RENAL MECHANISMS CONTROLLING COMPOSITION OF THE BODY FLUIDS[1]

DONALD D. VAN SLYKE

The Hospital of the Rockefeller Institute for Medical Research, New York, New York

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Our cells are bathed in a fluid the volume of which Peters (24) and his collaborators have measured and found to constitute about one-fourth of the body. Evolutionary physiologists like to say that, with regard to the concentration of its salts, the fluid represents the primeval ocean from which our ancestors emerged to venture existence on the dry land. Be that as it may, our cells have maintained a fastidious preference for a surrounding medium of constant and definite composition; and if they are outraged by brusque changes in this medium they refuse to perform their aliquot parts of the body's functions, and we become ill. If the insult is too great, some bloc of cells may so far refuse its duties that the entire organism can no longer carry on, and we die.

The composition of the inner fluid is maintained within necessary limits by a balance between velocities of inflow and outflow of water and solutes. The inflow comes from food and drink and from substances given off from the cells themselves, largely waste products of their chemical activities. The rates of inflow vary tremendously with the digestive and other activities of the body. The outflow is chiefly through the kidneys, and by them it must be so regulated that both the volume and the composition of the inner fluid are kept within limits that permit normal function of the cells and our own enjoyment of life.

The studies of renal behavior which I shall sketch this morning originated in a search for a chemical measure of kidney efficiency. The search was begun in the Rockefeller Hospital some score of years ago by Franklin C. McLean, who had been trained in physiology under Carlson at the University of Chicago, and who has now returned as a member of the faculty. McLean published the first American papers in the field (16, 17), and was then called to China to organize the Peking Union Medical School. After that the problem was continued by others at the Rockefeller Hospital. In its beginning, however, the problem was McLean's.

The studies that followed were made by an unusual succession of men,

[1] The Willard Gibbs Lecture, delivered in Chicago, Illinois, May 20, 1939.

most of whom are now making contributions to chemistry or medicine from their own laboratories or clinics. The work on the chemical factors of the problem was begun with G. E. Cullen, now in Cincinnati, was developed further by Hastings, now at Harvard, and was continued by Sendroy, who is now on one of your own faculties at the University of Chicago. Of the men who worked on the clinical and physiological sides of the problem in the early days were Austin and Stillman, then Salvesen, Lundsgaard, Møller and Kirk, later professors of medicine in Oslo and Copenhagen, Linder, now in Cape Town, and McIntosh, now in Montreal. Of our group your city has taken, not only McLean and Sendroy but also Leiter, Alving, and Benjamin Miller. To these men, and to Alma Hiller and C. P. Rhoads, who are still in the Rockefeller Hospital, are due the studies which we shall review.

First I shall venture to outline a bit of purely chemical work on the enzyme urease, which was a necessary preliminary to our attack on the kidney.

KINETICS OF UREASE

As the excretory product for the study of renal function, urea was the preliminary choice, because its excretion forms a major part of the work of the kidneys. A precise micro method was needed for determination of urea, and Cullen and I decided to use the enzyme urease, which splits urea quantitatively into ammonia and carbon dioxide. To control accurately the action of this enzyme we undertook a study of its kinetics (40, 48). This study led to evidence that the enzyme acts by combining in definite proportions with the urea, which after a definite mean time is thrown off as the hydrolytic products ammonia and carbon dioxide. Data on other hydrolytic enzymes,—invertase, maltase, lactase, emulsin, diastase, and arginase,—indicate that they act by combining with their substrates in a similar manner.

To the theory of alternating combination and decomposition we were led by the following observation: When a given amount of urease acted on solutions of varying urea concentration, the speed of hydrolysis increased with urea concentration up to a certain limit, as shown in figure 1. Beyond that further increases in urea concentration did not make the enzyme work any faster.

Our interpretation of this phenomenon was the following, roughly diagrammed in figure 2. Each urea molecule before it is decomposed first combines with the enzyme. Later, after a time interval, the urea is split and thrown off as its products, ammonia and carbon dioxide. The place of combination on the enzyme molecule is thereafter left vacant until another urea molecule makes contact. Then the decomposition is re-

peated. The time required for a single cycle is the sum of the time taken for another urea molecule to hit the vacated combining point, plus the

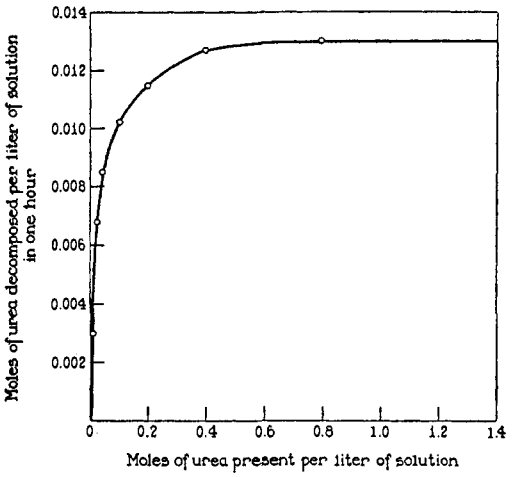

FIG. 1. Effect of urea concentration on rate of urea decomposition by urease (40)

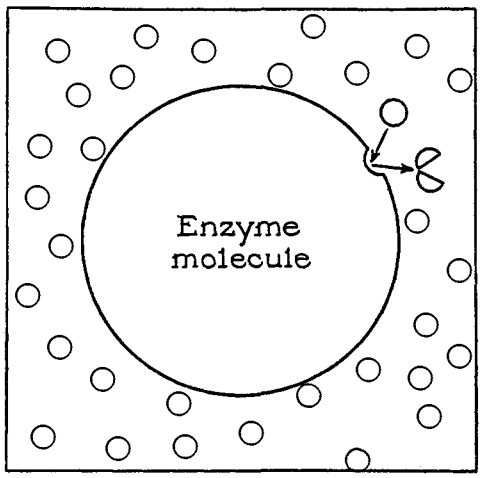

FIG. 2. Diagram of mode of action of the enzyme urease on urea. The urea molecules are represented by the smaller circles. One of them is indicated as having been split into two portions (ammonia and carbon dioxide), which are being ejected from the enzyme. Another urea molecule is indicated as about to take the place of the ejected one in combination with the enzyme.

time the enzyme then takes to split the urea molecule and eject the products. The more abundant the urea molecules are about the enzyme, the

shorter will be the probable path of the next urea molecule to the combining point on the enzyme, and hence the shorter will be the average time interval during which the enzyme is left uncombined and therefore inactive. If the urea concentration is increased enough, this inactive interval may become negligible compared with the interval used for the decomposition; the enzyme works all the time at full speed, because the unused intervals are negligible. Further increase in substrate concentration can cause no further increase in rate of hydrolysis. Under the conditions of the experiment shown in figure 1, full speed was approached when the urea concentration reached 0.6 molar.

