by this agency should complement and strengthen the basic research program and not be undertaken at the expense of the latter.

In the general area of applied research, NSB and PSAC would seem to hold essentially equivalent hunting licenses. But there is so much to be done, I fail to see this as a problem as long as the communication channels remain open. In any case, prediction becomes difficult because, in this area, NSB-NSF and PSAC-OST will be attempting to fill a vacuum which presently exists by virtue of the inadequacy of the efforts of those federal agencies which should have been responsible for fostering innovation in the civilian sector of our economy, that is, the Departments of Commerce, Justice, Interior, Labor, and the Post Office. We have yet to observe the approaches of the Departments of Transportation and Housing and Urban Development. As all of these learn to be effective science-using agencies, the need for much of the activity presently contemplated by PSAC and NSB may well subside.

This, then, is the pattern I foresee for the next several years: PSAC-OST will be largely concerned with policy and technological problems related to specific agency and interagency missions and affecting all phases of American life while NSB-NSF will be mainly concerned with the progress of science and science deucation including the problems of scientific manpower, science information, provision of research resources and the welfare and development of the institutions in which science and science education are conducted. Both bodies will continue to seek means by which science and technology may improve the human condition, but these opportunities are so diverse, unlimited, and challenging that we can only hope that this combination will prove equal to the total task.

**Note**

These opinions do not necessarily represent those of other members of NSB or PSAC. No official position in these regards has been taken by NSB, PSAC, NSF, or OST.

# Metabolic Aspects of Acid-Base Change

Interrelated biochemical responses, in the kidney and other organs, are associated with metabolic acidosis.

William D. Lotspeich

During normal metabolism certain animals, including man, produce large quantities of acid. Carbon dioxide, hydrated to volatile carbonic acid, is excreted mainly by the lungs. However, there are also produced considerable amounts of nonvolatile strong acids, particularly sulfuric and phosphoric, from the breakdown of phosphorous and sulfur-containing amino acids; these must be excreted by the kidneys. During some disease states in man, large quantities of either weak or strong acid accumulate in the body; during certain chronic lung diseases, for instance, carbon dioxide cannot diffuse rapidly out of the blood into the lung's air sacs, and the concentration of carbonic acid rises in the tissues; this condition is referred to as respiratory acidosis. During uncontrolled diabetes, when utilization of sugar is faulty, strong acids of intermediary fat metabolism accumulate in the body; this condition is called metabolic acidosis. For these reasons, the buffering of acid in the tissues and body fluids, its carriage in the bloodstream, and its excretion by lungs and kidneys represent important physiological and clinical problems.

Transport of strong acid in the bloodstream requires its neutralization with fixed cations, mainly sodium. In addition, it is apparent that not much of this acid can be excreted in the free form within the observed limits of urinary *p*H; thus most of it must be excreted, with its full complement of cation, in the form of an acid salt. It is apparent that if the cations implicated were those in limited supply, such as $Na^+$, $K^+$, $Ca^{++}$, or $Mg^{++}$, excretion of even the normal daily load of acid—not to mention the increased amounts during metabolic acidosis—would put an intolerable drain on these fixed reserves of cations. Therefore other means of excreting the acid must exist, and we now know that they do.

The kidney-tubule cells secrete hydrogen ions derived from metabolically produced carbonic acid, and ammonia derived from glutamine and amino acids. The secreted hydrogen ions, which represent the ultimate loss of the strong acid originally produced, are buffered either by phosphate or bicarbonate appearing in the glomerular filtrate from the blood, or by the ammonia made in the kidney cells and secreted into the filtrate as it passes down the tubules. Thus the strong acid is really excreted as the salt of a weak buffer acid or as the ammonium salt. For every mole of hydrogen so excreted, 1 mole of fixed cation is retained by the body, and to this extent the fixed cation reserves are protected. Our present concept of these mechanisms is portrayed in Fig. 1 (from *1*).

My purpose is to discuss some of the changes in kidney metabolism associated with excretion of acid, and the condition of metabolic acidosis. I also wish to point out some related changes in the biochemistry of other organs during metabolic acidosis, changes that illustrate the fact that the biochemical response to a metabolic acid-base change is a complex process involving the whole body in a way we are just now beginning to glimpse.

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## Renal Excretion of Ammonia

I will concentrate first on certain aspects of the system that produces and secretes ammonia in the kidney. It is well known that in man and certain other mammals the capacity to excrete ammonia not only increases sharply early in acidosis, but as this condition continues, a further, slower adapation also takes place in the kidney until practically the entire acid load is excreted as the ammonium salt. The nature of this slower adaptation is a remarkable feature of the renal response to acidosis.

An example of this adaptation in man is shown in Fig. 2. After 5 control days the subject induced in himself a metabolic acidosis by daily ingesting 10 to 15 grams of ammonium chloride for 5 days. Then followed a recovery period of 5 days. Ammonium chloride produces metabolic acidosis because a dose is equivalent to drinking an equivalent amount of HCl; it is metabolized in the liver to urea and HCl. Thus the increased excretion of ammonia results from the acid ingested. The rapid initial increase in loss of sodium, the gradual increase in excretion of ammonia, and the associated decrease in loss of sodium are all apparent here during the period of acidosis. I would like to consider the underlying nature of this adaptation in capacity to excrete ammonia during $NH_4Cl$ acidosis; its understanding has revealed several mechanisms of fundamental biologic significance.

One would perhaps first ask whether there may occur some sort of facilitation in the process of diffusion of ammonia across the luminal membrane of the tubular cell. We now believe that ammonia enters the tubular lumen by a simple diffusion process governed by the hydrogen–ion concentration gradient between cell and tubular urine, and by the proportion of the intracellular ammonia that exists as the more–lipid soluble, more-permeable, un-ionized free base at the prevailing intracellular *p*H (*2*). Some of the adaptive increase in excretion of ammonia could result from a favorable change in these factors that determine rate of ammonia diffusion into the luminal urine, but the available evidence suggests that it is unlikely that the whole thing may be explained in this way.

Another possible basis for the adaptation in excretion of ammonia would be an increased activity of the enzymes implicated in producton of ammonia within the tubular cells; indeed, such changes have been observed.

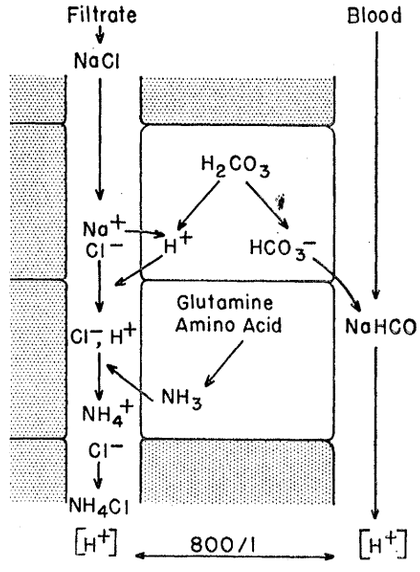



Fig. 1. Schematic representation of renal tubular synthesis and secretion of hydrogen ions and ammonia. [From Pitts (*1*)]

The major source of urinary ammonia is glutamine (*3, 4*); a system of at least two glutaminase enzymes catalyzes the production of free ammonia from this substrate (*5*). Slices of kidney from rats having chronic $NH_4Cl$ acidosis show increased capacity to produce ammonia from glutamine in vitro (*6*), and the same phenomenon has been observed repeatedly in homogenates and mitochondria prepared from kidneys of certain other chronically acidotic animals (*7, 8*). This change has been interpreted in terms of induced synthesis of the glutaminase enzymes (*9*); in the rat it generally parallels the increasing rate of excretion of ammonia (*7*). Thus the two have been seen in terms of cause and effect. Therefore, at least in certain species, some sort of adaptive increase in glutaminase enzymes probably plays a part. In the dog, however, there is no observable change in enzyme activity during chronic metabolic acidosis from $NH_4Cl$ (*10*), so other factors also must play a role.

