INCREASED RATE OF UREA FORMATION FOLLOWING REMOVAL OF RENAL TISSUE

EDWARD C. PERSIKE AND T. ADDIS

From the Department of Medicine, Stanford University School of Medicine

SAN FRANCISCO, CALIFORNIA

The experiments presented here show that the rate of urea formation is accelerated in rats during the first 24 hours after removal of three quarters or all of the total renal tissue, and that the greater the amount of kidney excised the greater is the quantity of urea formed. The observations were made on rats in which urea formation from food protein was excluded by subsistence on a calorically adequate diet containing no protein. Urea formation was calculated for consecutive 4-hour intervals, the amount of urea formed being derived from changes in the body urea content and from the quantity of urea excreted in the urine.

METHODS

Three hundred and ninety-two healthy female albino rats, selected at body weights of about 150 gm., were divided into 40 groups of approximately 10 rats each. The average body weight was 150.8 gm. with a standard deviation of 3.6 gm. Before the experiments, all rats were maintained on an adequate stock diet containing 17 per cent protein. After selection, the rats within each group were placed in individual cages and were fed a solution of 15 per cent glucose in water containing 0.4 per cent sodium chloride and vitamins of the B complex. After 48 hours of glucose feeding, 2 groups of unoperated controls were killed, 8 control groups were submitted to a sham operation in which both kidneys were exposed and handled, in 21 groups 75 per cent of the total renal tissue was removed, and in 9 groups a complete nephrectomy was performed. A small amount (less than 1 ml.) of intrabdominal bleeding occurs from the stump of the remaining kidney when 75 per cent of the total renal tissue is excised. Therefore, one kidney from each rat subjected to total nephrectomy was first incised and allowed to bleed briefly into the abdominal cavity before removal, in order to make the different operative procedures alike except for the amount of renal substance removed. Following operation, the rats were returned to individual cages, and the glucose feeding was continued until autopsy.

Two groups of sham-operated controls were killed at each of the following intervals after operation, at 4, 7, 16 and 24 hours. Three groups of animals from
which 75 per cent of the total renal tissue had been removed were killed at the end of the 1st, 4th, 7th, 12th, 16th, 20th and 24th hour after operation. Three groups of rats from which all kidney substance had been removed were killed 4, 16 and 24 hours after operation. The rats were killed under ether anesthesia by exsanguination. Aliquot portions of serum were joined to form one serum pool for each group. Urine collections were made immediately before each group was killed, and the individual collections were combined to make one urine pool for each group.

Urea was determined by the urease aeration method of Addis (1), all measurements being made in duplicate. The amount of urea formed during each period was estimated by adding to any urea excreted in the urine during that time the difference between the average urea content of the body found at autopsy and the average urea content of similarly treated groups that had been killed at the end of the preceding period. The urea content of the body was obtained by multiplying the mg. of urea per ml. of serum by the number of ml. of water in the body as calculated from the live body weight, assuming for this purpose that the total body water in a rat is 63 per cent of its body weight. Here we depend on Pace and Rathbun (2) who used the data of Ashworth and Cowgill (3).

Before operation, all the rats were approaching a minimum rate of urea formation. In the groups killed before operation, we found an average serum urea concentration of 9.0 mg/100 ml. Their body weight was 150 gm., and their total water content was estimated as 63 per cent of 150 or 94.5 ml. Their total urea content 48 hours after consuming nothing but glucose was thus $\frac{9.0 \text{ mg}}{100 \text{ ml.}} \times 94.5 \text{ ml.}$ or 8.5 mg. of urea. We say they were only approaching a minimum because we have found that when this diet is continued for more than 48 hours somewhat lower serum urea concentrations and lower total body urea contents may be obtained (4). We may assume, therefore, that during the next 24 hours, the period during which our observations were made, urea formation would ordinarily be very slowly falling. Here, then, is a minor error in our calculations, one which has the effect of under-estimating the increases in urea formation we found after operation.

It should be noted that the total urea content of each group was estimated at one time only, namely when they were killed. It may seem that we might have obtained more precise results if we had measured the serum urea concentration of each group at the beginning as well as at the end of each period. This, however, would have required tail cutting or cardiac puncture. Our experience has been that either of these procedures may at times, and in certain animals, induce wide deviations from the average behavior of undisturbed controls. Lippman has shown that this is true with respect to renal function in the rat (5). We have no data on the metabolic effects, but the effect of large hemorrhage on the rate of urea formation of nephrectomized rats has been studied by Engel and Engel (6).