To regulate the enzyme action at will one had to learn the effects of conditions on the two separate time intervals, for combination and for decomposition. The reaction was therefore formulated in such a way that the velocity constants governing the two intervals could both be measured.

$$\text{Time required for cycle} = \overbrace{\frac{1}{K_C U}}^{\text{Interval for combination of enzyme and urea}} + \overbrace{\frac{1}{K_D}}^{\text{Interval for decomposition of urea}} \tag{1}$$

where U = concentration of urea,

K_C = velocity constant of formation of enzyme–urea combination, and

K_D = velocity constant of decomposition of combined urea.

$$-\frac{dU}{dt} = \text{velocity of hydrolysis} \tag{2}$$

$$= \frac{1}{\text{Time required for cycle}}$$

$$= \frac{1}{\frac{1}{K_C U} + \frac{1}{K_D}}$$

When the urea concentration, U, is large,

$$-\frac{dU}{dt} = K_D \tag{3}$$

To obtain the time curve of urea decomposition, one integrates the differential equation and obtains:

$$\underbrace{t}_{\text{Time for lowering substrate concentration from its initial value, } A, \text{ to } U} = \underbrace{\frac{1}{K_C} \ln \frac{A}{U}}_{\text{Mean time spent by enzyme molecules uncombined}} + \underbrace{\frac{A - U}{K_D}}_{\text{Mean time spent by enzyme molecules combined with substrate}} \quad (4)$$

The constant K_D, indicating the rate of decomposition of urea after combination with the enzyme, was found by working with urea solutions so concentrated that the inactive periods of the enzyme were negligible, and K_D could be measured by equation 3. After K_D had thus been fixed, the combining constant K_C could be measured by equation 4 in urea solutions so dilute that the time intervals wasted in combining did take up a significant part of the total reaction time.

Thus it was found that K_C, which appears to indicate the relative combining speed of enzyme and urease, is diminished by other crystalloids in solution, either electrolyte or non-electrolyte: their molecules and ions apparently get into the way of the urea molecules and foil part of their attempts to hit the combining point of the enzyme. This effect is important in retarding hydrolysis of the last traces of urea, for it is then that the urea becomes so dilute that the combining speed assumes chief importance in determining the time required to complete the hydrolysis.

The effect of pH, as shown by figure 3, is altogether different on the two reactions. K_C, indicating the speed of combination, increases in a linear way with pH over the entire range from 5 to 9. In contrast, K_D of the decomposing reaction is quickest at pH 7, and slows down as the pH recedes from neutrality in either direction.

Whether the theory of hydrolytic enzyme action outlined above was correct or not, the study of factors affecting the two velocity constants, one predominant at high and the other at low substrate concentration, made possible a quick and exact method of analysis. To get the quickest hydrolysis of urea it became obvious that it is necessary to start the hydrolysis under conditions that favor the decomposing reaction, which predominates in the time consumption in the more concentrated solutions, and to finish under conditions that favor the combining reaction, which consumes increasingly greater proportions of the time as the urea concentration approaches zero. The hydrolysis starts most rapidly in neutral solution and approaches completion most rapidly in alkaline. Buffers are needed to control the pH, but they must be as dilute as possible in order

that their ions and molecules shall not interfere with the combination of enzyme and urea; otherwise the last part of the hydrolysis might be dragged out over a long time.

After these conditions were understood, and a crude but active dry preparation of the urease had been prepared by precipitation of soy bean extracts with acetone, a routine was developed in which urease and urea reacted quantitatively in one minute (39). The mixture was then acidified, and the carbon dioxide of the ammonium carbonate formed from the urea was extracted and measured by the gas pressure it exerted on a manometer. Results accurate to 1 part in 300 could be obtained in rapid routine analyses of 1-cc. samples of blood.

With the enzyme as a tool, we went on to study the action of the kidney.

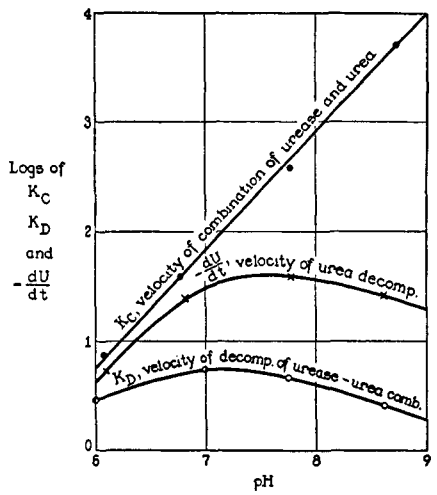

FIG. 3. Effects of pH on the two phases of urease action. Urea concentration, U, = 0.15 molar (47).

UREA CLEARANCE

It had already been shown in the laboratories of Marshall and Addis that the rate of urea excretion in man is proportional to the blood urea concentration. In other words, the amount of urea contained in a given volume of blood is excreted each minute. In normal men excreting abundant volumes of urine, the urea of about 75 cc. of blood is excreted per minute. We therefore say that the normal "blood urea clearance" averages 75 cc. per minute. Expression of the relations among blood concentration, renal excretion, and time, in a single unit capable of visualization (as the volume of blood cleared per minute of excreted substance), was introduced in 1928 by Møller, McIntosh, and Van Slyke (21), and has since been

applied by other authors to the excretion of various substances in renal studies.

