A PROGRAM FOR ACTION

The Government should accept new responsibilities for promoting the flow of new scientific knowledge and the development of scientific talent in our youth. These responsibilities are the proper concern of the Government, for they vitally affect our health, our jobs and our national security. It is in keeping also with basic United States policy that the Government should foster the opening of new frontiers and this is the modern way to do it. For many years the Government has wisely supported research in the agricultural colleges and the benefits have been great. The time has come when such support should be extended to other fields.

The effective discharge of these new responsibilities will require the full attention of some over-all agency devoted to that purpose. There is not now in the permanent Governmental structure receiving its funds from Congress an agency adapted to supplementing the support of basic research in the colleges, universities and research institutes, both in medicine and the natural sciences, adapted to supporting research on new weapons for both Services, or adapted to administering a program of science scholarships and fellowships.

Therefore I recommend that a new agency for these purposes be established. Such an agency should be composed of persons of broad interest and experience, having an understanding of the peculiarities of scientific research and scientific education. It should have stability of funds so that long-range programs may be undertaken. It should recognize that freedom of inquiry must be preserved and should leave internal control of policy, personnel and the method and scope of research to the institutions in which it is carried on. It should be fully responsible to the President and through him to the Congress for its program.

Early action on these recommendations is imperative if this nation is to meet the challenge of science in the crucial years ahead. On the wisdom with which we bring science to bear in the war against disease, in the creation of new industries and in the strengthening of our Armed Forces depends in large measure our future as a nation.

THE RENAL REGULATION OF ACID BASE BALANCE WITH SPECIAL REFERENCE TO THE MECHANISM FOR ACIDIFYING THE URINE. II

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(*Continued from page 54*)

While the negative aspects of these experiments are obvious, what positive facts are proven? As I mentioned before, there are only two sources of acid of significant magnitude in a protein-free filtrate of plasma, namely, monobasic phosphate and carbonic acid. Hence only these two acids appear in the glomerular filtrate in appreciable amounts. Their sum is only one fourth to one third of the observed excreted titratable acid. Therefore the renal tubules must have added no less than two thirds to three fourths of the excreted acid to the filtrate as it passed down the tubular lumen. There is reason from work of the school of Dr. Richards on the frog for believing that the tubules add all the acid which appears in the urine.

If the proximal and distal segments of the amphibian kidney and the mammalian kidney are homologous, as seems probable from comparative physiological studies, then acidification of the urine in the dog as in the frog should take place in the distal tubule. There are two types of cellular mechanisms, illustrated in the two diagrams in Fig. 3, which could bring about this process of acidification: first, a true secretory mechanism, illustrated on the left, by which acid in molecular form could be secreted into the tubular lumen; and second, a quasi-secretory tubular mechanism, illustrated on the right, which could bring about an exchange of hydrogen ions for sodium ions. At first glance these two mechanisms seem very dissimilar. However, our attempts to distinguish between them experimentally have convinced us that a distinction is largely academic, and with present methods, impossible of achievement. If molecular acid were secreted, that acid might be hydrochloric, as

POSSIBLE MECHANISMS FOR THE TUBULAR EXCRETION OF ACID

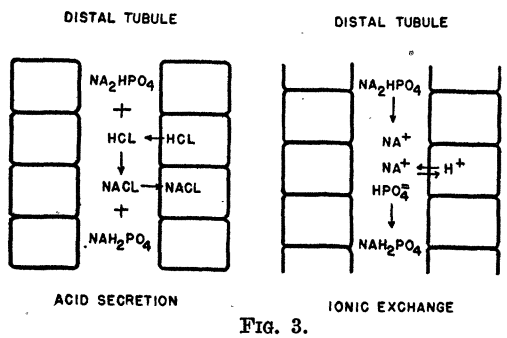

FIG. 3.

shown in this chart in the diagram on the left, or it might equally well be carbonic acid. Such acid could react within the tubule to transform dibasic sodium phosphate into the monobasic salt. In this process sodium chloride or sodium bicarbonate would be formed, and these salts might be expected to appear in the urine. In our experiments large quantities of titratable acid have been excreted, yet the urine has often contained neither chloride nor bicarbonate. Hence if any sodium chloride or any sodium bicarbonate were formed within the renal tubule, it must have been reabsorbed completely. Therefore it is apparent that even the acid secretory mechanism would to all intents and purposes accomplish an exchange of hydrogen ions for sodium ions. For certain indirect reasons which we need not consider here, we prefer the concept illustrated in the diagram on the right, namely, the direct exchange of hydrogen ions for sodium ions across the lumenal membrane.

