and in the dog this has been shown to increase the rate of sodium output (Wesson *et al.*, 1950); but a change in cardiac output was not excluded, nor was the possible effect of lowered colloid osmotic pressure in the plasma considered. When ECF volume is depleted by intraperitoneal infusions of concentrated protein, retention of water and chloride is observed, but here again the colloid osmotic pressure of plasma is altered (Cort, 1952). When plasma is infused, the renal excretion of sodium increases, without any change in either colloid or electrolyte osmotic pressure. These various observations make it fairly certain that increase in plasma volume will increase sodium excretion, and decrease in plasma volume will diminish it, but they leave it uncertain whether this effect is related to the plasma volume itself, to cardiac output and associated changes in renal hæmodynamics, or to the ECF volume as a whole. Clinically, one is impressed by the apparent indifference of the kidneys to extravascular accumulations of extracellular fluid, and one would be inclined to follow Borst in regarding cardiac output as a significant stimulus. This is probably not the whole explanation, however, as active reabsorption of

sodium by the tubule cells has been shown in cardiac failure, for example, and also in very moderate sodium depletion without obvious circulatory change.

The relative importance of hæmodynamic and hormonal factors in regulating sodium excretion is still disputable. Homer Smith and his school lay great stress on sodium load as the determinant of sodium excretion, but also allow the possibility of changes in tubular reabsorption under hormonal control (Smith, 1951). A few years ago, we were able to demonstrate one situation in which active tubular conservation of sodium was able to over-ride very considerable increase in the sodium load (Black, Platt and Stanbury, 1950). After a few days on a rice diet, sodium output in the urine was consistently less than 0·5 per cent of the filtered load over a range of sodium filtered from 4·5 to 29·8 mEq./min. Conversely, in Addison's disease sodium excretion remains high in spite of a diminished filtered load of sodium. Moreover, even in situations such as cardiac failure where diminished sodium load is demonstrable as one cause of sodium conservation, analysis of tubular performance suggests that an increased active reabsorption of sodium is also partly responsible. It is likely that adrenal cortical hormones are concerned in this, but we do not know the stimulus to adrenal activity, nor the precise hormone concerned.* Compound F has been shown to have a salt-retaining action, but it is uncertain whether it could account for the whole effect. The solution of this problem awaits more reliable methods for the assay of corticoids in blood and urine in sodium depletion and in œdema; studies on these lines would perhaps be more relevant than the many assays of ADH-like substances in the urine of œdematous patients, since excess of ADH would not account for sodium retention.

It would appear even from this short survey that renal handling of sodium is less understood than that of water;

*Recent work makes it seem likely that the main salt-retaining action of the adrenal cortex is mediated by the steroid "electrocortin" (Simpson *et al.*, 1953; *Experientia*, **9**, 33).

but potassium presents a still greater problem. There is some excuse for this, in that the main collection of body sodium perfuses the kidneys directly, and rapidly; while the bulk of body potassium is separated from the kidneys by the ECF, which contains little potassium. With sodium, it can be at least plausibly maintained that the serum sodium level, or perhaps the filtered load of sodium, is the main determinant of sodium excretion; and this would be teleologically justified, in that the serum sodium level is rather directly an index of the sodium content of the body. With potassium, however, the relationship between body potassium and serum potassium concentration is much less direct, as the serum potassium is influenced by the sodium balance, the presence of acidosis or alkalosis, and probably by changes in cell metabolism other than their potassium content. It is not, I think, possible at present to decide whether the stimulus regulating potassium excretion is the serum potassium level, or the amount of potassium in the cells. In favour of the importance of the serum potassium, we have the observations that insulin and glucose, which lower the serum potassium, but not the cellular potassium, decrease potassium excretion (Harrop and Benedict, 1923); and also that in diabetic ketosis, where there is a body deficit of potassium, but the serum potassium level is kept up by dehydration, the excretion of potassium remains high. On the other hand, Lowe (1953) has shown that the kidneys may excrete a urine with a lower potassium concentration than that of serum even in the presence of normal serum potassium levels; and Milne and I also found continuing potassium conservation at a stage of recovery from potassium depletion when the serum levels had returned to normal. Even in these two instances, where cellular potassium seems to have been important, we do not know whether concentration or total amount of cellular potassium was the effective stimulus, or whether the potassium content of the renal tubule cells had any special importance. In potassium-depleted rats, analysis of renal tissue gave no definite evidence of any considerable depletion of cellular