The group method we have used requires the use of a large number of rats, nearly 400 in this instance. We have adopted it as preferable to working with individuals, not only because we were thus enabled to avoid bleeding, but also because we believe that when as many as 40 comparable groups are used, we diminish the effect of individual variation. The group of 10 then becomes the individual,
and unexplained fluctuations in behavior are decreased. This, however, is not a random statistical group, but one in which each member of the group is known to have conformed with a series of particular requirements. During the collection of urine, the rat’s behavior and appearance had to be not unusual. The urine volume had to be not very much less than that obtained from the others. Finally, at the post-mortem examination made immediately after the urine collection had been completed, it was seen that no urine was left in the bladder, that there were no signs of circulatory failure, no gross organ anomalies, and that the centrifuged blood gave a clot volume neither unusually large or small. When any of these abnormalities were observed the urine and serum of that rat were discarded. Very few of our animals were rejected for any of the above reasons, but that was only because we had the good fortune to work with highly standardized healthy young rats. This is, indeed, a prerequisite for the safe use of such a group method because it is obvious that averages of only 10 concentrations and rates might be misleading if they included even one measurement greatly influenced by some unsuspected factor of an individual nature.

An essential element in the reliability of the results is the degree of precision
achieved in the collection of short time urine collections. The method we used has been described in detail elsewhere (7).

RESULTS

The serum urea concentrations and the rates of urea excretion for successive 4 hourly periods after operation are shown in figure 1.

In the controls the serum urea concentrations rise a little and the rates of urea excretion increase for the first 2 periods but thereafter both concentrations and rates fall to or below the preoperative levels.

![Graph showing average rates of urea formation](http://ajplegacy.physiology.org/)

Fig. 2. Average rates of urea formation (urine urea excretion per 4-hour period plus the difference between the body urea content at the beginning and at the end of the period, corrected to 130 gm. body weight).

In the groups from which 75 per cent of the renal tissue was removed the serum urea concentration is almost doubled within an hour after operation and then rises in a straight line for 16 hours and is thereafter maintained at a level four times higher than that of the controls. For the first 2 hours after operation there was complete anuria, and for the second 2 hours the rate of urea excretion was low, but from that time on urea excretion rapidly increased, equalling the control rate by the 7th hour, exceeding it by 100 per cent by the 14th hour and continuing at nearly double the control rate until the 24th postoperative hour.

The completely nephrectomized groups show a steeply rising serum urea concentration that increases steadily until, at the 24th postoperative hour, it is 17 times higher than the concentration of the groups from which 75 per cent of the kidney had been removed.
The amount of urea formed during each postoperative 4-hour period was calculated from the data given in figure 1, by adding to any urea excreted in the urine during that time the difference between the urea content of the body at the beginning and end of the period. The results of these calculations are given in figure 2.

During each successive period the rate of urea formation was greater in the totally nephrectomized groups than in those from which 75 per cent of the renal tissue had been excised, and in turn this latter rate was considerably greater than that of the controls. If we add the total urea formed over the 24 hours of observation we find that the nephrectomized groups formed 120 mg. of urea per rat, the 75 per cent groups 88 mg. per rat and the sham-operated controls 40 mg. per rat.

DISCUSSION

The significance of the urea formation figures we have given depends on the validity of the assumptions made in their calculation. It is conceivable that under the particular conditions we observed these assumptions might involve errors so large that they would invalidate our conclusions, not only quantitatively but even qualitatively. For this reason, but mainly because the measurement of the rate of urea formation may prove to be a useful tool in the field of protein metabolism, a discussion of the basis for the method should precede any conclusions.

We are indebted to Engel and Engel (6) for the first clear expression of the idea that we can derive the urea content of the whole body by multiplying the urea concentrations of the serum by an estimate of its total water content. This is possible because urea diffuses into every cell and every fluid of the body, distributing itself through the water of every tissue in equal concentration, a fact first demonstrated by Marshall and Davis in 1914 (8). It follows that if we determine the concentration of urea in 1 ml. of serum and multiply it by the number of ml. of water in the body we can approximate the urea content of the body at that moment. Engel and Engel also saw that if these measurements are repeated after an interval of time we can find to what degree the urea content of the body has increased or decreased. During this interval, urea has been leaving the body through the kidneys, the only place from which urea is excreted at a concentration higher than that in which it exists in the body water. Therefore, if the rate of urea excretion is determined we can get the amount of urea that is formed by adding to the excretion rate the increase or subtracting from it the decrease in the urea content of the body that has occurred during the time interval over which the rate is measured.

Admittedly the calculation can give us no more than a first approximation because the water content of the body is a quantity so hard to define. There is water that for many metabolic purposes is outside of the body, which is within it as far as urea is concerned. Urea diffuses into the large and variable volume of water that comes and goes between the body and the interior of the gastro-intestinal tract. Not all of the urea that enters the gut returns as such, for part or

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2 The degree to which urea concentrations may deviate from an exact equality of concentration in the water of each part of the body is not yet decided. See, for instance, the careful work of Rails (9).
all of it may be decomposed to ammonium carbonate by urease containing bacteria in the colon. This, of course, is no final loss because the liver will form urea again when the ammonia comes to it in the portal blood. However, the facility with which urea diffuses into the alimentary canal makes it necessary to take the water in the alimentary canal into account and this is done when we base our estimate of total body water on live body weight.