The ultimate source of hydrogen ions which are added to the glomerular filtrate in its passage through the renal tubule must be carbonic acid. No other source of acid of sufficient magnitude is available to the kidney. Wherever it is necessary to hydrate carbon dioxide to form carbonic acid in large quantities, one finds the enzyme carbonic anhydrase. Thus the red cell,[9] the pancreas,[10] the stomach[11] and the kidney[12] all contain this enzyme in high concentration. It is possible that the carbonic anhydrase found in the kidney may in some way be involved in the cellular processes concerned with acidifying the urine. A mode of experimental attack was suggested by experiments of Mann and Keilin,[13] who found that sulfanilamide in low concentration added to preparations of carbonic anhydrase *in vitro* completely inhibited the enzyme. If the enzyme of the kidney is concerned in the renal excretion of acid, then the administration of sulfanilamide should reasonably be expected to raise the pH of the urine and to decrease its titratable acidity.

A series of experiments were performed in much the same fashion as those described previously, *i.e.*, animals were made acidotic by the repeated feeding of hydrochloric acid and large amounts of phosphate were infused to raise the plasma concentration to 9 or 10 times the normal value. As is shown in Table 7, three control clearance periods preceded the administration of sulfanilamide. Three test clearance periods followed the administration of sulfanilamide, during which the effects of the drug on renal function could be observed. The changes produced by sulfanilamide in urine pH, fourth column, and in titratable acid, fifth column, were striking. The pH of the urine rose from 5.6 to 6.8 after the administration of sulfanilamide, and the titratable acid decreased to about one third of the control value. These are exactly the changes which we predicted from the *in vitro* studies of Mann and Keilin, and are reminiscent of the effects of sulfanilamide on gastric secretion of acid first demonstrated by Davenport.[14]

TABLE 7

THE EFFECT OF SULFANILAMIDE ON THE RENAL EXCRETION OF TITRATABLE ACID BY THE ACIDOTIC DOG. IN THIS EXPERIMENT THE MAJOR URINARY BUFFER WAS PHOSPHATE

Rate of glomerular filtration cc/min.	Plasma concentration		Urine pH	Titratable acid		
	Phosphate	Sulfanilamide		Observed	Calculated	
	mM/L.	mg per cent.		mEq./min.	mEq./min.	Per cent. of observed
69.8	9.35	...	5.55	0.435	0.431	99.1
74.3	9.48	...	5.57	0.466	0.467	100.2
74.8	9.67	...	5.61	0.478	0.471	98.6
76.9	9.90	54.0	6.67	0.234	0.243	103.8
75.6	10.1	66.0	6.77	0.199	0.207	104.0
71.6	10.7	78.4	6.81	0.177	0.186	105.0

The effects of sulfanilamide are even more striking in an experiment shown in Table 8, in which creatinine rather than phosphate served as the major urinary buffer. Large amounts of creatinine were infused to raise the plasma concentration to approximately 130 mgm per cent. and hence to cause the excretion of large amounts of this buffer. Creatinine is less effective as a urinary buffer than is phosphate, thus the control values for titratable acid were lower. The effect of sulfanilamide was to raise urinary pH almost to the level of plasma pH and to decrease the

TABLE 8

THE EFFECT OF SULFANILAMIDE ON THE RENAL EXCRETION OF TITRATABLE ACID BY THE ACIDOTIC DOG. IN THIS EXPERIMENT THE MAJOR URINARY BUFFER WAS CREATININE

