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The treatment of acute renal failure: a reevaluation of the role of testosterone and digitalis

Raymond William Turner
Yale University

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THE TREATMENT OF ACUTE RENAL FAILURE:
A REEVALUATION OF THE ROLE OF
TESTOSTERONE AND DIGITALIS

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Raymond William Turner

1958

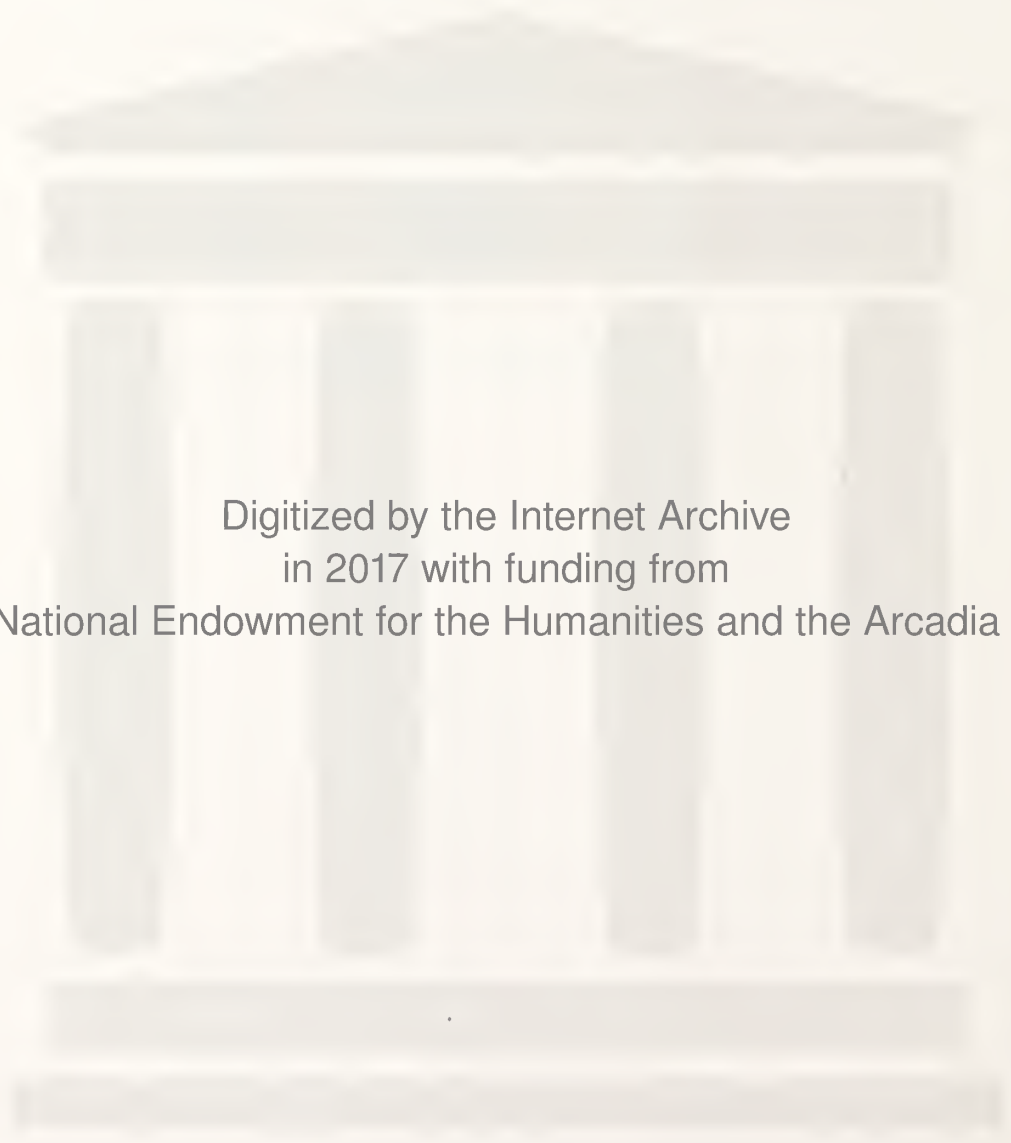
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THE TREATMENT OF ACUTE RENAL FAILURE:  
A REEVALUATION OF THE ROLE OF TESTOSTERONE AND DIGITALIS

By

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B.A. Amherst College, 1954

Thesis submitted to the faculty of Yale University  
School of Medicine in partial fulfillment of the  
requirement for the degree of Doctor of Medicine.