In our particular experiments there is a special criticism arising from the fact that we did not measure the body water but used an average calculated estimate. For preliminary purposes of relative orientation this may be permissible when the conditions are such that we need not suspect rapid changes in total body water. But in our experiments we had reason to think that the water content of the completely nephrectomized rats had increased. The tissues at the end of the 24-hour period seemed to be wetter than usual and there was some free abdominal fluid not found in the other groups. From an absolute point of view we therefore cannot place any reliance on the precision of the 120 mg. per rat urea formation we found over the 24 hours in the totally nephrectomized groups. But it is important to notice that the error is such that it would lead to an underestimate of the actual amount and that, a fortiori, the qualitative conclusion that there is an increase in urea formation in nephrectomized rats still stands. A constant error is, of course, incurred by treating serum as if it were water.

But to us the most important observation that can be made about the calculation of urea formation is that we can only hope for unambiguous results when the measurements are made under the conditions we used with respect to the removal of food protein from the gastro-intestinal tract. The production and absorption of amino acids in the gut is so variable and, under ordinary circumstances, so large, that unless this factor is eliminated it is difficult to assign any precise meaning to urea formation results. Only when we can be sure that none of the urea that is formed comes from precursors external to the body can we take the figures as an index of the rate of body protein catabolism. That, even then, it is a complete measure of body protein catabolism is still open to question, but that it measures a preponderating part of the total catabolism is at least a reasonable supposition. This, however, is no more than an addendum to the theoretical considerations presented by Ogden and Tripp (10).

If we accept our results as indicating, not quantitatively but qualitatively, that the more renal tissue we remove the greater is the rate of urea formation, we have to ask what reason there may be for this, to us, surprising finding. It is not an isolated observation, for several years ago it was shown in this laboratory that the rate of urea excretion was considerably increased in quarter kidney rats fed only glucose, an increase that was accompanied by an increase in serum urea concentration, so that there must have been an increase in urea formation (11). We cite these experiments because they extended over a period of 7 days. It would seem then that what we observe in the present experiments within a space of 24 hours, is more than a transient postoperative phenomenon. Yet it is contrary to the conclusions of Reid (12) who finds a decrease in urea formation in nephrec-
tomized rats which he attributes to the cessation of deamination in the kidney. Further, Mylon, Smith and Goldstein (13) show a decrease in urea formation in dogs with nitrogen retention induced by reduction in the quantity of renal tissue when amino acids are given. It may be that the increase in urea formation we find occurs only when the rate of protein metabolism has been reduced to a minimum before operation by giving no protein and a calorically adequate amount of glucose. In the present experiments we were not able to get precise measurements of the amounts of glucose consumed. We suspect that the variations in the formation of urea shown in figure 2 during the successive periods, particularly evident in the controls, may have arisen because many of the collections were made during the day when rats go to sleep and drink less than in the night. Even transient caloric inadequacy may raise the level of urea formation when protein metabolism is very low. For in experiments we shall report later we observed an increase within four hours after no food was given and this was more marked where water as well as glucose was withheld.

The first explanation of our findings that comes to mind is that the 3 degrees of urea formation are a consequence of three degrees of trauma in the operations. To us this seems inapplicable because in all cases a double laparotomy was performed, in all cases the kidneys were handled and in no case did any operation take more than a few minutes. It is true that of the 3, the 75 per cent removal was presumably the most traumatic, since half of the remaining kidney was excised. Yet in the simpler operation of double nephrectomy we find a greater increase in urea formation.

When Bondy and Engel (14) nephrectomized rats they found that the rate of urea formation rose until they died, but when the adrenals as well as the kidneys were removed the level of urea formation did not change. The simplest explanation of our results would be to suppose that some substance that accelerated protein metabolism was retained in the body as a consequence of the removal of renal tissue. That cannot be the only factor because the increase in urea formation occurs during the first four hours after operation, and has no relation to the degree of retention of urea. But it is still possible to view the total increase as the combination of an initial increase due to trauma, plus a later increase due to uremia. We adopt this as a provisional working hypothesis.

**SUMMARY**

On a diet of 15 per cent glucose in 0.4 per cent sodium chloride when urea excretion is approaching a minimum, the rate of urea formation in rats is increased during each 4-hour period of the first 24 hours after removal of 75 per cent of the total renal tissue, the increase being measured relatively to similarly traumatized control rats whose kidneys were left intact. Under the same conditions, a greater increase in urea formation follows the complete removal of both kidneys. During the first 24 hours after operation, controls with both kidneys intact form 40 mg. of urea per rat per 150 gm. body weight, rats left with a quarter of their renal tissue form 88 mg. and rats with no renal tissue produce 120 mg. of urea.
REFERENCES