## Renal Glutamine Extraction During Acidosis

Reference to Fig. 1 will show another possible explanation for the adaptation in capacity to excrete ammonia; this would entail an increasing rate of renal extraction of glutamine, and perhaps certain amino acids, from the arterial blood during acidosis. It is well known that in dog (*3*) and man (*11*) the renal extraction of glutamine is markedly increased during well-developed $NH_4Cl$ metabolic acidosis. In the rat, also, this phenomenon is already significantly apparent within 24 hours of $NH_4Cl$ acidosis (see Fig. 3, from some of our recent experiments; *12*).

Adult female rats were given $NH_4Cl$ by stomach tube (5 milliliters at 0.75*M*) twice on the first day; controls received an equal volume of tap water. Next morning one kidney was

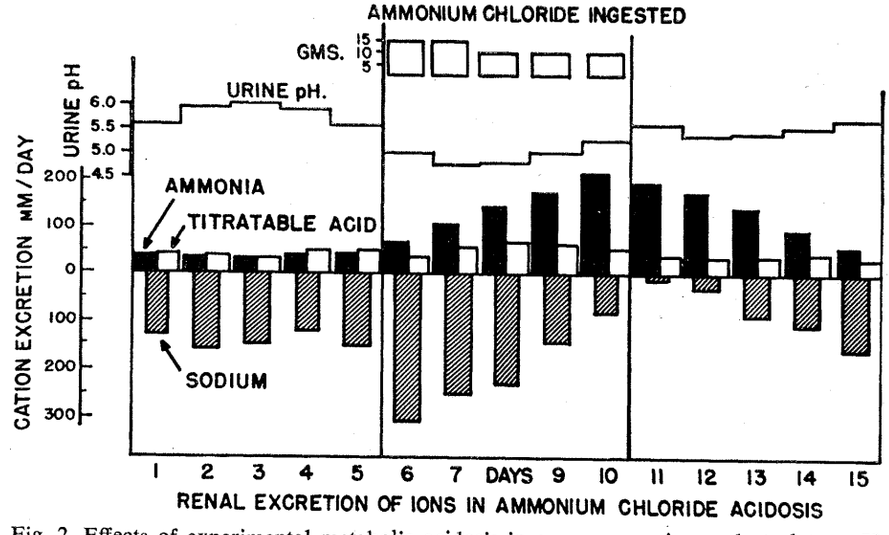



Fig. 2. Effects of experimental metabolic acidosis in a man on urinary electrolytes, *p*H, ammonia, and titratable acid. [From Pitts (*1*)]

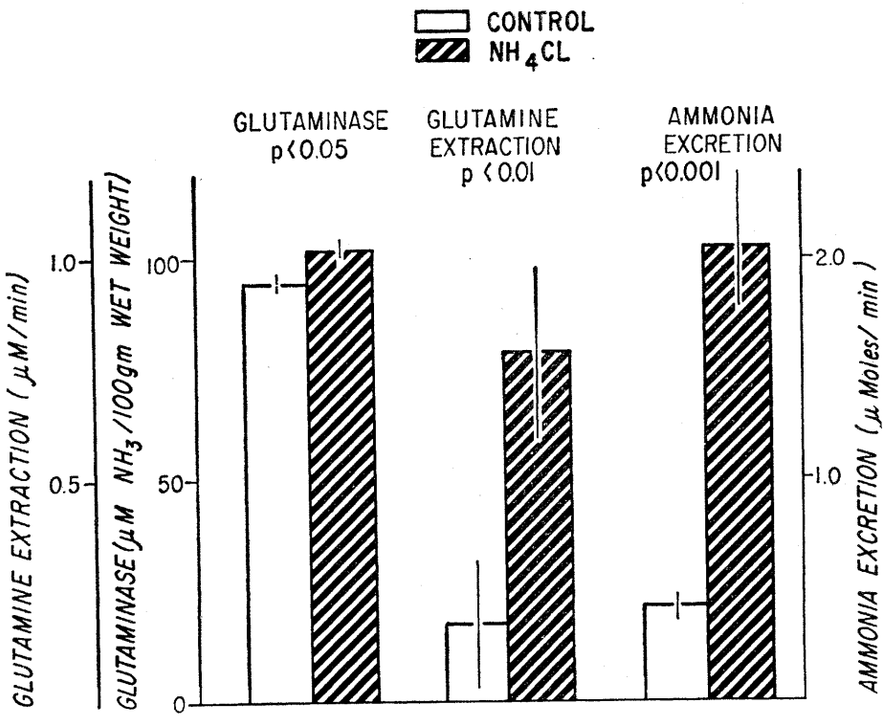
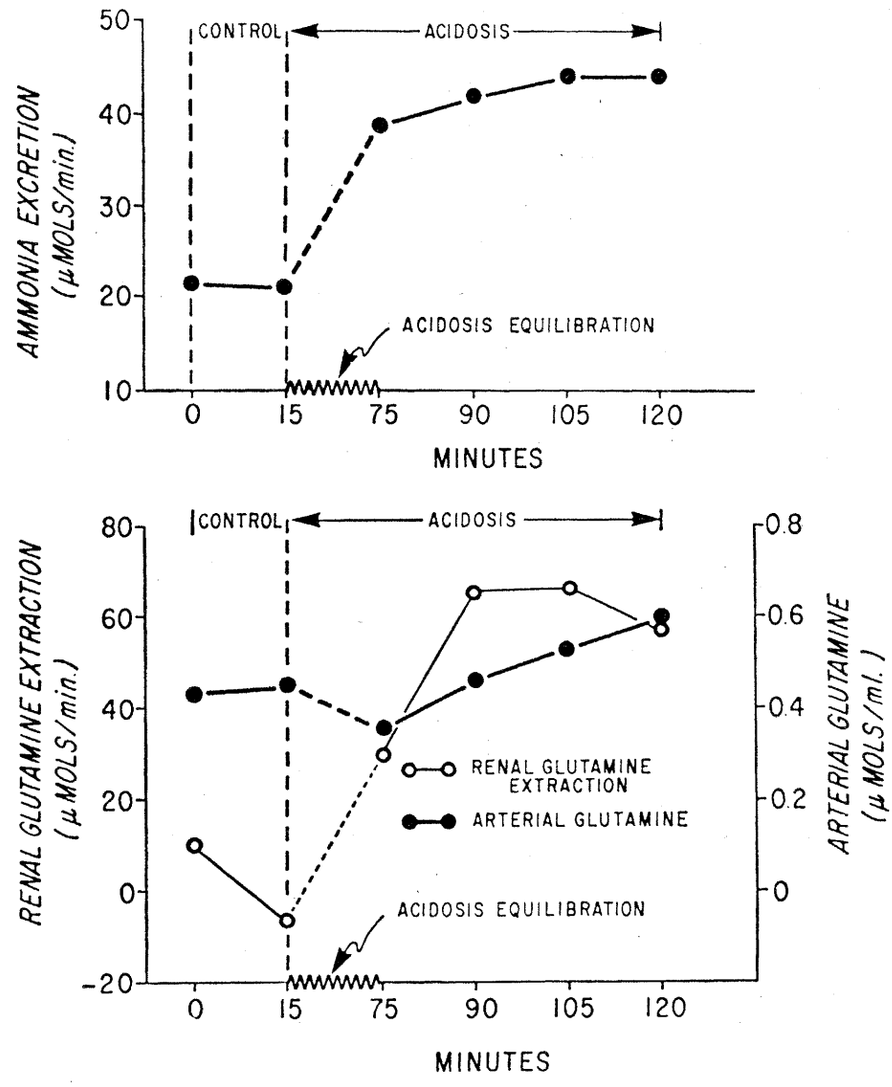



Fig. 3 (top left). Effect of 24-hour $NH_4Cl$ acidosis on the renal extraction of glutamine, rate of excretion of ammonia, and activity of kidney glutaminase I in the rat (*12*).




removed from each of the control and acidotic animals (under pentobarbital anesthesia), and the extraction of glutamine by the remaining kidney was measured; extraction was calculated from glutamine analyses of simultaneously drawn samples of femoral-arterial and renal-venous blood, and from the renal blood flow as measured by the Fick principle, by use of the renal extraction of Diodrast $I^{131}$ (*13*). Glutaminase-I activity was assayed from the removed kidney. It was apparent that, after only 24 hours of experimental metabolic acidosis, renal extraction of glutamine, excretion of ammonia, and glutaminase-I activity were all significantly increased. The rapidity of these changes in the rat is remarkable.