Rate of glomerular filtration cc/min.	Plasma concentration		Urine pH	Titratable acid		
	Creatinine	Sulfanilamide		Observed	Calculated	
	mg per cent.	mg per cent.		mEq./min.	mEq./min.	Per cent. of observed
96.7	131	...	5.76	0.148	0.154	104.0
96.6	128	...	5.76	0.150	0.152	101.4
89.4	126	20.2	5.95	0.094	0.091	96.8
100.0	129	27.4	6.29	0.050	0.049	102.0
93.8	135	36.4	6.69	0.019	0.021	110.0
90.6	138	43.7	7.16	0.003	0.003	100.0

[9] N. U. Meldrum and F. J. W. Roughton, *Jour. Physiol.*, 80: 113, 1933.

[10] H. vanGoor, *Arch. internat. physiol.*, 45: 491, 1937.

[11] H. W. Davenport, *Jour. Physiol.*, 97: 32, 1940.

[12] H. W. Davenport and A. E. Wilhelmi, *Proc. Soc. Exp. Biol. and Med.*, 48: 53, 1941.

[13] T. Mann and D. Keilin, *Nature*, 146: 164, 1940.

[14] H. W. Davenport, *Amer. Jour. Physiol.*, 133: 257, 1941.

observed titratable acidity of the urine almost to zero. These experiments are certainly consonant with the view that carbonic anhydrase is involved in some way in the mechanism for acidifying the urine.

Our concepts of the nature of this cellular mechanism are outlined in schematic form in Fig. 4. We have illustrated here a single cell from that part of the distal segment of the renal tubule which is concerned with acidification of the urine. On the left is the lumenal border of the cell in contact with the tubular urine. On the right is that border of the cell which is in diffusion equilibrium with the renal tubular blood. Carbon dioxide is produced within the tubular cell by oxidative metabolic processes and may also diffuse into the cell from the peritubular blood. This carbon dioxide is hydrated to form carbonic acid. The enzyme carbonic anhydrase serves to increase catalytically the rate of production of carbonic acid, but is of course not essential for the process of hydration. Thus when carbonic anhydrase is inhibited by sulfanilamide,[15] hydration of carbon dioxide and excretion of acid continue, although at a slower rate. Carbonic acid dissociates within the cell to form hydrogen ions and bicarbonate ions; the hydrogen ions are exchanged for sodium ions in the tubular lumen; and the sodium ions, accompanied by an equivalent number of bicarbonate ions, are reabsorbed into the tubular blood. While the details of this scheme are largely hypothetical, it has the virtue of explaining adequately the known facts concerning the excretion of titratable acid, namely, that hydrogen ions are added to the tubular urine by the renal tubular cells, that an equivalent amount of base is retained in the body, and that the enzyme carbonic anhydrase is in some way concerned in the process.

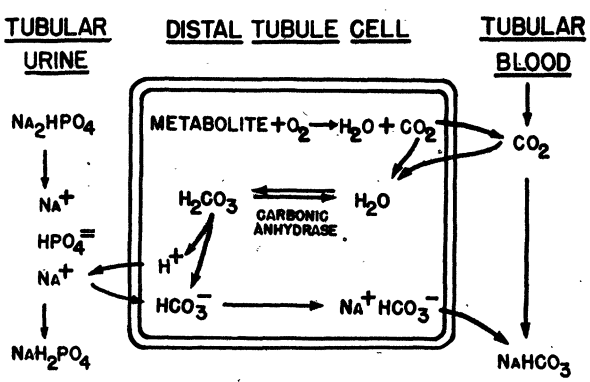

FIG. 4. Diagrammatic representation of the nature of the renal cellular mechanism for acidifying the urine. A single cell from the distal segment of the renal tubule is illustrated. From Pitts, R. F. and Alexander, R. S., *Amer. Jour. Physiol.*, 144: 239, 1945.

Acidification of the urine is a process which requires the expenditure of energy, *i.e.*, the exchange of ions will not proceed spontaneously. Thermodynamically, the energy expended to cause this movement of ions must at least equal the heat of neutralization of the acid which is excreted. Since biological processes are never 100 per cent. efficient, the actual cellular work done will be several times this value.

