A STUDY OF ACUTE RENAL INSUFFICIENCY[1]

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In the work reported in this paper we were concerned with factors which affect the survival time of totally nephrectomized rats. The purpose of this is to obtain knowledge about the causes of death in acute anuria and to get information which would be of value in the treatment of acute anuria. Such information can be applied in the treatment of chronic renal insufficiency and will also to some extent aid in the understanding of the mechanisms of the disturbances in this condition. One need only to refer to the recent review of Harrison and Mason (1) to see what a complicated condition chronic uremia may be. Acute anuria is undoubtedly much simpler and one is justified in considering each separately.

The method of investigation was simple. We nephrectomized groups of rats and determined the effects of different experimental conditions and agents on the survival time of the animals. We have attempted to vary only one factor at a time by having a group of control animals treated exactly as the experimental group except for the one variable that was being investigated. The variables studied were age, sex, food and the administration of certain substances which were suggested by the results of the feeding experiments.

Control groups and effect of sex and age

Our first concern was to determine the best experimental conditions, the variability in controls, and the effect of ordinary physiological variants such as age, sex, and nutritional state. For age and sex 4 groups were taken: Group 1, 96-day-old females; Group 2, 99-day-old males; Group 3, females over 200 days old; Group 4, males over 200 days old. All rats were fasted 24 hours before operation but were allowed water, of which they drank very little. No food or water was given after nephrectomy. Prior to this they had been on a stock diet. This contained yellow corn meal, whole wheat flour, dried milk and the necessary salts and vitamins. The average weights of the groups were as follows: Group 1, (20 rats), 84 grams; Group 2, (21 rats), 89 grams; Group 3, (20 rats), 161 grams; Group 4, (16 rats), 239 grams. The survival times are plotted in Figure 1. Groups 1 and 2 survived an average of 40 and 44 hours compared to 75 and 82 hours for Groups 3 and 4. It is quite apparent that young rats cannot survive total nephrectomy as well as adult rats. Sex has little effect on survival time, females living slightly shorter periods than males.

Preoperative fasting period

The effect of preoperative fast was determined in 3 groups of adult males. Group 4 (reported above) was fasted 24 hours, Group 5 was fasted 48 hours and Group 6 received the stock diet up to the time of operation. Neither food nor water was given after operation. Group 5, (18 rats), had an average weight of 260 grams and Group 6, (20 rats), 252 grams. The survival times are plotted in Figure 1. The average survival times for 0, 24- and 48-hours fast are 79, 82, and 94 hours respectively. Group 7 illustrates the effect of water administration after nephrectomy. Five cc. distilled water was given by stomach tube to these rats every 24 hours. There is a definite shift of the entire group towards shorter survival as compared to Group 4 and the averages of the 2 groups are 67 hours and 81 hours. Considering the closeness of the grouping in the 2 groups one is justified in concluding that water in this amount (about half the normal intake of rats) is harmful here. This group serves as our control for all experimental groups which were given solutions postoperative. The volume of the solutions given in these cases was about the same as that of the water in this group.

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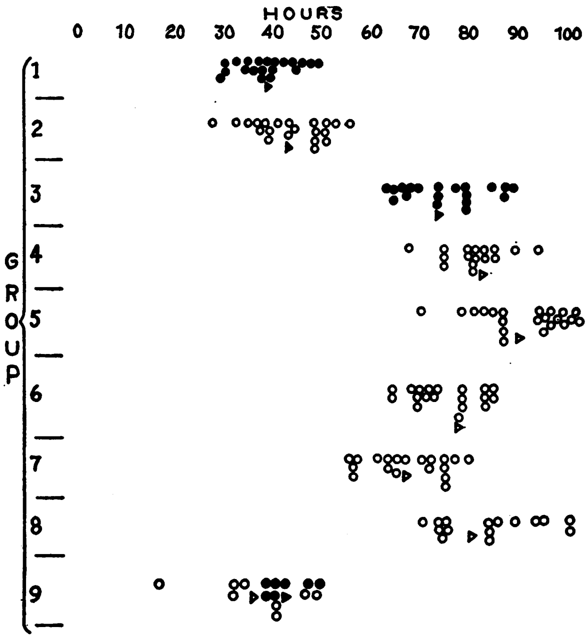