We were next interested to know how rapidly glutamine extraction changes during developing acidosis, and precisely how this function operates in an animal, like the dog, who shows no glutaminase change. The experiments designed to answer these questions were done by S. K. Addae, using large, female, mongrel dogs (*14*).

Unilateral nephrectomy first was performed through a flank incision 1 week before the study. On the day of the experiment, one femoral artery and vein were isolated, the artery was cannulated for blood collections, and through the femoral vein the right renal vein was catheterized for renal venous-blood collections. A general intravenous infusion was started in the external saphenous vein. Urine was collected from an indwelling bladder catheter. Renal extraction of glutamine was determined on this kidney from renal arteriovenous glutamine differences; renal blood flow, as it was in the rat. Glutamine was determined enzymatically by an *Escherichia coli* glutaminase method (*15*). The liberated ammonia was measured colorimetrically (*16*) after subtraction of suitable blanks.

In each experiment a moderate osmotic diuresis was first established by infusion of hypertonic sodium sul-

Fig. 4 (bottom left). Effect of acute metabolic acidosis on renal glutamine extraction, ammonia excretion, and glutamine concentration in arterial blood in the dog (*14*).

fate (720 milliosmolal) at a rate of 5 milliliters per minute. After a period of equilibration, a set of two control measurements was made. The infusion was then switched to one containing a mixture of $H_2SO_4$ and $Na_2SO_4$, in which the load of sulfate infused was the same as that given during the control periods. After 1 hour a series of additional measurements was made during the acutely developing acidosis. The results of such an experiment appear in Fig. 4. After 1 hour of infusion of acid, arterial *p*H had fallen from 7.44 to 7.23; $pCO_2$, from 40 to 32 millimeters of mercury. Thus a frank metabolic acidosis had been produced. This condition was associated with significant increase in extraction of glutamine during acidosis, when the arterial level of glutamine also rose slowly; we shall later return to this point. The results of these studies show that very rapid changes in renal extraction of glutamine can occur, and that extraction is related to the rising rate of excretion of ammonia during acute acidosis.

Increased extraction of glutamine, possibly some facilitation of diffusion of ammonia into the urine, and the adaptive increase in ammonia-producing enzymes—all may play roles in determining the rate of excretion of ammonia. They probably operate to differing degrees and in various combinations in different species, so that there is no unitary explanation for the adaptive capacity to increase excretion of ammonia.

During recent years I have become increasingly convinced that these changes associated with excretion of ammonia are probably only one part of a much more profound pattern of metabolic alteration in the kidney's response to acidosis. For this reason we have been carrying out many different studies of renal metabolism during metabolic acidosis, and I should now like to turn to some of these that have proven so fruitful and opened up a whole new field of research.

One of the areas that we decided to investigate was the hexose monophosphate shunt in the kidney. This metabolic pathway performs the direct oxidation of glucose in a wide va-

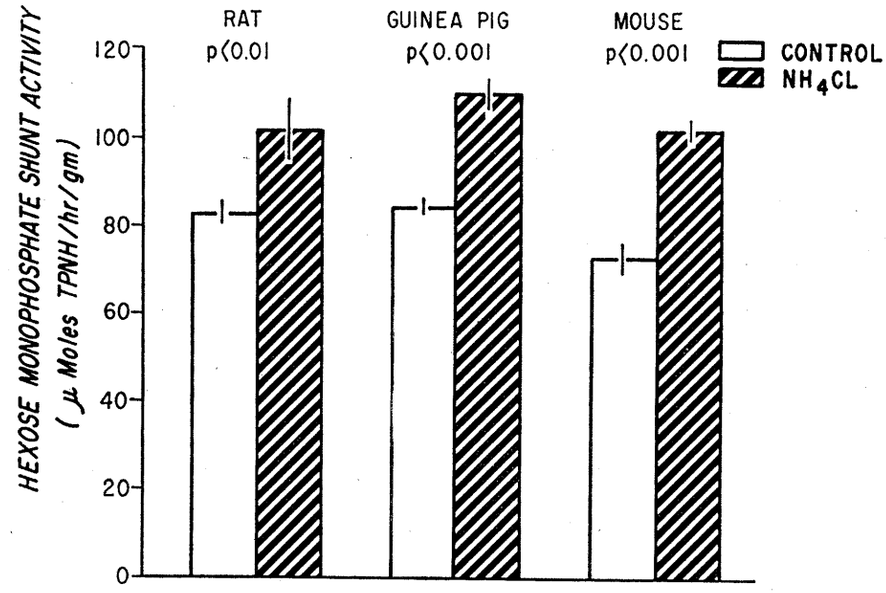



Fig. 5. Increased activities of hexose monophosphate–shunt enzymes in whole kidney of the metabolically aciodotic rat, guinea pig, and mouse. Enzyme activity was measured in dialyzed supernatant of centrifuged kidney homogenate by following rate of reduction of triphosphopyridine nucleotide at 340 mμ in a Beckman spectrophotometer in the presence of glucose-6-$PO_4$ (*40*).

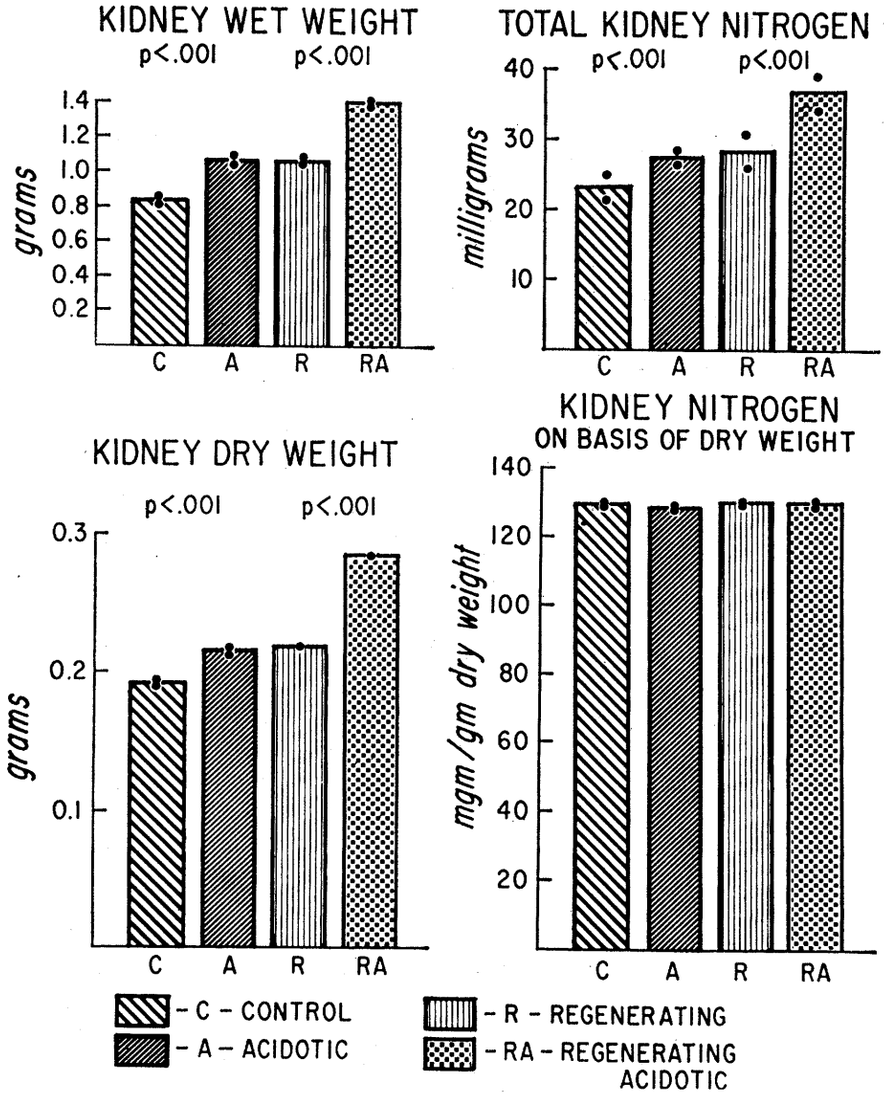



Fig. 6 (bottom right). Growth in rat kidneys resulting from metabolic acidosis produced by 7 days of drinking 0.28*M* $NH_4Cl$. Comparison and combination with hypertrophy in remaining kidney 7 days after unilateral nephrectomy (*19*).