Studies of urea clearance with different volume outputs of urine showed that as much as 75 cc. of blood per minute was cleared of urea only when the urine volume was fairly abundant, over 2 cc. per minute (2, 21, 22). Often the urine volume is less than this; in fact the average is about 1 cc. It was found that, as the urine volume fell below 2 cc. per minute, the urea clearance diminished in proportion to the square root of the volume, as exemplified by figure 4. With this relation established[2], when the urine

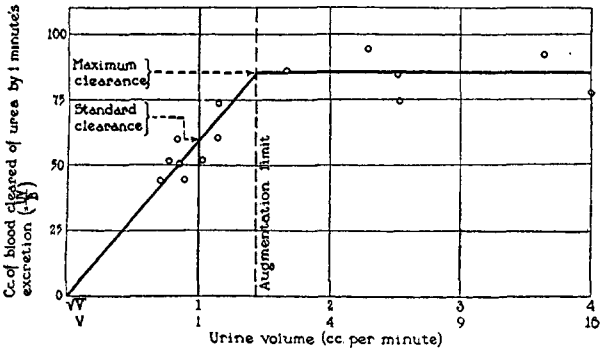

FIG. 4. Relation between the urine volume per minute and urea clearance. U and B indicate urea concentration in urine and blood, V the urine volume in cubic centimeters per minute. When the urine volume falls below the "augmentation limit" of about 2 cc. per minute, the clearance falls parallel with the square root of the volume. (From Møller, McIntosh, and Van Slyke (21).)

volume was low an empirical correction could be made for its effect on the clearance. The probable physiological cause of the effect will be discussed later.

[2] If the urine volume falls below 0.35 cc. per minute Chesley (3) finds that a maximum concentration of urine is reached; with further diminution in urine volume the urea clearance falls in direct proportion to the urine volume instead of to its square root. However, such low volumes are never reached in human urine except under most rigid conditions of water deprivation, or in pathological oliguria.

Dominguez (6), from data of previous authors (1, 21), developed the formula:

$$\text{Urea clearance} = M(1 - e^{-KV})$$

to express the effect of urine volume on the clearance (V = urine volume in cubic centimeters per minute, M = maximum clearance (about 75) approached at high V). This formula, although empirical, gives a curve which approaches the maximal clearance of 75 as an asymptote at high urine volumes, and approximates the square-root rule for volumes between 2 and 0.35 and the linear curve of Chesley for volumes below 0.35. Dominguez' formula has not been applied in clinical or physiological studies, but appears practicable.

Another factor for correction was body size. One could not expect a child to clear of urea as large a volume of blood per minute as a full-sized adult. Comparison of adults and children by McIntosh and Møller showed that the variation was a linear function of the body surface, like the variation in basal metabolic rate (15).

With the clearance thus developed, and corrected for variations in urine volume and body size, it could be applied to studies of renal disease. There it was found to provide a sensitive measure of renal efficiency. In progressive Bright's disease the clearance falls, as the excreting elements are destroyed, until only 3 or 4 cc. of blood are cleared per minute, instead of the usual 75. When the clearance falls to 3 or 4 cc., uremia sets in and death follows (47).

In acute nephritis, however, the clearance may fall almost to the uremic level, and then rise again to normal, with complete recovery (47). The excreting elements are injured, but not irreversibly. An empirical rule was found to hold in acute cases. If recovery is to occur, a rise in the clearance must begin within 4 months after the onset of the acute nephritis Complete recovery may take several months longer, but the upturn of the clearance must begin within 4 months, or as a rule the case will become chronic, and follow a course to fatal uremia. There have been just enough exceptions to prove the rule. I think that two cases in the past 10 years have had their clearances stay at their lowest levels for more than 4 months, and then have staged recoveries; both were children.

EXPLANATION OF CONSTANCY OF CLEARANCE WITH VARYING BLOOD UREA CONCENTRATION

To maintain the observed constant urea clearance of 75 cc. per minute when the blood urea is increased, say tenfold, one of two things must occur. Either the rate of blood flow through the kidney must increase tenfold, with extraction of a constant amount of urea from each cubic centimeter of blood, or else, if the blood flow stays constant, the amount of urea extracted from each unit volume of blood must rise tenfold, so that a constant *proportion* of the blood urea is extracted. Experiments to decide between these two mechanisms required comparison, with respect to urea content, of arterial blood with the venous blood leaving the kidney by the renal vein, in order to find what fraction of the blood urea was removed by the kidney.

We were for a long time stopped by the difficulty of obtaining blood from the renal vein without anesthesia or other unphysiological disturbance. The difficulty was finally overcome by Dr. C. P. Rhoads, who suggested that it might be possible to bring the kidney out to a position under the skin of a dog's back without injury, and suture it there in such a position

that the vein could be tapped by a needle put through the skin, as blood is drawn from the arm vein of a human subject in routine examinations. Rhoads volunteered to attempt the operations and they were completely successful (26). The "explanted" kidneys functioned exactly as they had in their usual positions, and Rhoads was able to needle the renal veins several times a day without inconvenience to the animal.

With this technique we found (45) that an average of one-twelfth of the urea was extracted from the blood of a dog as it flowed through the kidneys. This fraction remained the same, regardless of whether the concentration of urea in the blood was at the usual level of about 0.2 g. per liter, or was raised tenfold by urea feeding.

The blood flow could be calculated by comparing the amount of urea excreted per minute with the amount removed from each liter of blood perfusing the kidneys. For example, if the amount of urea excreted per minute by a dog is found by analyses of arterial and renal blood to be 0.3 of the amount extracted by the kidneys from a liter of blood, the renal blood flow is 0.3 liter per minute.

If in man, as in the dog, the kidneys extract one-twelfth of the urea from the blood that flows through them, the average man's urea clearance of 75 cc. per minute indicates a blood flow of 12×75 or 900 cc. per minute. Recent data on human subjects obtained by Chesley and Chesley (4) indicate that 900 cc. per minute is in fact about the mean renal blood flow in man.

The blood supply of the kidney is extraordinarily rich. The entire output of the heart in a resting man is estimated at 4 or 5 liters per minute. Of this about one-fifth goes through the kidneys. Assuming that the kidneys are 1/200 of the body weight, one calculates that, per gram of weight, they receive about thirty five times as much blood as the rest of the body under basal conditions.