potassium (Darrow *et al.*, 1953). If the amount of potassium in the body cells generally can in fact influence potassium excretion, we have no real knowledge of how it does so. Serum potassium changes are not constantly present; adrenal cortical hormones are likely to be concerned in potassium regulation, and have been shown to be important in the post-operative potassium diuresis (Moore and Ball, 1952).

When we compare the renal mechanisms effecting sodium and potassium excretion, an obvious difference appears in the ratio of excreted to filtered cation. With sodium, this is of the order of 1–2 per cent, with potassium 15–20 per cent. It is clear from this that decreased reabsorption would be adequate to account for say a tenfold increase in sodium excretion, but not for a similar increase in potassium excretion. When the number of nephrons is drastically reduced by disease, it is only by tubular secretion of potassium that a normal output can be maintained; and in such circumstances excretion of potassium in excess of the filtered load can be demonstrated. With normal kidneys, the predominant activity of the tubules in respect of potassium is clearly one of reabsorption; but Berliner and his group (1951) have shown rather clearly that this represents the net effect of simultaneous transport of potassium in both directions, so that some tubular secretion of potassium is part of normal tubular activity. They found an interesting relationship between potassium and hydrion excretion which led them to suggest competition between these two ions for exchange with that moiety of sodium which is available in the distal tubule for the Pitts cation exchange mechanism. Very recently, Mills and Stanbury (1953) have found evidence of a similar K-H reciprocity during diurnal variation in urinary acidification; and Mills and Thomas (1953) have also shown that the normal nocturnal excretion of an acid urine can be prevented by giving potassium salts, even potassium chloride. The intimate cellular mechanisms underlying these interesting changes in tubular behaviour have still to be unravelled; but it is apparent that the renal handling of potassium is both complex and flexible. The

ability to produce a urine very low in potassium is somewhat of a late response to potassium depletion, and the U/P ratio for potassium never approaches the very low sodium U/P ratio induced by very moderate sodium depletion; but this failure of conservation can be set against the very great ability of the kidney to set up a high U/P ratio from plasma, which is rather an unpromising raw material for potassium excretion. The toxicity of potassium in ECF makes an efficient mechanism for excreting this ion a biological necessity, while with sodium the natural risk is that of depletion, and the kidneys have not yet had time to devise a very good answer to ill-judged intravenous loads of sodium.

In addition to the many points of detail about which we need more information, it seems to me that there are two really major issues on which we have scarcely begun to acquire knowledge. One is the means whereby volume changes in ECF provoke changes in sodium excretion, the other the means whereby changes in the potassium content of body cells can influence the urinary output of potassium. When we stretch mechanistic explanations of salt conservation by the kidneys to their limit, we still do not really understand why a normal person can eat as much salt as his stomach will retain, whereas œdematous patients outwit all our devices of salt restriction. I am sceptical of the present tendency to make the kidney the culprit in all forms of generalized œdema, and would support J. P. Peters in stressing extra-renal factors in œdema formation. I cannot follow him in his recent attempt to ascribe the œdema of acute nephritis entirely to cardiac failure (Peters, 1953); though one may perhaps regard this as a fit rejoinder to those cardiologists who blame the kidney for congestive failure. There is no obvious direct clinical challenge, like that of œdema, to the second problem which I mentioned; but I do not doubt that its solution would bring clinical dividends since cellular potassium depletion is the accompaniment, though not necessarily the cause, of general cellular dysfunction. These wider problems of homeostasis should represent a stimulus to

nephrologists not to neglect entirely teleological considerations in their preoccupation with the details of renal performance. I suggest this rather timidly, for I do know that a wood is made up of trees, and trees of cells; but the patch of green on a map is also not without its uses.