riety of animal tissues. The important metabolic by-products of the hexose monophosphate shunt are ribose and reduced triphosphopyridine nucleotide (TPNH); ribose is used in nucleic acid synthesis, and TPNH is a crucial substance in lipogenesis and several other important biologic hydrogenations. The role of the shunt has received very little attention in the metabolism of the kidney.

Federico Dies, who undertook this study in my laboratory, discovered a remarkable increase, during metabolic acidosis, in the activity of the kidney shunt (*17*); this phenomenon in the rat, guinea pig, and mouse is shown in Fig. 5. The enzyme change is limited to not only the kidney but also its cortex. In the initial phases of metabolic acidosis there is a marked negative sodium balance, as we saw in the experiment of Fig. 2. This led us to look at the kidney hexose monophosphate–shunt activity in states of sodium depletion unassociated with acidosis, and several such states showed similar increase in the shunt of the kidney. Among these conditions were adrenalectomy, and sodium depletion produced by ingestion of a sodium-deficient diet plus chlorothiazide. Moreover, Dies found that, if adrenalectomized rats are allowed 1-percent NaCl to drink, the increase in activity in hexose monophosphate shunt is not so great.

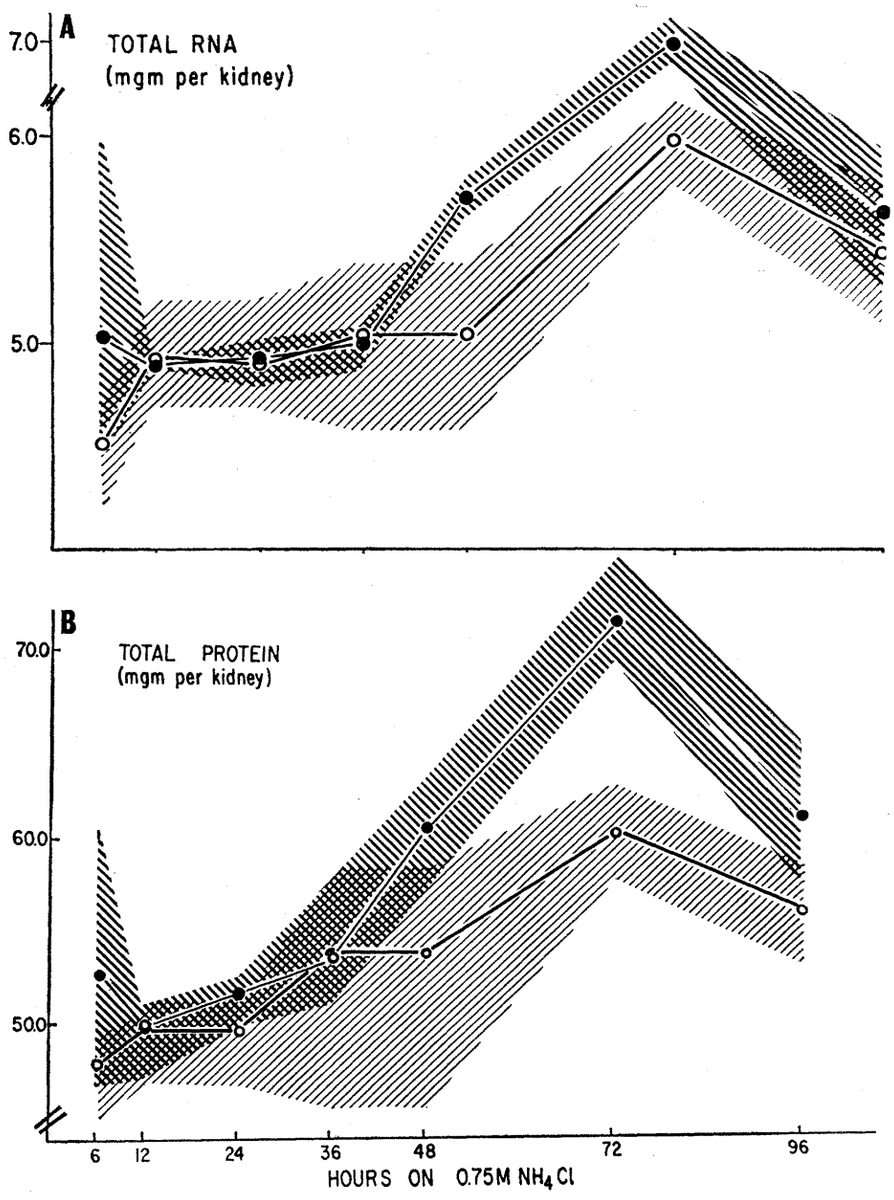



Fig. 7. Time course of RNA and protein contents of rat kidneys during $NH_4Cl$ acidosis. Rat received 5 ml of 0.75*M* $NH_4Cl$ twice daily by stomach tube. Controls drank tap water ad libitum. Each point represents the mean of the observations on three animals; the shaded envelopes show the spreads of the values obtained (*12*).

We do not know yet whether acidosis alters the shunt as a result of electrolyte imbalance in renal tissue, associated with this initial sodum loss, or whether an additional causative factor is in the acidosis itself. Nor do we understand the functional significance of the increase in hexose monophosphate in terms of what aspect of the renal regulation of acid-base balance it serves. In all the various states of electrolyte and acid-base imbalance in which we have observed the hexose monophosphate enzyme change, there is highly significant correlation between shunt activity and rate of titratable acid excretion. Thus the enzyme may play some rate-limiting role in the renal acid-excreting mechanism, in addition to the generally accepted scheme involving carbonic acid and carbonic anhydrase (Fig. 1).

The hexose monophosphate shunt produces reduced triphosphopyridine nucleotide which is an essential component of the reactions of lipogenesis; so the enzyme increase may be related to an acceleration in renal lipogenesis during acidosis. Dies has found increased turnover of lipid in the kidney during acidosis.

The rate of renal gluconeogenesis from glutamine, glutamate, or $\alpha$-ketoglutarate has been shown to be increased in slices of kidney cortex from acidotic rats (*18*). Reduced triphosphopyridine nucleotide functions in the "dicarboxylic acid shuttle" by producing malate from pyruvate, and then proceeding through oxaloacetic to phosphopyruvate. From this point, by way of a reversal of the Embden-Myerhoff reactions, synthesis of glycogen could be affected. In this way the hexose monophosphate shunt, by producing increased amounts of reduced triphosphopyridine nucleotide, could also function in this increased rate of gluconeogenesis during acidosis; also, gluconeogenesis could provide the glucose-6-phosphate needed to keep the shunt working at a greater rate.

These are some of the possible meanings of the hexose monophosphate change during acidosis. Although we do not yet have enough evidence positively to explain this interesting observation there are these several lines of research that in time should clarify its meaning.