Department of Internal Medicine  
Yale University School of Medicine  
April 15, 1958



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## INTRODUCTION

Although at the turn of this century and earlier, clinicians recognized that renal failure with oliguria was a condition sometimes superimposed upon such diverse pathological states as the acute episodes of Bright's Disease, fulminant cholera, cardiovascular collapse, mercury poisoning and surgical procedures, there was little insight into the mechanism and morbid implications of this urinary suppression (1, 2, 3,). The main attention was directed to the underlying emergency since it was with this that the afflicted often and quickly succumbed. In the event that the patient survived the first days of the initial insult, then the problem of the renal failure became a very real one. In the light of our present knowledge that acute renal failure, once established, is produced by a variable degree of damage to the tubular cells of the kidney, the earliest attempts at therapy for the condition seem to us misguided. They consisted in the use of diuretics and the forcing of fluids in the attempt to reinstate proper kidney function. This form of therapy presumed an intact and functioning tubule and eventuated in overhydration, hastening death by the production of pulmonary edema.

The development of modern methods of medical treatment (e.g. intravenous replacement therapy, antibiotics) has greatly decreased the mortality directly associated with the acute disaster producing renal failure. Also to be noted, is that some forms of modern therapy in themselves predispose to the development of anuria through tubular damage (e.g. injudicious sulfonamide administration, inaccurately matched blood transfusions). Consequently, it can be seen that the



clinician today finds himself vis a vis the problem of acute renal failure at least as often as were his forebears. However, this complication is no longer considered as dire a threat as it was 60 or even 10 years ago. Improved methods of diagnosis have allowed better definition of the pathological and biochemical derangements in acute tubular necrosis, permitting a more rational and successful approach to therapy.

Inasmuch as the acute renal deficiency involves failure of the kidneys for an unpredictable number of days to regulate the water and electrolyte equilibrium as well as to excrete the products of cellular metabolism, the more refined principles to be observed in its treatment may be briefly summarized:

- a) Avoidance of excessive salt and water administration.
- b) Suppression of cellular breakdown which results in accumulation of acids and potassium in the extracellular fluid.

In recent years the necessity for restricting salt and water has been extensively dealt with, emphasizing the strict limitation of these essentials to cover losses, both obvious and insensible, and taking into account endogenous water production (4, 5, 6, 7, 8).

Attention will be directed here to some approaches aimed at the second goal in the therapy of acute renal failure, that of inhibiting the accumulation of products of cellular catabolism with the resultant toxic effects upon the organism. In the anuric individual such products of cellular breakdown as non-protein nitrogen,



potassium and the acid metabolites: sulphates, phosphates, and urates, as would normally be excreted in the urine, accumulate in the extracellular fluid. If the "protein-sparing action" of non-protein calories is utilized in the form of carbohydrates or fats for energy requirements, the oxidation of endogenous protein and coincident cellular destruction can be minimized.

In his starvation experiments on normal human subjects, Gamble (9) pointed out that protein breakdown could be markedly reduced on an optimal diet of glucose alone.

In the situation of acute renal failure and the extremely high caloric requirements brought about by the stress of antecedent trauma, intoxication or infection, and the necessity for drastic limitation of the fluid vehicle to be used in the administration of calories, several investigators have recommended fat in addition to carbohydrate in the therapeutic regimen (10, 11).