PARALLEL VARIATION OF UREA CLEARANCE AND RENAL BLOOD FLOW

We have spoken of the blood urea clearance as though it were a constant value. A certain elasticity is characteristic of the normal kidney, however, and is evidenced by variations in the rate at which it works. A normal man, with abundant urine volume, clearing the urea of an average 75 cc. of blood per minute, may vary the figure between 60 and 100 cc. With dogs, still greater variations occur, and their kidneys can be greatly stimulated by feeding meat diets. As shown in Homer Smith's laboratory (36), if a dog on a cracker meal diet is changed to a meat diet the urea clearance may increase two- or three-fold. The question proposed itself: Is such a physiological increase in the clearance due to acceleration of renal blood flow, or is it due to a more complete extraction of urea from the blood

as it flows through the kidneys? The answer is shown in figure 5. The increase in the urea clearance was found to parallel the increase in blood flow through the dog's kidneys (46). The mean proportion of urea extracted from the renal blood remained at about 8.5 per cent.

FILTRATION–REABSORPTION THEORY OF RENAL EXCRETION

All the results outlined fit the filtration–reabsorption theory of urine excretion. I shall pause to outline this theory, for it assists one to tie together the facts just discussed and to fit into the picture others to be brought out later.

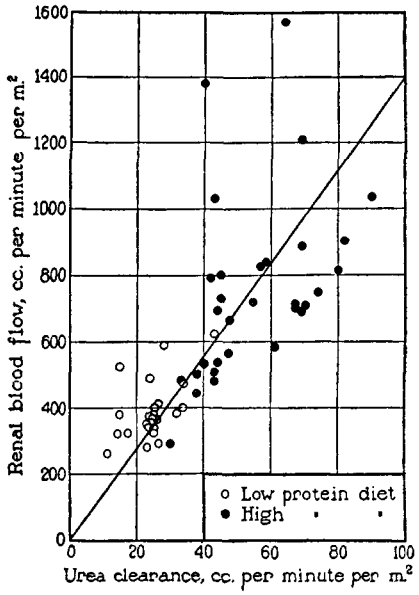

FIG. 5. Parallelism between urea clearance and renal blood flow in dogs. (From Van Slyke, Rhoads, Hiller, and Alving (46).)

The physiologist, Ludwig, realized that the glomerulus was adapted to filtration, and proposed the first filtration–reabsorption theory. He suggested that as blood passed through the capillary loops in the glomerulus (figure 6) some of the water with its dissolved non-colloid solutes was filtered out into the globular space outside the capillary tuft. The filtrate then passed down the tubule, where it was conceived to be concentrated by reabsorption of water.

When the chemistry of blood and urine became better known, however, it became obvious that simple reabsorption of water could not explain the differences between blood and urine concentrations of different solutes.

For example, the ratio, concentration in urine: concentration in blood plasma, averages about 50 for urea and for glucose only about 0.2 (41). For such differences Cushny (5) advanced the hypothesis that the tubules reabsorb different constituents of the glomerular filtrate with varying degrees of completeness, and that in general the tubules reabsorb those substances which the body needs to retain, and refuse more or less completely to reabsorb substances of which there is a surplus, or which are

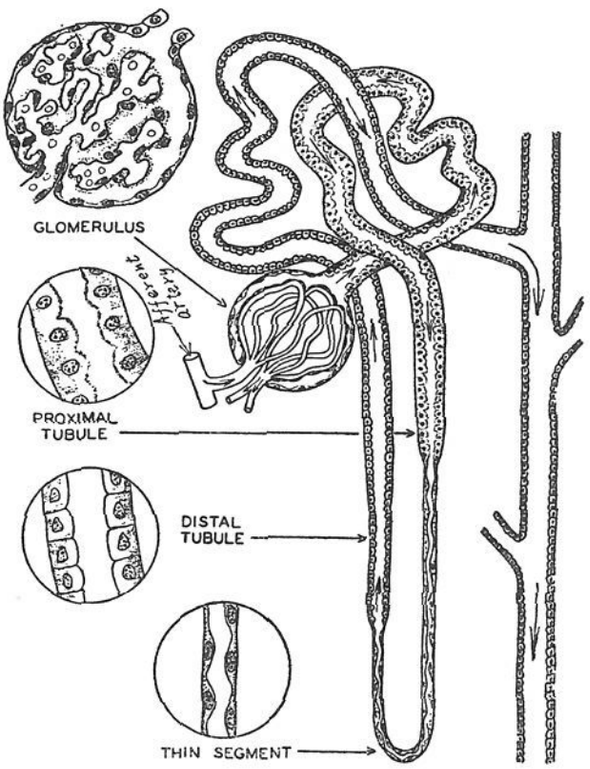

FIG. 6. Diagram of a nephron (from Homer Smith (36)). Blood enters the glomerulus from the afferent artery, flows through the capillary loops of the glomerular tuft, and emerges by the efferent vessels. From these the blood circulates about the tubules in a network of vessels not shown in the diagram. The fluid filtered from the blood in the glomerulus passes down the proximal and then the distal tubule (in the direction shown by the arrows), and has its composition altered by tubular reabsorption of water and some of the solutes, so that it enters the collecting tubule as elaborated urine.

waste products to be gotten rid of. This theory has been brilliantly confirmed by recent work of several laboratories, but in particular those of A. N. Richards (27, 28, 31, 32), Smith and Shannon (34, 35, 36, 37, 38), and Marshall (12, 13).

Richards has tapped single glomeruli of the frog's kidney with microscopic glass pipets, and with his collaborators, Wearn, Hayman, Bordley, Walker, and others (31), has developed ultramicro methods for analysis of

samples of 1 or 2 cmm. of the glomerular filtrate thus obtained. They have found that it is in fact a true filtrate, with urea, uric acid, chloride, creatinine, glucose, and pH in approximately the same concentrations found in the plasma. That the mammalian glomerulus filters in the same manner as demonstrated for the frog's glomerulus has not been proved in the same direct way, but the similar structure makes a similar filtration probable, and all the facts accumulated by exact methods are in harmony with the filtration mechanism for the mammals.