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DISCUSSION

BULL: I have a Table to show one mechanism which is probably *not* the signal for sodium retention. The possibility exists that the receptor

Table I

THE EFFECT OF LOWERING CEREBROSPINAL FLUID PRESSURE ON H_2O, ELECTROLYTE AND CREATININE EXCRETION

	Change in excretion	*P*	*Change detectable at P=0·05*
Creatinine .	+7·5 per cent	0·2<P<0·3	13·3 per cent
Chloride . .	−4·6 per cent	0·6<P<0·7	20·8 per cent
H_2O . . .	+10·4 per cent	P=0·05	10·4 per cent
S. G. . . .	−0·0025	0·2<P<0·3	0·0041

sensitive to changes in total body water or sodium may lie in the head. Barbour and I thought of the possibility that it might be a baro-receptor of some sort, and we lumbar-punctured a number of patients and lowered their CSF pressures to half their initial values. Before this was done they had urine collections for a number of periods to get a baseline and then for a period afterwards. The net result, within the limits that we were able to achieve, is seen in the right-hand column. There was no significant change except in the case of water excretion, where there was a slight increase—the reverse of what one would expect teleologically. We are inclined to attribute that increased urine flow to possible psychological factors. At any rate, there is no significant change in chloride excretion within the limits of about ± 20 per cent.

HELLER: I should like to say a word in connection with the use or abuse of the expression "volume receptor." This term seems to me rather badly chosen because, if we go down to the cell level, surely a stimulus can only be transmitted by some physical or chemical change in the internal environment of the receptor cells. An iso-osmotic change in total body water is neither, and hence to speak of volume receptors seems to me meaningless. Another remark I should like to make, which may seem rather pedantic, is that Dr. Black has said that "classically" the site of action of ADH has been assumed to be in the distal convoluted tubule. If "classical" is, as customary, used in the sense of "historical," I thought that the first hypothesis was that of E. K. Marshall (1933), who suggested that the hormone acts on the loop of Henle. Admittedly, there is not much evidence for that, it is merely a deduction from comparative physiological investigations, but Marshall's assumption is perhaps slightly less or, at worst, as hypothetical as any other theory which has been formulated later. I think what it really amounts to is that we do not know where the hormone acts and hence the field for Dr. Wirz is entirely free.

LEWIS: I would like to ask Dr. Merrill and Prof. Borst what they think of the antidiuresis of quiet standing? What do they think the mechanism is? I believe that if you could tell us how you think that acts, it might guide us, perhaps, as to your views on the regulation of volume.

MERRILL: I can say very simply that I have no clear idea whatever, but if I must postulate a thesis I would say it occurred as a result of redistribution of blood which then necessitated a change in intravascular tone. Then by some pressure receptor a response is initiated, mediated perhaps through the hypothalamus, which we know does that sort of thing, which makes the renal tubule, if you will, or renal dynamics change in such a fashion as to retain sodium.

BERLINER: It occurs in the denervated kidney.

BRADLEY: Yes, but in association with changes of blood pressure. That seems to me the important fact. I, for one, am unwilling to accept the notion that we are dealing here with "volume" receptors rather than pressure receptors. There are marked changes in blood pressure throughout the whole arterial system with changes in posture. We tend to think of arterial pressure in man as the pressure in the recumbent person. When one stands upright, however, arterial pressure remains

unchanged at the level of the diaphragm, or a little bit above it, but all pressures above that point ("zero reference plane") fall and the pressures below it rise. The pressure we measure in the brachial artery may remain unchanged but that does not mean that there is no change elsewhere. Moreover a widespread vasoconstrictive response is demonstrable, operating not to maintain cardiac output but to maintain blood pressure. So, rather than emphasizing cardiac output as Dr. Borst does, I am inclined to view the arterial pressure as the operative stimulus which must be maintained constantly in order to provide for perfusion of some area at a constant rate. Volume control would then follow automatically.