## Renal Hypertrophy in Metabolic Acidosis

During part of a study of renal regeneration in the rat I have recently observed that production of chronic metabolic acidosis in the rat causes true increase in the rate of growth of the kidneys (*19*); indeed, this growth is of the same order of magnitude as is growth in the remaining kidney after unilateral nephrectomy. Furthermore, acidosis and unilateral nephrectomy are additive in their effects on kidney growth (Fig. 6). In both situations the proportional increases in wet weight, dry weight, and tissue nitrogen indicate that this kind of renal hypertrophy represents true growth of new tissue. Analyses of these kidneys show that their contents of RNA and protein are significantly increased within from 24 to 48 hours of initial ingestion of $NH_4Cl$; Fig. 7 shows the time course of these changes.

This sequence of events closely resembles the way in which certain hormones affect synthesis of protein in their target organs. In several situations it has been shown that certain hormones have direct action on genes by derepressing them and allowing DNA-dependent synthesis of RNA to proceed at an accelerated rate. This was the revolutionary conclusion from Karlson's first observations on ecdysone (*20*), and the same thing has been seen with various different hormones: for example, the actions of estrogen on the rat uterus (*21*), aldosterone on the toad bladder (*22*), growth hormone on the liver (*23*), testosterone on the prostate (*24*), and adrenocorticotrophic hormone on the adrenal cortex (*25*).

These conclusions have rested heavily on the use of antibiotics that are known to block the reactions of protein synthesis at certain specific points: an example is actinomycin D, which in low doses blocks DNA-dependent synthesis of messenger RNA (*26*). Therefore, when actinomycin D blocks a hormone-induced stimulation of protein synthesis, it is concluded that the hormone affects the sequence of protein-synthetic reactions at the gene level.

With these thoughts in mind Elebute and I wondered whether $NH_4Cl$ acidosis might likewise "uncover" or derepress a gene site in kidney cells, and so allow synthesis of new RNA and cell protein to be accelerated. For this reason we decided to study the effects of actinomycin D on this $NH_4Cl$–induced synthesis of protein in the kidney. We also decided to see whether actinomycin D would affect the induced changes in glutaminase enzymes and rate of excretion of ammonia during acidosis. Thus, we thought, we might be able to determine whether the RNA and protein changes are related in any way to the increased excretion of ammonia during acidosis.

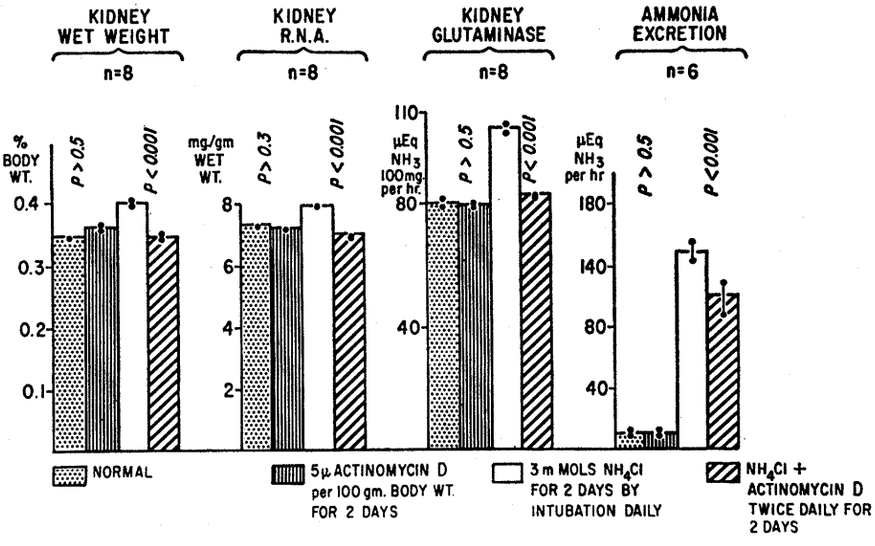



Fig. 8. Inhibition by actinomycin D of the changes in kidney RNA, glutaminase I, and ammonia excretion during $NH_4Cl$ acidosis in the rat (*28*); see text.

## Actinomycin D and the Acidotic Kidney

At the high doses of actinomycin D (300 to 500 micrograms) sometimes used, rats become very toxic, the peritoneal cavity contains much blood-tinged fluid, and the specificity of the effect on synthesis of RNA becomes questionable (*27*). For this reason we first established a minimal dose of actinomycin D that would have effect without producing such gross signs of toxicity: it proved to be 5 micrograms per 100 grams of rat, intraperitoneally twice daily for 2 days in adult female rats of 200 to 250 grams. Figure 8 shows experimental results with normal and acidotic rats by use of this dose schedule (*28*).

One group of rats were made acidotic by intragastric administration of 5 milliliters of 0.75*M* $NH_4Cl$ twice daily for 2 days; the control group received equal amounts of tap water. Another group were given actinomycin D intraperitoneally in saline; they appeared perfectly normal, ate well, and excreted the same amounts of urine as did controls; renal blood flow also was unaffected by this treatment. The fourth group received both $NH_4Cl$ and actinomycin D. On the evening of the 2nd day the bladders of all rats were emptied by manual suprapubic pressure, and the rats were placed in metabolism cages for 12-hour collection of urine. Next morning, bladders were emptied again, cages and funnels were washed with distilled water, volumes of urine and washings were measured, and urine ammonia was determined by the Conway microdiffusion method (*29*). The rats were then decapitated and exsanguinated. Upon opening the peritoneum we always noted a small amount of blood-tinged fluid, but much less than is caused by the larger doses of actinomycin D. Both kidneys were quickly removed, decapsulated, blotted free of blood on filter paper, and weighed on a torsion balance. One of each pair of kidneys was used for assay of glutaminase I (*19*); the other, RNA (*30*).

Figure 8 shows the usual increases in excretion of ammonia, kidney RNA, wet weight, and glutaminase during $NH_4Cl$ acidosis of this magnitude. Actinomycin D alone, in the nonacidotic rat, had no measurable effect on any of these variables, but in the acidotic rats it prevented altogether the increase in RNA and the attendant hyper-

Table 1. Effect of actinomycin D (AD) on RNA of the remaining kidney after unilateral nephrectomy in the adult rat (six rats in each group). Differences between sham-operated controls and operated rats are significant $p < .05$; differences between operated and operated and treated with actinomycin D also are highly significant $p < .01$.

| Treatment | Renal RNA (mg/g, wet wt) |
|---|---|
| Sham operation | 6.09 ± 0.12 |
| Uninephrectomy | 6.27 ± .07 |
| Uninephrectomy + AD | 5.77 ± .12 |

trophy; it also reduced significantly the magnitude of the glutaminase change and the increased rate of excretion of ammonia during acidosis. This experiment indicates that $NH_4Cl$ does indeed stimulate synthesis of DNA-dependent RNA in the kidney, and that this process has something to do—directly or indirectly—with the change in excretion of ammonia seen during $NH_4Cl$ acidosis.

These results are partly in agreement and partly at variance with those of Goldstein (*31*). His experiments were of quite similar design but had important differences: he gave actinomycin D at 20 micrograms per 100 grams to male rats twice within 24 hours, and at the same time gave more than four times the dose of $NH_4Cl$ that we did. He found that increase of both types of renal glutaminase was blocked by this dosage of actinomycin D, but that acceleration of the rate of excretion of ammonia during acidosis was not affected. The latter difference may relate to his much higher dosage of $NH_4Cl$; although he ascribed the blockage of enzyme changes to inhibited synthesis of DNA-dependent RNA, he did not measure renal RNA during his experiments.

## Compensatory Renal Hypertrophy

One experiment (Fig. 6) made it apparent that the renal hypertrophic effects of metabolic acidosis and unilateral nephrectomy are additive. In order further to compare the two processes, we decided to study the effects of the same dose of actinomycin D (used on the acidotic rats) on the compensatory hypertrophy in the remaining kidney after unilateral nephrectomy. Adult female rats weighing 200 to 250 grams were used. Controls were sham-operated through a midline back incision under light ether anesthesia. Another group were unilaterally nephrectomized in the same manner. After their recovery from anesthesia, actinomycin-D treatment was started on six of the operated animals; the dosage and schedule of treatment were the same as for the acidotic rats. On the morning of the 3rd day the right kidneys of the sham-operated controls, and the remaining right kidneys of the uninephrectomized rats, were removed quickly after decapitation and exsanguination. After decapsulation, and weighing on a torsion balance, each whole kidney was homogenized rapidly in 5 milliliters of 0.154*M* KCl, to which was added 5 milliliters of 8-percent perchloric acid. RNA was then determined as before (*30*); the results appear in Table 1.