The failure of this acid-excreting mechanism in nephritis is obviously one cause of the acidosis which characterizes the terminal stages of this disease. In chronic diffuse glomerulonephritis the number of functional nephrons is of course markedly reduced. Ordinarily one considers that the reduced excretory capacity of the kidney and the consequent retention of phosphate and sulfate in the blood stream adequately account for the acidosis. I wonder, however, if in addition to the obvious morphological lesion in this disease, there may not also be a biochemical lesion of the renal tubular cells. By this I mean that the disease process might adversely affect the functional capacity of the tubular cells of the remaining nephrons. It might do so by reducing their ability to do the work involved in acidifying the urine, or by reducing the efficiency with which they perform ionic exchanges. Furthermore it is possible that the disease process might bring about a reduction in the intracellular concentration of carbonic anhydrase, and thus specifically reduce the capacity of the tubular cells to produce hydrogen ions at a rapid rate.

In the remaining time at my disposal I should like to discuss briefly the factors which determine the rate of excretion of titratable acid and the bearing which these factors have on the renal response to acidosis in diabetes. There are three major factors which determine the rate of excretion of titratable acid by the normally functioning kidney. These include first, the quantity of buffer excreted; second, the degree of acidosis; and third, the physiochemical properties of the urinary buffer.

In the experiments now to be described as in the previous ones, dogs were rendered acidotic by the daily feeding of dilute acid. The degree of acidosis of the animal in the experiment illustrated in Fig. 5 was moderately severe, as is indicated by a plasma carbon dioxide combining power of 22 vol. per cent. The normal for the dog as for the human is between 50 and 60 vol. per cent. A series of solutions of neutral sodium phosphate of increasing concentration were infused intravenously, causing the animal to excrete progressively increasing amounts of buffer over a range of one tenth to eight tenths of a millimol

[15] According to a recent study of Davenport (*Jour. Biol. Chem.*, 158: 567, 1945), the sulfanilamide concentrations attained in our experiments (20 to 78 mg per cent.) should inhibit 99.93 to 99.97 per cent. of the carbonic anhydrase present in the kidney. However, the minute fraction of the enzyme which remains active (0.03 to 0.07 per cent.) probably accounts for the residual acid excretion which we have observed.

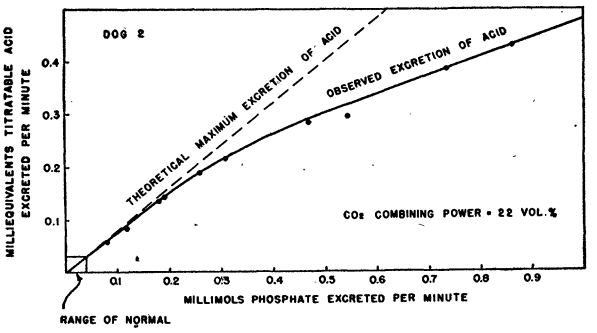

FIG. 5. The relation between the rate of excretion of titratable acid and the rate of excretion of phosphate in the acidotic dog.

of phosphate per minute. In this and in the succeeding two figures we have plotted the milliequivalents of titratable acid excreted per minute against the millimols of buffer excreted per minute. It is apparent that the rate of excretion of titratable acid progressively increased with the increase in the rate of excretion of phosphate. The dashed line labelled the *theoretical maximum excretion of acid* is that amount which would have been eliminated if in each instance the animal had produced urine of maximal acidity, *i.e.,* urine of pH 4.8. Actually you see that the greater the excretion of phosphate the greater is the deviation from the theoretical maximum rate of excretion of acid. In other words, the more phosphate that is presented to the kidney, the less completely does the kidney utilize its full buffer potentialities. The maximum observed rate of acid excretion in the experiment shown in Fig. 5, namely, 0.431 milliequivalents per minute, is equivalent to the excretion of 6,200 cc of N/10 acid per day. In the lower left-hand corner of the chart is a small rectangle which indicates the range of excretion of phosphate and titratable acid in normal man. It is apparent that the quantity of titratable acid which the kidney can excrete under stress is far greater than the quantity which is normally excreted, and that a major factor which limits acid excretion is the quantity of buffer available in the urine.