In accord with this mechanism our results with urea may be explained as follows: As the blood flows through the glomerulus (figure 6) a fairly constant fraction of the water is filtered out, with its dissolved urea. The limit of the fraction of plasma water filtered appears to be set chiefly by the balance between the blood pressure within the glomerular capillaries, which forces the filtration, and the opposing osmotic suction of the plasma proteins, which tends to keep water from leaving the plasma. As the blood flows from the afferent artery through the glomerular capillaries the blood pressure presumably decreases, and the osmotic suction of the plasma proteins rises as they become more concentrated from loss of filtered water. To judge from the capillary pressure measurements of Landis (11) and the known osmotic activities of the proteins, it appears probable that the two forces may approach equilibrium at about 25 mm. of mercury pressure before the end of the capillary is reached. In this manner a fairly constant proportion of the plasma water may be filtered.

PROPORTION OF PLASMA WATER FILTERED

In testing the conception of glomerular filtration the question arose: What fraction of the plasma water is filtered out as the blood passes through the glomeruli? If all the urea that is filtered were excreted, one could estimate the fraction of blood water filtered as equal to the fraction of urea removed from the renal blood. However, there is reason to believe that some of the filtered urea diffuses back into the blood with the water reabsorbed from the tubules. For study of the filtered fraction an excretory substance was needed which would not be reabsorbed. It was provided by the anatomist, Gersh (7). He found that when ferrocyanide was excreted by rabbits it could be made visible by precipitation as Prussian blue, and could be seen in the glomerular filtrate and the tubular fluid. However, none appeared in the cells that line the tubules. It appeared that, unlike urea, ferrocyanide passes down the tubules without at all diffusing back into the blood. That the kidney of the dog behaves in the same way towards ferrocyanide was shown later by Gersh and Benjamin Miller, whom Gersh kindly invited to his laboratory, which was then at the Johns Hopkins University, for the necessary experiments.

There were two other substances which, because their clearances were known to be higher than the urea clearance, seemed perhaps able to escape reabsorption in the tubules. One of these was creatinine, which had been suggested by Rehberg (25) as a non-reabsorbed substance. The other was the complex carbohydrate inulin, which Richards, and Smith and Shannon, had found to be excreted by dogs with the same clearance as creatinine.

Consequently we injected ferrocyanide, creatinine, and inulin, dissolved together, into the veins of dogs and observed the proportions that were extracted from the blood plasma by the kidneys (42, 43). For these observations the dogs prepared by Rhoads (26) with explanted kidneys were used, and the percentage extraction was measured by the decrease in plasma concentration of each substance as the blood passed through the kidneys. Such a small proportion of the filtered water escapes reabsorption that the volume of the plasma is practically unchanged by passing

TABLE 1

Extraction of ferrocyanide, creatinine, inulin, and urea from blood plasma by the dog kidney

MATERIAL	NUMBER OF DETERMINATIONS	MEAN EXTRACTION PERCENTAGE	STANDARD DEVIATION
Ferrocyanide	25	18.8	5.5
Creatinine	36	19.9	3.8
Inulin	21	22.3	7.9
Urea	37	8.3	2.0

through the kidneys, and the fall in concentration of dissolved substances in the plasma could be taken as an approximate measure of the proportion removed by the kidneys.

The results (see table 1) showed that all three substances, ferrocyanide, inulin, and creatinine, were extracted from the plasma to the same extent,—about 20 per cent of each on the average was removed as the blood passed through the kidneys.

We believe that the most probable interpretation of these results is that about 20 per cent of the plasma water and its dissolved crystalloids are filtered out in the dog's glomeruli.

PASSIVE REABSORPTION OF UREA FROM TUBULES

That the kidney removes urea from the blood less completely than creatinine or ferrocyanide seems due to back-diffusion of part of the filtered urea into the blood of the tubular network as the filtrate passes down the tubules. That the kidney permits part of a useless excretory product to

diffuse back in this manner must be attributed to an imperfect development of the organ's function. It is what Metchnikoff called one of the anomalies of nature. The kidney would be a more perfect organ if the tubular cells were entirely impermeable to urea, so that all that is filtered passed out in the urine, as in the dog is the case with ferrocyanide and inulin and creatinine. Comparison of the extraction percentages of these substances with that of urea, however, indicates that, even when urine volumes are large enough for maximal urea clearance, about 40 per cent of the filtered urea diffuses back into the blood[3] and has to be brought around to the kidney and filtered again before it is finally gotten rid of (42, 43).

The back-diffusion of urea appears to occur in two phases: a first phase which accompanies reabsorption of the first 90 per cent of the water of the glomerular filtrate, and a second phase which accompanies reabsorption of whatever part of the last 2 per cent of the filtered water is reabsorbed. The absorption of the first 90 per cent of the 120 cc. of water filtered per minute one may call, with Homer Smith, "obligative reabsorption," since it appears imperative in the normal kidney, which seldom lets the urine flow rise above 12 cc. per minute, even under the pressure of water drinking or diuretics. The backflow of this reabsorbed water into the tubular blood must be rapid, and it sweeps with it about 40 per cent of the filtered urea. Further reabsorption of water does not appear to take any more urea with it, however, until the urine volume is contracted to about 2 cc. per minute, since the urea clearance shows no definite change with urine volume over the range of the latter from 12 cc. to 2 cc. per minute (figure 4, horizontal part of the curve). However, when contraction of the urine volume extends below 2 cc. per minute the ratio of urea concentration in the distal tubular lumen to urea concentration in the blood goes above 30, and along this steep osmotic gradient urea begins again to diffuse back into the blood in amounts which increase rapidly with the degree to which the urine is further concentrated (figure 4, sloping part of the curve).