An experimental evaluation of the comparative effects of iso-caloric diets of carbohydrate, fat, and protein in nephrectomized rats was carried out by Masson, Corcoran, and Page (12), taking as criteria of benefit blood urea nitrogen levels and survival time. They were able to show by these criteria that a carbohydrate diet is somewhat more beneficial than a fat diet and that both are far superior to a protein diet or to administration of no calories at all. These authors also demonstrated prolonged survival of nephrectomized rats administered testosterone or desoxycorticosterone acetate along with a carbohydrate diet.





Since there has been considerable experimental evidence that testosterone, in addition to its androgenic properties, has a potent anabolic effect, producing prolonged retention of nitrogen, phosphorus, and potassium in the intact animal and human (13, 14, 15, 16, 17), it seemed reasonable to assume after Masson's demonstration that this anabolic action of testosterone could offer protection against cellular breakdown to patients with acute anuric uremia even in the face of the associated profound metabolic derangements and altered diet which did not exist in the original hormone studies.

In subsequent years, there has been but little report in the literature of the use of testosterone in the therapy of acute renal failure with the result expected from the implications of Masson's work. A report of 3 cases in the French literature is available which demonstrated a decrease in the progress of azotemia in renal shutdown treated with 80 mg. of testosterone a day (18). Encouraging reports of the use of testosterone in the therapy of chronic renal failure are more easily found with the observation that its administration produces a substantial retention of nitrogen and potassium and a decrease in the level of the blood urea nitrogen together with a "positive sense of well-being" on the part of the patient (19, 20). Merrill (8) and Bull (5) recommend its use in renal failure, acute and chronic.

Hyperkalemia, one of the more important complications of continuing anuria, has a depressant effect on muscular activity and may produce paralysis and cardiac arrest. Kolff (21) has demonstrated that administration of insulin with infusion of very large amounts



of glucose will result in a decrease in the serum potassium over a 24-hour period. In the anuric individual, it is probable that potassium is induced to move from the extracellular to an intracellular site.

There has been considerable discussion concerning protection by potassium against digitalis-induced cardiac arrhythmias, as reviewed by Lown (22). In digitalis intoxication, any of the salts of potassium will abolish manifestations of drug overdose (23, 24). In many circles, the reverse proposition is also held to be true: that digitalis will counteract the myocardial toxic effects of excess serum potassium, particularly in the situation of acute renal failure. It is difficult to find published documentation of this thesis.

In 1940, Zwemer and Lowenstein (25) reported experiments with normal, intact rats, mice, and cats, showing that very high doses of cardiac glycosides (strophanthin, ouabain, and digitalin) previously administered, protected the animals against lethal amounts of infused KCl. In another series of experiments, it was observed that normal serum potassium could be significantly decreased over a 24-hour period after an injection of strophanthin. Apparently, it was on the basis of this evidence that digitalis has been regarded as a possible adjunct to the therapy of hyperkalemia in acute renal failure.

In the series of experiments to be reported, we were interested in observing the differential protein-sparing effect of varying amounts of carbohydrate calories and in re-evaluating the role of



testosterone in experimental acute renal failure in the rat. Particular attention was directed at potassium metabolism, and the effect of the cardiac glycosides against hyperkalemia and its toxicity in the context of anuria was investigated.



## MATERIALS AND METHODS

The animals used were male albino rats ranging in weight from 250 to 375 grams. Nephrectomy was performed in two stages, 7 days apart, removing the organs through lumbar incisions under ether anesthesia. The kidney capsule was stripped before removal to avoid damage to the adrenal glands. Until the time of the second nephrectomy, the animals were fed ad libitum on regular laboratory chow and water. The rats were weighed at the time of each operation and at 24-hour intervals following the second procedure. During each 24-hour period subsequent to the second nephrectomy each rat received 10 cc of water by gavage (approximately equivalent to 3 cc per 100 square cm of body surface, (after Masson's method (12))), in two divided doses in which was contained a known number of calories in the form of glucose. Tail blood was drawn in 0.5 cc aliquots for determination of blood urea nitrogen and serum potassium at the time of the second operation and at 24-hour intervals thereafter. These measurements were done by the micro-diffusion analysis of Conway (26) and with the Baird flame photometer, respectively. Each determination was done in duplicate.