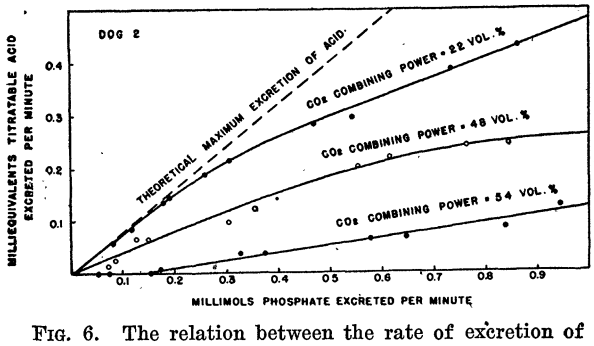

FIG. 6. The relation between the rate of excretion of titratable acid and the serverity of the acidosis in the acidotic dog.

The second factor determining the rate of excretion of titratable acid is the degree of acidosis. Three experiments similar to the one just presented were performed on a single dog. The results obtained in these experiments are summarized in Fig. 6. In the different experiments the degree of acidosis was varied by altering the total amount of acid fed to the animal. The carbon dioxide combining power noted on each experimental curve indicates the severity of the acidosis. A combining power of 54 vol. per cent. is within the range of normal for the dog. A combining power of 22 vol. per cent. is indicative of a moderately severe acidosis. It is apparent at any given rate of excretion of phosphate that the more severe the acidosis, the greater is the excretion of titratable acid. It is interesting to note that the more severe the acidosis, the more nearly the kidney approaches the theoretical maximum rate of excretion of acid. Since our trained dogs are valuable animals, we did not feel justified in pushing the acidosis further in an attempt to force them to do their theoretical best.

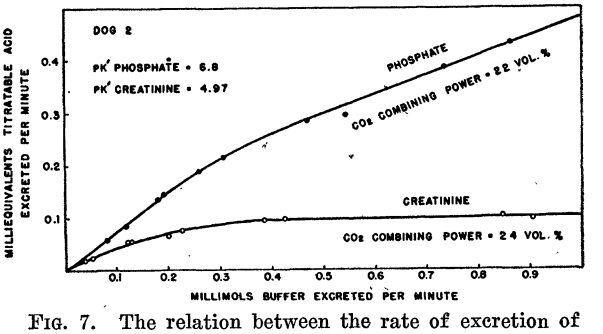

FIG. 7. The relation between the rate of excretion of titratable acid and the strength of the buffer acid excreted in the acidotic dog.

The third factor determining the rate of excretion of titratable acid is the strength of the buffer acid excreted. A convenient designation of the strength of a weak acid is its pK′ value. The pK′ of an acid is the pH which is observed when the acid is exactly half neutralized. Therefore the lower the pK′, the stronger is the acid. The physiologically important hydrogen of phosphate is the second one, and its pK′ is 6.8; *i.e.,* when half of the monobasic phosphate in a solution is neutralized to the dibasic form, the pH of the mixture is 6.8. The pK′ of creatinine is 4.97; *i.e.,* when half of the creatinine is in the form of free base and half is in the acid form, say of creatinine hydrochloride, the pH of the mixture is 4.97. Accordingly monobasic phosphate is a much weaker acid than is creatinine hydrochloride. From Fig. 7 it is apparent that phosphate is a far better urinary buffer than is creatinine, and that when equi-molar quantities of these two buffers are presented to the kidney, the excretion of acid is far greater with phosphate than with creatinine.