It is apparent that within 48 hours of unilateral nephrectomy there is already significant increase in concentration of RNA in the remaining kidney. Actinomycin D in such dosage significantly blocks this change and the attendant kidney growth, as it does in the kidneys of acidotic animals.

Thus, in this sense at least, the hypertrophic processes in the kidneys of $NH_4Cl$–acidotic rats resemble those in uninephrectomized rats; in both they seem to entail early acceleration of synthesis of DNA-dependent RNA. But the two types of growth also differ in certain respects: for instance, there is no change in glutamine extraction and no increased utilization of the carbon skeleton of glutamine in the case of unilateral nephrectomy —as there is during acidosis (see below). Furthermore, neither glutaminase nor hexose monophosphate–shunt enzymes are changed in the remaining kidney after unilateral nephrectomy; nor does this kidney show increased capacity to excrete ammonia and titratable acid. These last two observations lend further support to the idea that these two enzyme changes specifically serve aspects of the renal response to acidosis rather than the renal-growth process in general.

Table 2. The effect of $NH_4Cl$ acidosis in the rat on the specific activity of four components of the kidney 1 hour after injection of L-glutamine-$C^{14}$. Controls numbered five; rats with acidosis, four. The glycogen, prepared by the usual KOH–digestion and alcohol-precipitation method (*35*), still contained considerable amounts of protein after prolonged dialysis against water. Thus the specific activity of the glycogen fraction may reflect, at least partially, contaminating protein.

| Component | Specific activity (cpm/mg) | | | Significance ($p$) |
|---|---|---|---|---|
| | Controls | With acidosis | Change (%) | |
| Protein | 152.3 ± 11.2 | 209.2 ± 4.6 | + 37 | < .01 |
| Nucleic acids | 6.18 ± 2.0 | 33.5 ± 5.7 | + 442 | < .02 |
| Lipid | 8.92 ± 1.7 | 23.7 ± 2.8 | + 166 | < .02 |
| Glycogen | 132.4 ± 24.4 | 279.4 ± 16.3 | + 110 | < .01 |

Table 3. Effect of unilateral nephrectomy in the rat on the specific activities of three tissue components of the remaining kidney 1 hour after injection of L-glutamine-$C^{14}$. Tissues tested 4 days after nephrectomy; five rats in each group.

| Component | Specific activity (cpm/mg) | | | Significance ($p$) |
|---|---|---|---|---|
| | Controls | After nephrectomy | Change (%) | |
| Protein | 95.5 ± 18.5 | 96.6 ± 7.4 | + 1.2 | < .5 |
| Nucleic acids | 66.6 ± 13.0 | 41.2 ± 5.8 | − 38.1 | < .01 |
| Lipid | 2.86 ± 0.22 | 3.37 ± 0.37 | + 17.8 | < .5 |

## Renal Metabolism of Glutamine

Glutamine, as we have seen, is one of the major substrates extracted by the kidneys from renal arterial blood, and we usually think of its renal metabolic utilization solely in terms of meeting the requirements for production of urinary ammonia; but little or no attention has been given to the assimilation of its carbon skeleton by the kidney. One would suspect that substrate extracted by an organ at the rate of extraction of glutamine by the kidney would play some more general role in that organ's metabolism. Also its accelerated rate of extraction during acidosis, the attendant hypertrophic process, and increased gluconeogenesis from glutamine (*18*) suggest a possibly increased rate of utilization of the carbon skeleton of glutamine during acidosis.

To test this assumption, two series of experiments were performed with $C^{14}$-labeled L-glutamine (*32*). Adult female rats were made acidotic by their drinking 0.28*M* $NH_4Cl$ ad libi-

tum for 7 days; controls drank tap water. On the morning of the 8th day all rats were given, by intraperitoneal injection, either 10 or 20 microcuries of L-glutamine-$C^{14}$ (*33*). One hour later the animals were rapidly decapitated and exsanguinated. Both kidneys of each were quickly removed, decapsulated, blotted dry, and weighed on the torsion balance; determinations of the following were then made on them: wet weight, protein (*34*), nucleic acids (*30*), glycogen (*35*), and lipids (*36*). Each of these fractions was determined chemically by the method indicated; solutions of each were then dried, plated, and counted in a gas-flow counter.

Table 2 shows the results of one series of experiments of this type, which were exactly confirmed by a second series. The specific activity of $C^{14}$ is much higher in all fractions of kidney from the acidotic animals—especially in the lipid and nucleic acid fractions. These findings mean that the carbon skeleton of glutamine, as well as its amide and amine nitrogens, is utilized at a greater rate by the kidney of the acidotic animal. Thus the increased renal extraction seen during acidosis reflects metabolic changes involving glutamine that go well beyond the requirements for increased excretion of ammonia.

For comparison, Table 3 shows data from an exactly similar experiment on the incorporation of glutamine carbons into the several tissue components of the remaining kidney 4 days after unilateral nephrectomy. There is no significant difference in any of the tissue components here, with the exception of nucleic acids, in which specific activity is reduced. The finding means that this kind of hypertrophy does not involve increased utilization of glutamine for the synthesis of major tissue components; it also correlates with our observation that extraction of glutamine by this remaining kidney is not changed as it is during acidosis.

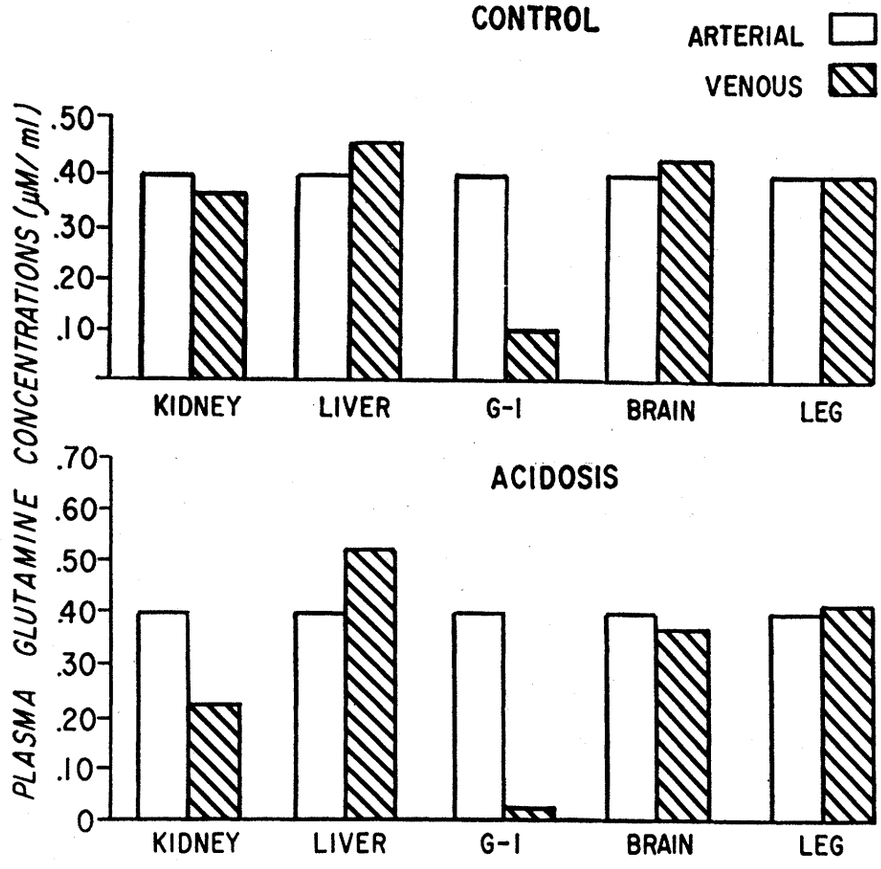



Fig. 9. Arteriovenous glutamine concentrations across several organs of the dog, as affected by acute sulfate acidosis; observations were made during the dog experiment shown in Fig. 4. The height of each open bar represents the mean of the control periods; of each striped one, the mean of observations during the acid infusion (*14*). *G-I*, gastrointestinal tract.