The protocol was divided into three series, each including its own set of controls:

- Series I a. Four rats received no calories in 10 cc water.
- b. Four rats received 4 glucose calories (1 gram of glucose) in 10 cc water.
- c. Four rats received 10 glucose calories (2.5 gram of glucose) in 10 cc water.





Series II. All rats received 10 glucose calories in 10 cc water.

- a. Fifteen rats received 10 mg. daily of Testosterone propionate in peanut oil, intraperitoneally, beginning 24 hours before the second nephrectomy.
- b. Seven rats treated similarly, injected with peanut oil alone.

Series III. All rats received 10 glucose calories in 10 cc water.

- a. Four rats received no cardiac glycoside.
- b. Six rats injected with 0.25 mg Ouabain intramuscularly at the time of the second nephrectomy (equivalent to the strophanthin dosage of Zwemer (25)), followed by injection of 0.125 mg daily thereafter.
- c. Four rats injected with 0.05 mg Digitoxin at the time of the second nephrectomy, followed by 0.01 mg daily thereafter.
- d. Four rats injected with 0.1 mg Digitoxin at the time of the second nephrectomy, followed by 0.02 mg daily thereafter.



## RESULTS

Fed ad libitum after the first nephrectomy, the rats gained 10 to 15 per cent of their original weight in the seven day interim before the second operation. Animals who did not so thrive were discarded. Twenty-four hours following the second procedure the weights were decreased by 10 to 15 per cent and remained stable throughout subsequent weighings.

At the time of the second nephrectomy blood taken for urea nitrogen and potassium determinations revealed normal values: 20 to 30 mg per cent and 4.0 to 5.5 mEq/L respectively.

All values are summarized as the mean of each group with the standard deviation. Unless otherwise stated below, there was no significant difference between the distribution of values of the experimental and control groups ( $p > 0.05$ ).

Time of survival (in hours after the second nephrectomy) is correct to  $\pm$  4 hours.

In Series I it will be noted that the rats fed only water showed a more rapid progress of azotemia (significant at 24 and 48 hours after nephrectomy) than did animals receiving 4 to 10 glucose calories with their water. The glucose-fed animals survived longer than the others. Although it appears that the blood urea nitrogen may have been somewhat lower and survival longer in animals administered 10 calories than shown by the ones receiving 4, there was no statistical difference between the two. In all cases, serum potassium rose daily, but did not vary in any consistent way with caloric intake.



In Series II, the progress of the azotemia was less rapid in the testosterone treated animals than it was in the control group. This is suggestive 24 hours after nephrectomy, and there is a significant difference between the two groups at 48 hours. Although there is no statistical difference, there is a suggestive prolongation of life noted in the testosterone treated rats. Again, there is no apparent effect of treatment on serum potassium.

In Series III, it can be seen that neither serum potassium concentration nor survival was influenced by the administration of cardiac glycosides.

Reference to Figure I will reveal that the serum potassium concentration at 48 hours is not related to survival time, nor can survival time be related to weight as illustrated in Figure II. Only values of rats receiving 10 calories daily in 10 cc of water are plotted.