The bearing of these results on the renal excretion of acid in diabetic acidosis is rather direct. Thus the uncontrolled diabetic excretes large quantities of buffer in the form of beta-hydroxybutyrate and acetoacetate; as much as 500 to 1,000 millimols of these buffer substances may be excreted in 24 hours. You recall that the rate of excretion of titratable acid in our experiments was directly related to the rate of excretion of buffer. Likewise the severity of the acidosis is often very great in the uncontrolled diabetic and plasma carbon dioxide combining powers as low as 10 to 20 vol. per cent. are not uncommon. Again you recall that the rate of excretion of titratable acid in our acidotic dogs was directly related to the severity of the acidosis. Both factors, the high rate of buffer excretion and the severity of the acidosis, combine to stimulate the renal excretion of titratable acid in the diabetic. Accordingly the titratable acid of the urine may rise to as high as 1,500 cc of N/10 acid per day. But beta-hydroxybutyric acid and acetoacetic acid are relatively strong acids in comparison with monobasic phosphate, and are therefore poor urinary buffers. In fact, they are stronger buffer acids than is creatinine hydrochloride, which in our experiments was shown to be a rather poor urinary buffer. You recall that the dog, in a state of acidosis comparable to that of the diabetic, and excreting quantities of phosphate comparable to the quantities of ketone bodies excreted by the diabetic, eliminates as much as 6,000 to 7,500 cc of N/10 titratable acid per day, *i.e.*, some 4 to 5 times the quantity eliminated by the diabetic. This quantitative difference is certainly related to the strength of the buffer acid excreted and not to a greater effectiveness of the acid-eliminating mechanism in the dog. Since the capacity of the renal tubules to excrete relatively strong acids such as beta-hydroxybutyric and acetoacetic in free titratable form is limited, the kidney must excrete them in large part combined with fixed base. Accordingly the alkali reserve of the body is progressively exhausted, and terminally the acidosis of diabetes may become extreme.

In conclusion let me summarize briefly our concepts of the renal mechanism for excretion of titratable acid, and the nature of the aberrations of this mechanism in disease. A slightly alkaline filtrate of plasma containing buffer acids in combination with sodium ions is formed in the glomeruli. In the passage of this alkaline filtrate through the distal segments of the renal tubules hydrogen ions formed within the tubular cells are exchanged for sodium ions in the tubular urine. The urine becomes acid by the conversion of buffer salts to free buffer acids, and base is saved by the reabsorption into the renal venous blood of sodium ions along with equivalent numbers of bicarbonate ions.

The quantity of base which is saved, or conversely the quantity of free titratable acid which is excreted by this mechanism, is determined by three major factors: first, the quantity of buffer available for the kidney to operate upon; second, the strength of the buffer, *i.e.*, how strongly it resists giving up its sodium ions in exchange for hydrogen ions; and third, the severity of the acidosis, which is of course a measure of the need of the body for the conservation of its basic constituents.

Under normal conditions the capacity of the kidney to exchange hydrogen ions for sodium ions is sufficient to permit the excretion of the fixed metabolic acids without depletion of the body stores of fixed base. But in diabetes the excessive production of metabolic acid so overwhelms the renal mechanism that it is incapable of full compensation, and progressive acidosis develops. One reason for the failure of compensation is the rather unfavorable physiochemical properties of two of the buffer acids formed in this disease, beta-hydroxybutyric and acetoacetic acids. Both acids are too strong for the kidney to operate on effectively.

In nephritis the mass of functional renal tissue is reduced. Therefore each remaining unit must carry more than its normal share of the burden of acid elimination. Furthermore, it is probable that the disease process has reduced the capacity of these remaining units to excrete acid as compared with the capacity of normal units. Perhaps it has done so by restricting the chemical work which the renal tubular cells can do, or perhaps the disease process has specifically reduced the cellular content of the vital enzyme carbonic anhydrase.

I am sure that much of what I have said concerning the overall aspects of the renal regulation of acid base balance is familiar to you all. However, I have tried to emphasize the dynamic aspects of the problem, for acid-base regulation is most certainly concerned with the moment to moment maintenance of balance. If I have given you a more fluid concept of the problem, I have accomplished my major aim. My secondary aim has been to show you how recently developed methods of study of the kidney may be applied to the solution of basic problems of renal physiology, and how they in turn may throw light on certain abnormalities of function in disease. But I admit frankly that more questions have been raised concerning renal physiology in nephritic and diabetic acidosis than have been answered. It is our hope, by continuing our studies on patients with these two diseases, that we shall be able to provide a direct answer to some of the more puzzling of these clinical physiological questions.