## Extrarenal Glutamine Metabolism

Ever since it was first noted that the kidney extracts more glutamine from the renal arterial blood during metabolic acidosis, it has also been observed that the level of glutamine in arterial blood does not fall during this state; indeed it may even rise slightly (*37*) (Fig. 4). This fact suggests that accelerated extraction of glutamine by the kidney somehow signals its increased production elsewhere in the body, the most likely site being the liver. For this reason we studied the arteriovenous differences in glutamine across a number of organs of the dog during acute metabolic acidosis (Fig. 9; *14*), and the glutamine content of the same organs in the rat during $NH_4Cl$ acidosis (Fig. 10; *14*).

Since measurements of blood flow across the dog's organs were not made during these experiments, the arteriovenous glutamine differences are qualitative indicators, rather than quantitative measurements, of glutamine extraction or production by each organ.

The arteriovenous difference in glutamine across the kidney reflects its uptake under conditions of unchanged blood flow; uptake is greatly increased during acidosis. In the liver the situation is just the reverse: In the control state, the arteriovenous difference in glutamine indicates a small net production of glutamine which becomes greater during sulfate acidosis, meaning that the rate of production of glutamine by the liver is markedly increased, even during such an acute sulfate acidosis.

The arteriovenous difference across the gastrointestinal tract derives from glutamine determinations on femoral arterial and portal venous blood, and represents organs drained by the portal vein, including the gastrointestinal tract itself as well as spleen and pancreas, but not the liver. This measurement indicates an uptake of glutamine by these viscera that is markedly increased during acidosis. This observation has not been recorded before, and is of sufficient interest to warrant further study.

The brain shows slight production of glutamine in the control, with a shift to a barely significant uptake during acidosis. Leg muscle shows no uptake during either normal or acidotic state.

The findings on tissue glutamine in the rat (Fig. 10) corroborate and strengthen the meaning of the arteriovenous glutamine studies on the dog: the results show that during acidosis the

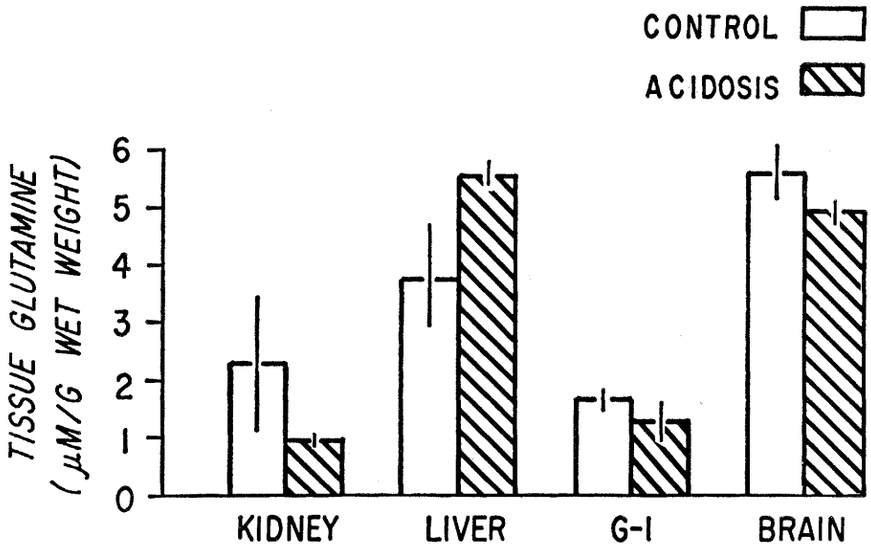



Fig. 10. Tissue glutamine content of four organs of normal and $NH_4Cl$–acidotic rat. Bars represent the means for six animals; limits depicted are the standard deviations (*14*). *G-I*, gastrointestinal tract. Average contents (micromoles per gram, wet weight) for normal and acidotic rats were, respectively: (kidney) $2.3 \pm 1.2$ and $1.0 \pm 0.1$ ($p < .001$); (liver) $3.8 \pm 0.9$ and $5.6 \pm 0.3$ ($p < .001$); (*G-I*) $1.7 \pm 0.2$ and $1.3 \pm 0.3$ ($p < .2$); (brain) $5.6 \pm 0.5$ and $4.9 \pm 0.2$ ($p < .01$).

renal level of glutamine falls as the kidney extracts more of it and assimilates it more rapidly. This observation is confirmed (*38*). Concomitantly, the hepatic level of glutamine rises during acidosis—again supporting the conclusion that its rate of hepatic production is increased.

Addae's studies of the glutamine-synthesizing enzymes of the liver have shown that they are significantly increased in the rat liver by the 5th to 7th day of $NH_4Cl$ acidosis (*14*). Thus there must be two phases to the liver's response to acidosis: The first is a rapid release of stored glutamine into the blood, or a rapid increase in its synthesis without an induced change in glutamine synthetase activity—which is probably the case in the experiment illustrated in Fig. 4. The second is a slower increase in rate of hepatic synthesis of glutamine, involving an enzyme change.

On the basis of our experiments, the nature of the feedback information that prompts the liver to greater production of glutamine in response to increased uptake of glutamine by the kidney and certain of the splanchnic viscera so far remains unknown. We have observed, as one would expect, that the portal venous concentration of ammonia is consistently elevated in the $NH_4Cl$–acidotic animal; ammonia is one of the substrates for synthesis of glutamine. Duda and Handler have shown that the nitrogen of $N^{15}$-labeled ammonium lactate, injected into the rat, is rapidly incorporated into liver glutamine before appearing in the liver's other nonprotein nitrogenous components (*39*). This finding suggests that the rate of entry of ammonia into the liver must affect the rate of hepatic synthesis of glutamine. Therefore the level of portal venous ammonia, as it varies from the normal to the acidotic state, may play an important role in setting the rate of hepatic synthesis of glutamine in response to its varying rate of utilization by the kidney and splanchnic viscera.

## Summary

The state of metabolic acidosis involves changes of a varied and subtle nature in other organs as well as in the kidney. This fact has been illustrated in metabolic studies with glutamine, a major substrate of the kidney and of various other organs.

In addition to the well-described increase in renal glutaminase enzymes, the hexose monophosphate–shunt enzymes also are much more active during metabolic acidosis; this phenomenon is limited to the kidney; its exact meaning remains speculative, but its possible relation to renal excretion of acid, lipogenesis, and gluconeogenesis during acidosis has been discussed.

In the kidney there are metabolic changes associated with ammonium chloride acidosis that affect the basic mechanisms of gene-directed growth. There is a renal regenerative process during this kind of acidosis that resembles in some respects the compensatory hypertrophy in the remaining kidney after unilateral nephrectomy, from which it also differs in important ways. Lastly, we must now regard the role of glutamine in renal metabolism as an affair that goes well beyond the specific needs of formation of ammonia during the normal and acidotic states. Glutamine enters the general metabolic mill of the kidney, its carbon skeleton is incorporated into all the major tissue components, and it is an important source material for gluconeogenesis in the kidney; all of these renal functions of glutamine are increased during metabolic acidosis.

Thus there is a fruitful field of exploration ahead, not only in the biochemical aspects of the renal response, but also in the metabolic interrelations among different organs during various types of acid-base change. This article, in concentrating on metabolic acidosis, gives only a glimpse of a broader picture to come.