MERRILL: I think that does fit in with the observations of Dr. Hickam who found in severe postural hypotension that that response occurred, not as a result of marked decrease in cardiac output, but as a failure of vascular compensation.

BRADLEY: Since a vasoconstrictor response occurs following the assumption of the upright position and since we can show that there is a similar rapid onset of vasoconstrictor response within the kidney, it is very hard for me to believe that a change in filtration does not also play a rôle in addition to hormonal factors.

LEWIS: Are there not two factors when you stand up? First there is the change in filtration rate with the readjustment of the circulation, such as you are talking about—

BRADLEY: Every patient we have studied has had a fall in filtration rate.

LEWIS: Yes, but then the antidiuresis of quiet standing comes on progressively in the next hour, doesn't it? I am not suggesting that the filtration rate goes on falling.

BRADLEY: We have seen the urine flow decrease immediately in our cases. It is true that urine flow continues to fall, and that following return to the recumbent position it may not return to the control levels. The antidiuresis may persist and I agree with you that more than one factor is involved.

LEWIS: The degree is so different. If you stand a patient with a water diuresis up, there is a little drop, due to the drop in filtration rate, a drop in the sodium load, presumably an osmotic drop. But if he goes on standing up, there is a big drop which suggests that some other mechanism comes into play, which is quite different from the one you are talking about, which happens only in the first place.

RAASCHOU: There is always an elevation of the diastolic pressure in the upright position.

BRADLEY: Not above the shoulders.

MCCANCE: Is it certain that there is always a fall in glomerular filtration rate when one stands up? In my experience there is not.

BRADLEY: In our experience—nearly always.

LEWIS: We think there is too.

MCCANCE: When we worked on this subject in Germany we did not always find one, but we did find an outstanding fall in the volume of urine produced. (see *M.R.C. Sp. Rep. Ser. No.* 275.)

BORST: According to my experience in normal subjects the urine output does not show a marked reduction by the act of getting out of bed and being up and about. If this were so, we would all have nocturia. However, in patients and in convalescents change of posture may be of great influence on water and salt excretion. It is not so easy to assess whether it also produces a depression in glomerular filtration rate. At the moment the patient changes from the recumbent to the upright position the dead space is filled with diluted urine. At the time that the first specimen after being up is collected there remains in the kidney and in the pelvis a highly concentrated urine. That may simulate a fall in clearance of even 25 per cent. But the next specimens of urine show an increase in the output of creatinine to the control level in spite of a continuing position. Therefore the first fall in creatinine excretion may not be real. If corrections are made, the fall in the clearance may disappear completely (see Fig. 18 p. 281). However, in some cases there is also a real fall, which seldom exceeds 15 per cent. Most workers focus attention on those cases. I just think that cases with a constant glomerular filtration merit special consideration. They prove that the salt retention connected with insufficient circulation is selective and cannot be regarded as a mere result of an impaired renal function.

The typical reaction of the kidney to an insufficient circulation and its far-reaching implications in all fields of clinical medicine can be studied without determining inulin clearances. The work of Dr. Molhuysen which I mentioned yesterday is an example. If a clinician finds a retention of extracellular fluid his interest should not be mainly focused on glomerular filtration rate and on adrenocortical function. First of all he should investigate whether there are factors tending to depress the circulation. He cannot ignore the dominating effect of a reduced circulation just because he is unable to estimate the fall in cardiac output. Perhaps I may illustrate that with an example. Recently we studied a patient with Laennec's cirrhosis who had a good diuretic response to the slow and continuous intravenous re-infusion of his own ascitic fluid. Suddenly the output of sodium and to a lesser extent of potassium fell; creatinine output was unaffected. The patient had no complaints, only his pulse rate was slightly increased. Two days later he passed tarry stools. Re-examination of the urine specimens, which had been kept in the ice-box, revealed that the output of urea and indican rose sharply simultaneously with the decrease in sodium excretion. The depression of the circulation that had been so clearly indicated by the retention of sodium apparently resulted from the bleeding of an œsophageal varix.