The two phases of urea reabsorption appear to be due, as pointed out by Smith and Shannon (36), to different processes. The second phase, because of the great concentration gradient between the filtrate and the blood, seems attributable to diffusion pressure breaking through the resistance of

[3] The data of table 1, showing only 8.3 per cent decrease in plasma urea concentration as the blood passes the kidneys, compared with 20 per cent for ferrocyanide, inulin, and creatinine concentration, would seem to indicate that not 40 but 60 per cent of the filtered urea gets reabsorbed. However, part of the urea extraction indicated by plasma analysis is masked by quick transfer of urea from red blood cells to plasma when water is reabsorbed in the tubules. When correction is made for this transfer, reabsorbed urea is estimated at 40 per cent (43). The transfer does not occur with creatinine, inulin, or ferrocyanide (43).

the tubular cells. The first phase, however, occurs when the concentration gradient is relatively small, and appears to be in part due to some other contributing force, such as the mechanical sweep of the back-drawn water. However, in both phases urea moves from higher concentrations in the filtrate to lower ones in the blood; there is no active propulsion at any time against a urea concentration gradient, and hence reabsorption of urea in both phases may be termed *passive* insofar as the activity of the tubular cells is concerned. Uric acid appears to be partly reabsorbed by similar passive diffusion.

ACTIVE REABSORPTION BY THE TUBULES

In contrast to urea and uric acid, glucose can be actively pushed by the tubular cells back into the blood, even *against the concentration gradient*, for the process continues after the glucose in the tubular lumen becomes less concentrated than in the blood. Such absorption is called *active*. Besides glucose, water, sodium, potassium, chloride, and other blood crystalloids of which certain amounts must be maintained in the body, are taken back by active, selective reabsorption from the tubular lumina when necessary to prevent the body's supply from falling below the physiological optima. For such substances the cells of the tubular walls act as force pumps, driving the substances back into the blood, when necessary against concentration gradients. Richards and his collaborators (30, 32), by ultra-analyses of fluids drawn from successive segments of amphibian tubules, have mapped the parts where different filtrate substances are reabsorbed.

KINETICS OF ACTIVE REABSORPTION. SIMILARITY TO ENZYME KINETICS

In the conception of this active function of the tubules an important step has been made by Shannon and Fisher (34, 35). By increasing the concentration of glucose in the glomerular filtrate Shannon and Fisher (35) found that the rate of reabsorption could be increased up to a certain limit. Beyond this maximum further increase of glucose in the tubular lumen could not make the sugar pass back into the blood any faster, and it began to escape into the urine. With regard to glucose reabsorption the tubular cells were then acting at full speed, like the urease in figure 1 when the urea concentration exceeded 0.6 molar. Shannon and Fisher's quantitatively formulated explanation was that the glucose combines reversibly in the cells with a hypothetical carrier substance, from which the sugar is later freed to pass into the blood on the other side of the cell. This hypothetical process is analogous to that by which urease and other hydrolytic enzymes appear to combine with their substrates, and after an interval to eject them, in this case as hydrolytic products. Shannon's curves of the

process of glucose reabsorption are of the same character as the curve of urease action in figure 1.

TUBULAR EXCRETION

Besides reabsorbing, the tubular cells can pass materials in the opposite direction, from the blood into the tubular lumen. This process is tubular excretion. When certain substances are injected into the blood, they are almost completely extracted from the plasma that passes through the kidneys. Such is the case with an organic iodine compound called "diodrast" and with the dye phenol red. Marshall (12) has shown that it would not be possible to filter more than a slight fraction of this dye, because 80 to 90 per cent circulates loosely combined with the proteins in the plasma and is non-filterable. Yet the kidneys remove 60 per cent, 70 per cent, or more of it from the plasma. The only way in which this could occur is by an excretory process which is active, like the active reabsorption of glucose, and it appears possible only in the tubules. The tubular cells thus appear to act as two-way carriers, passing water, glucose, and certain other substances against osmotic gradients from the tubular fluid back to the blood, and passing other substances, such as diodrast and phenol red, from the blood into the tubular lumen. Tubular excretion does not ordinarily seem to play an important rôle in maintaining the normal composition of the body fluids of mammals. It is a reserve function, that can come into play in unusual emergencies, as when such a foreign substance as phenol red or diodrast enters the circulation. In some salt water teleosts, such as the toadfish, however, it is the only mode of renal excretion: these fish have lost their glomeruli (8, 37), and perform all their renal excretion by extrusion of substances from the tubular walls (13, 36). They have developed this mode of excretion, while the mammals and many lower animals have developed glomerular filtration, the tubules becoming specialized chiefly in reabsorption of the substances that must be retained. Shannon (34) has shown that his theory for active tubular reabsorption by combination and dissociation applies equally well to active tubular excretion.

COMPARISON OF UREA CLEARANCE WITH DIODRAST CLEARANCE IN MAN

If any constituent is completely removed from the renal blood and excreted by the kidneys, the "clearance" of that substance will be equal to the total volume of blood flowing through the kidneys per minute. There is evidence that tubular excretion enables the normal human kidney to extract the organic iodine compound, diodrast, completely, or almost completely, from the renal blood. Therefore the diodrast clearance has been used by Smith, Goldring, and their collaborators (2, 36, 37, 38) as a measure of the renal blood flow in man. Urea clearances were measured

at the same time. The renal blood flow was caused to vary by such drugs as adrenaline and theophylline. It was found that the urea clearance was unaffected by variations in estimated renal blood flow, when the latter was calculated as equal to the diodrast clearance.

This experimental result is the opposite of that obtained in our laboratory by comparing urea clearance and measured blood flow changes in dogs, when the blood flow variations were produced by adding or withholding meat in the diet. In our dog experiments we found that the urea clearance was not independent of the renal blood flow, but was directly proportional to the flow, the percentage of urea extracted from the renal blood remaining constant despite gross variations in blood flow and clearance. From the constant extraction percentage, it would appear that in our experiments the renal blood probably perfused the glomeruli at a constant pressure, even when the renal blood flow varied, and that the most probable mechanism for varying the flow without the pressure was by opening and closing varying proportions of the glomerular capillaries. Such an opening and closing had been seen by Richards (29) in the capillaries of the frog's glomerulus. In contrast, the results of Smith, Goldring, and their collaborators are most readily explainable on the assumption that the renal blood flow is controlled, not by the opening and closing of capillaries, but by the contraction and expansion of the efferent artery leading from the glomerulus. In such control the same arterial contraction that slowed the blood flow would produce a back-pressure, which would increase the proportion of plasma water and solutes filtered in the glomerulus; as much filtrate per minute might thus be filtered with a slow blood flow as with a rapid one. Smith in fact, concludes (38): "The renal blood flow in man is apparently controlled by the efferent arterial tone."