SUMMARY OF THE DATA

The Effects of Caloric Intake, Testosterone, and Digitalis on the Blood Urea Nitrogen, Serum Potassium and Survival Time of Nephrectomized Rats

| Series | No. of Rats | Daily Calories | Treatment              | Day 0 Weight | Day 1            |                | Day 2            |                | Surv. Hrs. |
|--------|-------------|----------------|------------------------|--------------|------------------|----------------|------------------|----------------|------------|
|        |             |                |                        |              | BUN <sub>1</sub> | K <sub>1</sub> | BUN <sub>2</sub> | K <sub>2</sub> |            |
| I      | 4           | 0              | -                      | 293±19       | 130.7±11.6       | 9.7±.6         | 214.4±20.2       | 11.1±.3        | 54.5±2.4   |
|        | 4           | 4              | -                      | 276±22       | 97.5± 2.5        | 10.7±1.6       | 162.1± 7.9       | 12.4±.4        | 66.8±6.8   |
|        | 4           | 10             | -                      | 273±25       | 96.1± 2.6        | 11.4±1.1       | 157.7±10.1       | 11.5±1.2       | 68.3±14.3  |
| II     | 15          | 10             | Testosterone           | 320±42       | 97.1±15.9        | 8.7±.9         | 162.5±20.9       | 10.5±1.3       | 72.4±16.2  |
|        | 7           | 10             | -                      | 315±44       | 105.9±13.9       | 8.4±2.2        | 198.3±13.9       | 10.3±1.6       | 63.6± 5.7  |
| III    | 4           | 10             | -                      | 288±26       |                  |                |                  | 11.2±.2        | 64.5± 7.7  |
|        | 6           | 10             | Ouabain                | 331±17       |                  | 9.7±.9         |                  | 11.8±1.2       | 62 ± 5.8   |
|        | 4           | 10             | Digitoxin<br>(0.05 mg) | 335±30       |                  | 9.1±.4         |                  | 10.3±.06       | 62 ± 5.6   |
|        | 4           | 10             | Digitoxin<br>(0.1 mg)  | 285±24       |                  |                |                  | 11.5±1.3       | 61.5±6.4   |





The Effect of Varied Caloric Intake on Blood Urea Nitrogen,  
Serum Potassium and Survival Time in Nephrectomized Rats

Series I

|   | Weight         | BUN <sub>0</sub> | K <sub>0</sub> | BUN <sub>1</sub> | K <sub>1</sub> | BUN <sub>2</sub> | K <sub>2</sub> | BUN <sub>3</sub> | K <sub>3</sub> | Survival Hours |
|---|----------------|------------------|----------------|------------------|----------------|------------------|----------------|------------------|----------------|----------------|
| a |                |                  |                |                  |                |                  |                |                  |                |                |
|   | R <sub>1</sub> | 280              | 25.2           | 5.0              | 144.2          | 9.9              | 241.9          | -                |                | 52             |
|   | R <sub>2</sub> | 285              | 27.4           | 5.6              | 116.2          | 8.9              | 11.3           | -                |                | 53             |
|   | R <sub>3</sub> | 320              | 26.9           | -                | 128.8          | 10.2             | 207.8          | 10.6             |                | 56             |
|   | R <sub>4</sub> | 285              | 25.2           | -                | 133.4          | 9.9              | 213.9          | 11.2             |                | 57             |
| b |                |                  |                |                  |                |                  |                |                  |                |                |
|   | R <sub>1</sub> | 265              | 20.7           | 6.3              | 95.0           | 10.6             | 154.8          | 12.7             |                | 64             |
|   | R <sub>2</sub> | 255              | 23.8           | 6.3              | 95.6           | 10.4             | 173.3          | 11.8             |                | 60             |
|   | R <sub>3</sub> | 305              | 22.4           | 6.3              | 99.9           | 10.5             | 159.0          | 12.6             | 167.7          | 76             |
|   | R <sub>4</sub> | 280              | 22.4           | 7.2              | 99.4           | 11.1             | 161.3          | 12.6             |                | 67             |
| c |                |                  |                |                  |                |                  |                |                  |                |                |
|   | R <sub>1</sub> | 280              | 21.0           | 6.5              | 98.3           | 12.4             | 170.0          | 12.1             |                | 54             |
|   | R <sub>2</sub> | 270              | 21.6           | 6.6              | 94.7           | 9.8              | 150.9          | 10.0             | 169.1          | 79             |
|   | R <sub>3</sub> | 300              | 26.9           | 6.3              | 99.1           | 11.6             | 161.8          | 12.7             |                | 58             |
|   | R <sub>4</sub> | 240              | 25.5           | 6.9              | 92.4           | -                | 148.2          | 11.3             | 140.8          | 82             |