FIG. 1. SURVIVAL TIME IN HOURS OF DIFFERENT GROUPS OF THE NEPHROTOMIZED RATS

Each black circle represents one female rat, each white circle one male rat. Triangles represent averages.

Group 1. 96-day old females—fasted 24 hours before operation.
Group 2. 99-day old males—fasted 24 hours before operation.
Group 3. Adult females—fasted 24 hours before operation.
Group 4. Adult males—fasted 24 hours before operation.
Group 5. Adult males—fasted 48 hours before operation.
Group 6. Adult males—not fasted preoperative.
Group 7. Adult males—fasted 24 hours preoperative; 5 cc. distilled water every 24 hours by stomach tube postoperative.
Group 8. Adult males—fasted 24 hours preoperative; 5 cc. 20 per cent glucose by stomach tube postoperative.
Group 9. Adult males and females—fasted 24 hours, then fed meat for 1 hour (5 grams average eaten) before operation.

Group 8 illustrates the action of glucose feeding after nephrectomy. Five cc. 20 per cent glucose was given by stomach tube to these rats every 24 hours. The grouping and average survival time here are practically the same as the control Group 4. But since these rats received almost as much water as those of Group 7 we have probably a combined action here of the harmful water effect and a beneficial glucose effect so that they counteract each other. One is justified in concluding that glucose itself (not as a solution) is beneficial.

All these groups illustrate the low degree of variability of survival time within the prescribed conditions. It is evident that one can obtain survival times with remarkably little variation if one defines the physiological condition of the group very closely. This shows that the standard nephrectomized rat (done in groups of 10 to 20) can be a very useful preparation for testing factors that may bear on kidney insufficiency. Our control results show that age has a very definite relationship to survival time but that sex and the length of the preoperative fast have only a slight effect. Postoperative water administration has a slight but definite harmful action and postoperative glucose feeding has a definite small beneficial effect.

Experimental groups

In our experimental groups we used adult rats throughout. We first studied the effect of meat feeding prior to operation. Fifteen rats of both sexes were fasted 24 hours and were then given boiled rabbit meat of which they ate, on an average, 5 grams each. The average survival time was 39 hours (limits 17 to 49 hours) which is in marked contrast to controls fed the stock diet (Group 6) or fasted 24 hours preoperative (Group 5). Meat obviously contains a principal toxic to nephrectomized rats.

Our next step was to determine whether toxic agents are present in the urine of normal animals and, if so, in what amount. There is, of course, the possibility that the kidney may detoxify substances as the liver does. To test this we attempted to administer to nephrectomized rats the amount of urine normally excreted per unit of time by a normal rat under definite conditions. We collected the urine from a group of 10 adult rats for 3 days. The total volume was then divided by the number of days times the number of rats times the average weight of the rats in centigrams. This gave a figure expressing the volume of urine per day per 100 grams adult rat, which we may refer to as one urine unit. This urine was then given to the experimental rats periodically in an amount obtained by multiplying the weight in centigrams by the urine unit.