The diametrical difference between our results with the dog and those of Smith and Goldring with man, concerning the relation of urea clearance to renal blood flow, may be due (*1*) to the difference in technique of estimating renal blood flow, or (*2*) to an actual difference between the mechanisms of the human and canine kidney,[4] or (*3*) to the fact that the renal blood flow

[4] The kidneys of the dog and man do not behave entirely alike with regard to every function. While it appears that in both species the inulin and creatinine clearances (taken without administration of creatinine in the case of man) (20) are equal to the glomerular filtrate, ferrocyanide shows an altogether different behavior in man. Whereas in the kidney of the dog or rabbit both histochemical and functional studies indicate that ferrocyanide is excreted without any reabsorption by the tubules, in man Miller and Winkler (19) found that ferrocyanide was reabsorbed to about the same extent as urea, *viz.*, 40 per cent or more. The cells of man appear to be less resistant to penetration by ferrocyanide than those of either animal. One sign of this is the failure of the tubules of man to prevent back-diffusion of filtered ferrocyanide. Another is that injected ferrocyanide is of the order of tenfold as toxic for man as for the dog; it appears to penetrate the cells of the tissues as well as of the tubules in man more readily than in the dog.

variations in our dogs were induced by diet, while the variations in Smith and Goldring's human subjects were induced by other stimuli, *viz.*, drugs or water diuresis. It may be that under the same stimuli the dog would show the same behavior noted by Smith and Goldring in man. Whether the dog will do so, is one of the questions not yet answered. A positive answer would indicate that the dog under different stimuli could use at least two different vascular changes to vary his renal blood flow, and would lead one to anticipate a similar versatility in man.

MAINTENANCE OF BODY NEUTRALITY BY REGULATION OF BICARBONATE EXCRETION

Besides excreting waste products, the kidney must maintain the neutrality of the body, which produces usually acid, but sometimes alkali, in excess. The regulation by excretion of acid or alkali at need is so sensitive that the pH of the blood seldom varies beyond the range 7.30 to 7.45.

TABLE 2

Estimated ordinary 24-hr. filtration and excretion of bicarbonate, free carbonic acid, and phosphate by man

(From Sendroy, Seelig, and Van Slyke (33))

SUBSTANCE	GLOMERULAR FILTRATE, 170 LITERS AT pH 7.4		URINE, 1.5 LITERS AT pH 6.1	
	Concentration	Total amount	Concentration	Total amount
	millimoles per liter	*millimoles*	*millimoles per liter*	*millimoles*
HCO_3	25	4200	2	3
H_2CO_3	1.3	200	2	3
PO_4	1	170	43	65

The manner in which the kidney produces at will acid or alkaline urine may be deduced from experiments by Sendroy and Seelig (33), showing that bicarbonate is actively reabsorbed, although free carbonic acid is not. Bicarbonate concentration in the urine was found to vary from four times to one-twentieth of the bicarbonate concentration in the blood plasma. The highest bicarbonate concentrations were in the most alkaline (pH 7.5) urines, and the lowest in the most acid (pH 4.9) urines. *Bicarbonate can be so completely reabsorbed that in the more acid urines practically none at all is present.* As seen from table 2, of the bicarbonate filtered only about 1/200 ordinarily appears in the urine, and in the most acid urines as little as 1/2000 may appear. Bicarbonate is the chief form of reserve buffer alkali in the body fluids, and it is by the kidney's power to reabsorb it from the glomerular filtrate that loss is prevented.

Free carbonic acid, on the other hand, was found from one to five times as concentrated in the urine as in the blood, but never less concentrated.

Phosphate is the only other important blood buffer that appears to be filterable. About three-quarters of that filtered appears to be reabsorbed. The effect of its reabsorption on preservation of the phosphate content of the body fluids is vital, but the effect on preserving neutrality appears negligible compared with bicarbonate reabsorption.

Of the urinary constituents causing the acid reaction that is ordinarily present, *viz.*, pH 6 ± 1, acid phosphate, as shown by Henderson and Palmer (9), is the most important. The large amount of phosphate present, compared with the HCO_3^- and H_2CO_3, appears due to the immensely less complete reabsorption of PO_4.

It does not appear probable that reabsorption of alkaline phosphate and excretion of the unreabsorbed acid phosphate into the urine account for the acidity of the latter. If the fall of urinary pH below blood pH were attained by selective reabsorption of HPO_4^{--}, phosphate excretion would be expected to diminish as the urine became more acid. On the contrary, in conditions of acidosis, where the urinary pH is low, the excretion of total phosphate is not smaller, but greater, than usual.

One is led to the conclusion that the acid phosphate in the urine is chiefly formed by reaction in the tubules of alkaline phosphate with free carbonic acid. Phosphates and carbonates are in equilibrium according to the equation

$$\frac{HPO_4^{--}}{H_2PO_4^-} = 0.2\frac{HCO_3^-}{H_2CO_3} \qquad (5)$$

As bicarbonate is withdrawn from the tubular fluid, the ratio $HCO_3^-:H_2CO_3$ is decreased, and in consequence reaction with alkaline phosphate occurs,

$$H_2CO_3 + HPO_4^{--} = HCO_3^- + H_2PO_4^-$$

with lowering of the ratio, $HPO_4^{--}:H_2PO_4^-$, in accordance with equation 5.

It appears accordingly that, although the acidity of the urine is due chiefly to its acid phosphate, the presence of the phosphate in acid rather than alkaline form is due to the manner in which bicarbonate is reabsorbed in the tubules, leaving a relative excess of free carbonic acid to react with the alkaline phosphate. Continued reabsorption, active and passive, removes nearly all of both the bicarbonate and free carbonic acid, but leaves the acid phosphate.