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K. J. FRANKLIN: I make these closing remarks, I imagine, in a fourfold capacity; first as a member of the Executive Committee of the Renal Association which has been associated with the Ciba Foundation in the arrangement of this Symposium; secondly as an Officer of the Royal Society of Medicine which is receiving us later in the day: thirdly

as one interested in the kidney; and fourthly as one who likes, in conjunction with his major teaching and research activities, to study the way in which physiology and allied disciplines have developed, are developing, and are likely to develop.

When Dr. Wolstenholme invited me some months ago to make the closing remarks at this meeting, I thought that it would devolve on me to present a précis of the proceedings. Since then, fortunately for me if not for them, that task has been passed on, in more extensive form to Drs. Oliver, Taggart, Pitts, Alwall and Borst (*Lancet*, 18 July, 1953). It will therefore suffice if later in my talk I include a few general observations.

I am thus freed to go straight to a matter which is obviously in the minds of all of us and which has already been spoken of by some, namely, our great indebtedness to the Trustees of the Ciba Foundation, to its most efficient Director, and to his very competent assistants. Dr. Wolstenholme himself deserves, I think, a very special tribute. Organizing a small international conference of three dozen members is in some ways a more exacting business than organizing a large-scale one, for on the proper selection of those to be invited depends the scientific and social success of the meeting. I feel sure that none of you will find fault with one at least of his selections in this particular instance and I imagine that you will also agree with the other thirty-five. It is fitting also to mention here the indebtedness of the Renal Association to the Foundation and to him, for all the Associations' meetings have taken place here ever since its inauguration in March, 1950.

No Director, to return to the main topic, can succeed without a loyal and efficient staff and I propose to single out three ladies for special honourable mention. These are Miss Cameron, who has been taking notes of what has been said and who will be concerned later with the editorial work that is to result in our book; Miss Bland, who assisted in much of the preliminary organization and has seen that the wire recorder has revolved duly (except when Prof. Baldwin insisted that his *ex cathedra* statements were not to be regarded as pontifical); and Miss Evans, who has been in charge of the catering arrangements. In Brillat Savarin's book, "Physiologie du Gout ou Méditations de Gastronomie Transcendante," is the sentence: "Convier quelqu'un, c'est de se charger de son bonheur pendant tout le temps qu'il est sous notre toit," and we can assure Miss Evans that she has amply discharged this duty.

Let me, with those due acknowledgements most cordially made, pass to some other points. The first international meeting which I attended was the Edinburgh Congress of Physiologists just over thirty years ago (incidentally the one at which Richards and Wearn demonstrated their technique), and I was struck then, as I have been at every international meeting which I have attended ever since, by the really inspiring atmosphere of such conferences, an atmosphere which we have experienced at this present one, shortly alas to close. One reward of the scientist is his actual discovery of something never known before, and that is really indescribable. But another reward comes in such meetings as

the present, when for a while he or she lives with fellow-scientists from different countries, becomes supra-national and enjoys an exhilarating freedom from ordinary cares and a precious spiritual and intellectual communion with normally widely-separated colleagues, with whom he has perhaps corresponded but whom he has never met or has met too seldom.