One urine unit per centigram body weight was given to the experimental rats periodically by stomach tube or intraperitoneally. Three groups were studied. The first group (called A) received urine from fasted rats, the second (B) from fasted rats allowed to drink 10 per cent sucrose ad lib., and the third (C) from rats allowed to eat meat ad lib. The experiment is summarized in Table I. There was no significant

TABLE I

Effect of administration of normal rat urine on survival time of nephrectomized rats

Group	Received urine from rats	Number of doses received	Time between adminis-trations	Average survival time
			hours	*hours*
A.	Fasted	2	30	49
B.	On sugar diet	2	20	52
C.	On meat diet	2	10	12

difference between results with the stomach and intraperitoneal modes of administration. The urine of meat-fed rats obviously contains highly toxic material. The other urines are much less toxic. The ammonia content of the urine of the meat-fed animals was found to be much higher than that of the other groups. This compound has to be considered separately from other urine constituents since the ammonia found in the urine does not necessarily come from ammonia circulating in the blood and tissues, but is formed to a large extent by the kidney (probably from urea) and is also formed in the urine after it leaves the kidney as a result of bacterial action (To minimize this, we added to the collecting tubes a little chloroform which was evaporated off before the urine was administered). When an amount of ammonia (given as NH_4Cl solution) equal to the amount present in the urine given to (C) was given to other nephrectomized rats it lowered the survival time to 39 hours (average of 8 animals). We repeated the experiment with the "meat" urine, treating it first with permutit to remove ammonia. Six nephrectomized rats were given 2 doses (as defined above) of this 20 hours apart. Average survival time was 34 hours. The same procedure was carried out with the urine of rats fed casein, and this urine was given to 10 nephrectomized rats in 2 doses 20 hours apart. The average survival period was also 34 hours as compared with the control period of 67 hours (Group 7). The ammonia content of these permutit-treated urines was very low. However we gave the same amount of ammonia (as NH_4Cl) to 7 nephrectomized rats and obtained an average survival period of 79 hours. The urine of meat- and casein-fed rats is definitely toxic and this toxicity is in no way due to ammonia.

Our next attempts were to fractionate the urine of meat-fed rats in order to track down the source of this toxicity. We attempted a rough separation between organic and inorganic constituents by evaporating the urine to dryness *in vacuo* and extracting the residue with isobutyl alcohol. This reagent will take up many of the organic constituents and leave most of the inorganic salts. The residue, after this extraction, was taken up in water, treated with permutit, and given by stomach tube to nephrectomized rats in 2 doses (on the urine unit basis described above) 20 hours apart. The group (8 rats) had an average survival time of 29 hours. The isobutyl alcohol-soluble fraction (after evaporating off the alcohol and taking up in water) was given to 10 nephrectomized rats (on the same unit basis) in 2 doses 20 hours apart. They had an average survival time of 68 hours. These results suggest that the chief toxic factor is inorganic.

To test out the toxicity of the inorganic part of the urine, we evaporated the urine of meat-fed rats to dryness and ashed the residue. This was taken up in water and given by stomach tube to 10 nephrectomized rats in 2 doses 20 hours apart. Each dose represented the ash from the urine excreted in one day by a normal meat-fed rat of the same weight as the exeprimental animal. The average survival time was 29 hours.

This ash was analyzed and each "unit" dose was found to contain 17 mgm. potassium and 7 mgm. sodium. Two doses, 20 hours apart, containing these amounts of these elements (given in solution as chlorides) were given to 7 nephrectomized rats by stomach tube. The average survival time was 26 hours. This experiment was repeated without the sodium on 12 rats that had an average survival time of 24 hours. It is apparent that sodium in this quantity is without action and that the harmful agent in the ash of the urine of meat-fed rats is potassium. The toxicity of potassium seems to be roughly proportional to the amount given. Potassium is not markedly toxic when given in smaller amounts. When only one dose of the above solution (17 mgm. potassium per 100 grams of rat) was given to 5 rats, the average survival time was 57 hours, and when 6 mgm. per 100 grams of rat were given the average survival time (8 animals) was 69 hours.