There are times when more alkali than acid is produced in the combustion of foods, or is absorbed as administered sodium bicarbonate, and the kidney is then called upon to get rid of alkali instead of acid. It appears to do this simply by reabsorbing the bicarbonate somewhat less completely

than usually, so that from a fraction of a gram to 2 or 3 g. per hour is let escape into the urine. Since glomerular filtration is calculated to remove 12 to 15 g. of bicarbonate per hour from the blood, the greatest observed bicarbonate excretions can be easily attained merely by decreasing the reabsorption.

MAINTENANCE OF BODY NEUTRALITY BY AMMONIA EXCRETION

To prevent acidification of the body, the kidney has, besides bicarbonate reabsorption, another mechanism in the ability to form ammonia. Nash and Benedict (23) showed that the urinary ammonia is formed in the kidney. Its precursor is presumably either the urea or the amino acids brought to the kidney by the blood. *In vitro* evidence of deaminase in kidney tissue favors the amino acids (10), but the only *in vivo* experiments yet tried have failed to show that the kidneys remove amino acids from the blood (14). Whichever is the source, the ammonia is produced from a neutral precursor.

The apparent manner in which this ammonia is manipulated by the kidney to preserve the body's neutrality may be outlined as follows, in accord with the filtration–reabsorption theory: Assuming, for purposes of illustration, that the invading acid is sulfuric acid, it will decompose body bicarbonate by the reaction,

$$H_2SO_4 + BHCO_3 = B_2SO_4 + CO_2 + H_2O^5$$

B representing fixed base, chiefly Na and K.

The ammonia is probably extruded as ammonium bicarbonate into the glomerular filtrate as the latter passes down the tubule. The filtrate to which the ammonium bicarbonate is added already contains all the diffusible ions of the plasma, including those of the invading sulfuric acid. Consequently, with others, the ions B^+, NH_4^+, SO_4^{--}, and HCO_3^- are present in the tubular lumen. Some of the tubular cells have, as we have seen, the ability to absorb alkali bicarbonate. These selective cells absorb HCO_3^-, together with equivalent amounts of alkali cations, and let the NH_4^+ and SO_4^{--} pass into the urine. Probably reabsorption of $BHCO_3$ occurs in the distal limb of the tubule, as Richards (32) finds that the filtrate turns acid in this limb of the amphibian nephron.

[5] More logically, perhaps, the equations could be written in ionic form as $H^+ + HCO_3^- \rightleftharpoons H_2CO_3$, etc. We have, however, retained the somewhat archaic forms in order to hold in sight the essential rôle of the alkali cations in making possible the existence of the buffer anions. If the cations normally balancing HCO_3^- and $(Protein)^-$ anions were lost from the body, restoration of the buffer effects of the bicarbonate and proteinate would become impossible.

The process may be represented as follows:

B_2SO_4	+	NH_4HCO_3	=	$2\,BHCO_3$	+	$(NH_4)_2SO_4$
(Filtered from blood plasma into glomerular filtrate)		(Formed in kidney and secreted into filtrate in tubular lumen)		(Reabsorbed from tubular lumen and retained in body)		(Excreted in urine)

The net result is to replace B_2SO_4 in the body with $BHCO_3$. The regenerated $BHCO_3$ not only acts to restore the bicarbonate reserve of the body; it also acts, by shifting the equilibria of such reactions as

$$BHCO_3 + H\ (Protein) \rightarrow B\ (Protein) + H_2CO_3$$

to restore alkali to other buffers. This restoration continues until all the buffers of the body, and with them the pH, are restored to the normal state which they enjoyed before the invasion by sulfuric acid.

When the kidney is severely damaged in nephritis the abilities both to regulate bicarbonate excretion and to form ammonia decrease, and acidosis may result (9, 44).

BOUNDARIES OF PRESENT KNOWLEDGE OF RENAL FUNCTION

We have seen that renal maintenance of the volume, composition, and neutral reaction of the body fluids is explainable by developments of the filtration–reabsorption theory of Cushny and Richards, according to which the filtered substances are divided into three classes: (*1*) those which, like glucose, are completely or almost completely reabsorbed from the tubules; (*2*) those which, like water and the electrolytes, are reabsorbed as completely as may be necessary to maintain normal amounts and concentrations in the body; and (*3*) the waste products, such as urea, creatinine, and uric acid, which are not at all actively reabsorbed, although some of them slip back into the blood to varying extents by passive diffusion. The relative efficiency with which each of the substances in this third class is excreted is indicated by the volume of blood cleared of substance per minute by the kidneys.

In addition to their selective absorption from the glomerular filtrate, we have seen that the tubular cells probably elaborate the urinary ammonia, and excrete it into their lumina in exchange for alkali that they reabsorb from the glomerular filtrate. Ammonia formation and reabsorption of alkali bicarbonate appear to be the two chief mechanisms by which the kidneys maintain the neutrality of the body fluids.

Under conditions of stress the tubules furthermore appear able to assist

by direct excretion in ridding the blood of certain injected dyes and other foreign substances.

The work of Shannon has shown that in active tubular reabsorption (as of glucose) and tubular excretion (as of phenol red) the process of transfer of substance through the tubular cells appears to be accomplished by successive combination and dissociation. The mechanism in its kinetics appears to be strikingly similar to that noted for a number of hydrolytic enzymes.

One may say that the action of the glomerulus as a filter is established beyond a reasonable doubt, and that the factors controlling the speed and completeness of the filtration seem fairly well evaluated. The problem of the immediate future seems to be that of attaining some similar knowledge of the physicochemical mechanisms involved in the selective action of the tubules. This is an extraordinary function, influenced by more factors than can be estimated at present. Among these are the hormones. For example, when the pituitary fails to secrete a certain hormone or hormones, the kidneys fail to retain water to a normal degree, and diabetes insipidus results, with excretion of water up to 30 or 40 liters per day. And when the cortin of the adrenals fails, the kidneys let the sodium concentration of the plasma fall and the potassium concentration rise, until these changes alone are sufficient to threaten life. One might feel justified in abandoning explanation of tubular function to the vital forces that can never be explained. Yet advances in tubular physiology, made in the laboratories of Richards, Marshall, Smith, Shannon, and others, let one hope that the future may further clarify the mechanism that governs the volume and composition of the inner sea in which we live.

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