### References and Notes

1. R. F. Pitts, *Federation Proc.* **7**, 418 (1948).
2. J. Orloff and R. W. Berliner, *J. Clin. Invest.* **35**, 223 (1956).
3. D. D. Van Slyke, R. A. Phillips, P. B. Hamilton, R. M. Archibald, P. H. Futcher, P. H. Hiller, *J Biol. Chem.* **150**, 481 (1943).
4. R. F. Pitts, L. A. Pilkington, J. C. M. deHaas, *J. Clin. Invest.* **44**, 731 (1965).
5. A. Meister, *Physiol. Rev.* **36**, 103 (1956).
6. B. M. A. Davies and J. Yudkin, *Biochem. J.* **52**, 407 (1952).
7. F. C. Rector, Jr., D. W. Seldin, J. H. Copenhaver, *J. Clin. Invest.* **34**, 20 (1955).
8. L. Goldstein and E. H. Dearborn, *Proc. Soc. Exp. Biol. Med.* **93**, 284 (1956).
9. L. Goldstein and C. J. Kensler, *J. Biol. Chem.* **235**, 1086 (1960).
10. F. C. Rector, Jr., and J. Orloff, *J. Clin. Invest.* **38**, 366 (1959).
11. E. E. Owen and R. R. Robinson, *ibid.* **42**, 263 (1963).
12. C. Bignall and W. D. Lotspeich, unpublished.
13. J. Bergstrom, H. Bucht, B. Josephson, *Scand. J. Clin. Lab. Invest.* **11**, 71 (1959).
14. S. K. Addae and W. D. Lotspeich, *Federation Proc.* **25**, 203 (1966).
15. A. Meister, S. P. Colowick, N. O. Kaplan, in *Methods in Enzymology* (Academic Press, New York, 1957), vol. 2, p. 380.
16. A. L. Chaney and E. P. Marbach, *Clin. Chem.* **8**, 130 (1962).
17. F. Dies and W. D. Lotspeich, *Amer. J. Physiol.*, in press.
18. R. E. Fuiz, D. Goodman, D. E. Kamm, G. F. Cahill, A. Marble, *J. Clin. Invest.* **44**, 1049 (1965).
19. W. D. Lotspeich, *Amer. J. Physiol.* **208**, 1135 (1965).
20. P. Karlson, *Perspectives Biol. Med.* **6**, 203 (1963).
21. G. C. Mueller, J. Gorski, Y. Aizawa, *Proc. Nat. Acad. Sci. U.S.* **47**, 164 (19[illegible]
22. T. H. Hamilton, *ibid.* **49**, 373 (1963).
G. Porter, A. R. Bogoroch, I. S. Edelman, *ibid.* **52**, 1326 (1964).
23. G. P. Talwar, S. L. Gupta, F. Gros, *Biochem. J.* **91**, 565 (1964); A. Korner, *Biochem. Biophys. Res. Commun.* **13**, 368 (1963).
S. Liao and H. G. Williams-Ashman, *Proc. Nat. Acad. Sci. U.S.* **48**, 1956 (1962).

25. R. V. Farese and W. I. Reddy, *Endocrinology* **73**, 294 (1963).
26. E. Reich, R. M. Franklin, A. J. Shatkin, E. L. Tatum, *Science* **134**, 556 (1961).
27. M. Revel, H. H. Hiatt, J. P. Revel, *ibid.* **146**, 1311 (1964).
28. O. Elebute and W. D. Lotspeich, unpublished.
29. E. J. Conway, *Microdiffusion Analysis and Volumetric Error* (Crosby Lockwood, London, 1950).
30. D. Glick, Ed., *Methods of Biochemical Analysis* (Interscience, New York, 1954), vol. 1, p. 290.
31. L. Goldstein, *Nature* **205**, 1330 (1965).
32. F. Dies and W. D. Lotspeich, unpublished. The experiment I reported was repeated and the results were confirmed by B. Guyer.
33. Obtained from Schwarz BioResearch, Inc., as L-glutamine $NH_2C^{14}$.
34. A. G. Garnall, C. J. Bardawill, M. M. David, *J. Biol. Chem.* **177**, 751 (1949).
35. W. Z. Hassid *et al.*, in *Methods in Enzymology* (Academic Press, New York, 1957), vol. 3, p. 34.
36. Lipids were determined as follows: the perchloric acid precipitate of the homogenized kidney was successively extracted with acetone, chloroform and ethanol at 1 : 1, and finally ether. The combined extracts were then partially dried and extracted five times with ether and chloroform at 6 : 1. These combined washes were dried and weighed in tared flasks as the lipid fraction.
37. R. F. Pitts, *Amer. J. Med.* **36**, 720 (1964).
38. R. W. Shalhoub *et al.*, *Amer J. Physiol.* **204**, 181 (1963).
39. G. D. Duda and P. Handler, *J. Biol. Chem.* **232**, 303 (1958).
40. G. E. Glock and P. McLean, *Biochem. J.* **55**, 400 (1953).
41. Supported by grants from NIH (AM03601), the American Heart Association, the Life Insurance Medical Research Fund, and the Ernest L. Woodward Fund. Some work was done with Federico Dies, S. K. Addae, and Oyinade Elebute. I thank Charlene Bignall and R. M. Dies for technical assistance; Bernard Guyer and John Hamburger who worked as summer students; and N. G. Brink, Merck, Sharp and Dohme Co., for supplying actinomycin D.

# Biographical Predictors of Scientific Performance

## Criteria of scientific performance and creativity can be predicted from biographical information.

Calvin W. Taylor and Robert L. Ellison

This paper will present a summary of research on the use of biographical information to predict various criterion measures of successful performance and accomplishments in science (*1*). In our studies of the relationship of biographical information to success in science, over 2,000 scientists have filled out one of our 300-item multiple choice questionnaires. The majority of this work has been conducted in conjunction with (*2*) the National Aeronautics and Space Administration (NASA).

The term "biographical information" is open to some possible misinterpretation when applied to the measuring instrument, the Biographical Inventory, (hereafter called the BI), which has been used in these studies. The BI contains a wide variety of questions about childhood activities, experiences, sources of derived satisfactions and dissatisfactions, descriptions of the subject's parents, academic experiences, attitudes and interests, value preferences, and self-descriptions and evaluations. The items thus encompass a wide variety of information and are not limited to a narrow definition of what could be included within the rubric of biographical information. By using such a broad approach, one potentially can attempt to measure not only previous life history experiences and past environmental effects on a person, but also to assess the outcome or manifestation of the hereditary environment combination as it is personified in the individuals studied.

The intent in these studies was to exploit the biographical approach and thus determine and more fully understand the experiences, backgrounds, opinions, self-images, and attitudes which would aid in differentiating the highly productive and creative scientists from those who were less productive and creative. When the biographical characteristics, experiences, and self-descriptions were identified, the practical goal was to utilize these characteristics in developing an easily administered and scored biographical inventory which would aid in the identification of scientific talent at the college level. Hopefully, the inventory could be rewritten for the early high school level and used as a vocational guidance instrument, so that high school students who had scientific potential could be encouraged to further their development.

When this study was initiated in 1959, biographical information was considered to be one of the most promising means of identifying creative scientific talent. Previous research from a variety of investigators had indicated that biographical information was a potentially promising technique for the identification of creative scientific talent, although no one had made a definitive attempt to exploit this potential (*3, 4*). The approach had, however, demonstrated its usefulness in a variety of other settings for predictive purposes; for example, identifying successful salesmen, predicting college success, identifying leadership ability in the Army after World War II, and others.

Two studies were especially useful in laying the foundation for the later use of the biographical approach in the studies of NASA scientists. These were by Ellison (*5*), who tried out a large number of biographical items, and by Taylor, Smith, Ghiselin, and Ellison (*6*), who conducted an intensive criterion study and later administered a series of predictors including a biographical inventory. In both studies the initial validities found between the empirically keyed biographical scores and the corresponding criterion were extremely high, (.70 to .94). No cross validation was attempted in either of these two preliminary studies because of the relatively small sample size but the best items from both studies were identified and retained for future use in the NASA project. However, a priori scoring keys for the biographical responses worked very well on

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