It is apparent that potassium is toxic to nephrectomized animals and undoubtedly a definite part of the toxicity of meat in this condition is due to this element. But the entire toxicity cannot be ascribed to it. Examination of the results will show this. As stated previously, under effect of feeding, the group of animals fed meat immediately before nephrectomy, ate an average of 5 grams and the average survival time was 39 hours. The average potassium intake per rat with this meat would be 15 mgm. and the average per 100 grams of rat would be 10.2 mgm. The effect of intake of such an amount of potassium would be expected to give a survival time between that of the 2 groups mentioned above (6 mgm.—69 hours and 17 mgm.—57 hours) or something like 63 hours as compared to the actual survival of 39 hours. On the other hand, examination of our results with regard to the toxic factor in the permutit-treated urine of meat-fed animals reveals that potassium is the main source of the toxic activity there. These two statements are not necessarily irreconcilable. All the toxic agents of meat may not be present in the permutit-treated urine of meat-fed rats. The kidneys may dispose of some of the toxic products of meat in other ways than just by excretion of them. Then again ammonia may play an important rôle in this toxicity, and finally it is possible that the permutit removed other toxic materials along with the ammonia.

DISCUSSION

Our purpose in this paper is not to solve with any simple formula the riddle of uremia. A reading of the reviews of Harrison and Mason (1) and of Fishberg (2) will convince one that the problem is very complicated and will probably need years of careful systematic research to unravel completely. Primarily we wish to present here a method and to show its fitness for the investigation of certain phases of this problem and its use in obtaining information of value in the therapy of renal insufficiency. The low variability in the survival time of the standard nephrectomized rat under any well-defined set of conditions makes it easy to determine whether any given factor or procedure is harmful or beneficial in renal insufficiency. One has the advantage of simplicity in dealing with acute insufficiency rather than chronic insufficiency since the chronic state is definitely more complicated than that following nephrectomy. This method should be of value too in studying those mechanisms present in both acute and chronic uremia.

Our results with the urine-fed animals indicate that the detoxifying function of the kidney is carried out to a considerable extent by excretion of the toxic products in the urine. That certain of the ordinary products of normal metabolism are toxic and are eliminated as such in the urine follows from our observation that the urine of fasted animals shortens the life of the experimental animals. It is not quantitatively so, however, since, when we gave the urine of normal fasting rats excreted during 48 hours to the nephrectomized rats, it did not shorten their lives 48 hours but 17 hours (these animals received about the same amount of water with the urine as did the control group, *i.e.*, their average survival times were 50 hours as compared to the control group's 67 hours). We cannot, then, think of the toxic products of normal metabolism entirely as simple poisons that are produced at a certain rate and excreted in the urine as such; nor can we think that if they are dammed back when the kidney is not excreting them they will kill the animal when their concentrations reach a critical value. The mechanism is not as simple as that and we intend continuing our investigation of this phase of the problem.

Our results indicate that potassium is toxic to nephrectomized rats; that meat feeding is also harmful, and that part of this action of meat is attributable to the potassium it contains. The urine of meat-fed rats is highly toxic and the chief poison in it (after permutit treatment) is potassium. Our meat-fed rats from which we were collecting urine ate per day an average of twice as much meat as did the meat-fed nephrectomized rats during one hour before operation. If these latter animals had eaten as much meat as the former, undoubtedly their life would have been much shorter due to the combined action of the large amount of potassium and of the other toxins.

SUMMARY

The low variability of the survival time of the standard nephrectomized rat makes it an excellent test object for the study of the toxic mechanisms in renal insufficiency and for the study of the efficacy of different therapeutic measures in this condition.

The conditions for the test animals must be very closely controlled as certain physiological factors affect this survival time. Immature rats have definitely shorter survival times than the adult. Fasting brings about a slight prolongation of life. Water (in an amount approximating the ordinary intake of the rat) is deleterious when given after nephrectomy. Glucose is beneficial.

Meat (fed immediately before operation) is definitely toxic.

The urine of fasted rats contains toxic materials but the amount of these poisons is not enough to account quantitatively for the entire toxic condition which supervenes after nephrectomy.

Potassium is harmful and is responsible for part of the toxicity of meat.

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