At past international Physiological Congresses, it has been the custom to select someone with a wide command of languages to demonstrate his prowess in the final meeting by making a speech in which, if possible, he included a sentence or two in the language of each of the attending members. I thought of trying to imitate this precedent today and analysed the membership. I found there were thirteen delegates from outside Great Britain, namely six Americans, two Swiss, and one each from Belgium, Denmark, France, Holland and Sweden. Thirteen is regarded by some as an unlucky number, but it is not so in my personal experience and the present meeting only confirms my view. With regard to the language business, however, despite two periods totalling a year and a half of residence in the United States, and despite the fact that I am a Deputy Sheriff in Harris County in Houston, Texas, I can only claim to understand American and not to speak it. I am also diffident about speaking in French, except to my students, who on occasion have to put up with my efforts and have no court of appeal. Dutch I can read but am chary of speaking, and I recall that Rudolph Magnus was similarly diffident, for only after twenty years of lecturing in the Pharmacological Institute at Utrecht did he find himself speaking one day in Dutch instead of German and thereafter continued in the former. That leaves only Swedish and Danish, and the little knowledge gained in Stockholm in 1926 in International Congress time, and at the last Physiological Congress three years ago in Copenhagen, has deserted me. So I will not attempt to emulate at this conference the various efforts made by Prof. Barger and other linguists at the Congresses which I have attended. Instead, I will say how very greatly I, for one, have appreciated the expositions and discussions by experts to which I have, in the last day or two, listened. With my main renal interest lying in the kidney's vascular system and circulation, I have not felt entitled to do anything but absorb, rather than secrete or excrete, but I must pay tribute to the outstanding interest which the proceedings of the conference have aroused in one who has a general interest in the kidney and its rôle in the organism as a whole.

When we consider that it is only one hundred and eleven years since Bowman made known for the first time the essential structure of the kidney and adumbrated his idea of its functioning, and that Carl Ludwig brought out *his* first statement (in a doctorate thesis) of *his* views in that year, we may well wonder at the advances revealed during our meeting. Through the work of Dr. Oliver, Dr. Darmady, Prof. Sheehan and others the structure of the organ in diverse conditions is becoming ever clearer, and we have also had widespread evidence about functioning in general, and more detailed biochemistry in particular, from both normal and clinical aspects.

The advances as a whole can only be described as spectacular, but we have at the same time to confess that they are but a beginning, that the often brilliant concepts and painstaking and imaginative investigations of which we have had examples given to us at this meeting have not yet, in many instances, led to final conclusions, and that the kidneys, contributing so small a proportion of the total body weight, are still in large measure organs of mystery. It is obvious from the discussions, for instance, that comparative anatomy and comparative biochemistry are largely virgin fields which it would be profitable to explore farther.

This confession of partial ignorance should not be a cause of regret but only a stimulus to further effort. If I may tell a short story: Two people were walking in Ireland somewhere, I think towards a place called Ballyhulish, and after they had walked five miles and thought it was about time they were getting somewhere, they saw a passer-by and they asked him "How far to Ballyhulish?" and he said "Five miles over there." They walked on another five miles and found another person—still no Ballyhulish—so they asked this man, "How far to Ballyhulish?" and he said "Five miles over there." And one turned to the other and said "Well, at least we can say we are holding our own." I think in the matter of the kidney we are not only holding our own but making progress.

I should like to tell one more story, quite a brief one. The then Assistant Commissioner of Police in this metropolis went to visit a prison, and after his tour round the prison he was asked if he would like to address the convicts. He said he would. They all assembled in a hall and he started off by calling them "Dear friends." Then he thought that was possibly not the right way for an Assistant Commissioner of Police to address convicts, so he changed it to "Fellow citizens," only to remember that they had lost their civic rights. His final effort was: "Well, anyway it's nice to see so many of you together, and I hope it won't be long before we meet again." That sentence, inappropriate in his address, is appropriate in mine. It is nice to see so many nephrologists together, and I hope it won't be too long before we meet again.

AUTHOR INDEX

Plain numbers indicate a contribution by the author, either in the form of a paper or to the discussions.

Numbers in italics indicate a reference to the author's work.

SUBJECT INDEX

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