a) Rats received no calories in 10 cc water

b) Rats received 4 calories in 10 cc water

c) Rats received 10 calories in 10 cc water



The Effect of Testosterone Propionate on Blood Urea Nitrogen, Serum Potassium and Survival Time in Nephrectomized Rats

Series II

a

|                 | Weight | BUN <sub>0</sub> | K <sub>0</sub> | BUN <sub>1</sub> | K <sub>1</sub> | BUN <sub>2</sub> | K <sub>2</sub> | BUN <sub>3</sub> | K <sub>3</sub> | Survival Hours |
|-----------------|--------|------------------|----------------|------------------|----------------|------------------|----------------|------------------|----------------|----------------|
| R <sub>1</sub>  | 430    | 24.9             | 6.2            | 93.8             | -              | 164.1            | 9.1            | 236.8            | 9.9            | 92             |
| R <sub>2</sub>  | 360    | 29.2             | 6.4            | 116.5            | 10.2           | 146.7            | 13.0           |                  |                | 70             |
| R <sub>3</sub>  | 320    | 21.8             | 4.8            | 123.2            | 7.7            | 202.2            | 12.5           |                  |                | 60             |
| R <sub>4</sub>  | 390    | 26.0             | 5.3            | 98.8             | 7.6            | 152.9            | 9.5            |                  |                | 72             |
| R <sub>5</sub>  | 310    | 28.3             | 5.0            | 112.0            | 8.3            | 181.2            | 10.8           |                  |                | 56             |
| R <sub>6</sub>  | 300    | 25.2             | 4.7            | 121.2            | 7.8            | 200.0            | 10.3           |                  |                | 58             |
| R <sub>7</sub>  | 280    | 24.6             | 5.6            | 93.5             | 8.0            | 153.7            | 9.5            |                  |                | 70             |
| R <sub>8</sub>  | 270    | 27.2             | 5.7            | 89.6             | 6.9            | 167.2            | 9.4            |                  |                | 66             |
| R <sub>9</sub>  | 300    | 30.0             | -              | 82.6             | 7.2            | 171.6            | 10.7           |                  |                | 72             |
| R <sub>10</sub> | 305    | 21.0             | -              | 72.5             | -              | 170.8            | 8.3            |                  |                | 70             |
| R <sub>11</sub> | 310    | 20.2             | 4.4            | 111.4            | -              | 142.0            | 11.8           |                  |                | 60             |
| R <sub>12</sub> | 305    | 25.5             | 4.9            | 89.7             | -              | 140.0            | 10.7           |                  |                | 62             |
| R <sub>13</sub> | 310    | 25.2             | 5.4            | 80.6             | -              | 169.3            | 10.1           | 216.7            | 10.8           | 108            |
| R <sub>14</sub> | 315    | 23.2             | 4.4            | 87.1             | 8.3            | 134.1            | 10.8           |                  |                | 66             |
| R <sub>15</sub> | 300    | 23.2             | 4.7            | 84.4             | 8.7            | 142.5            | 10.9           | 201.9            | 11.9           | 104            |

a) Rats received 10 calories in 10 cc water, treated with testosterone propionate



The Effect of Testosterone Propionate on  
 Blood Urea Nitrogen, Serum Potassium and Survival  
 Time in Nephrectomized Rats

Series II  
 b

|                | Weight | BUN <sub>0</sub> | K <sub>0</sub> | BUN <sub>1</sub> | K <sub>1</sub> | BUN <sub>2</sub> | K <sub>2</sub> | Survival Hours |
|----------------|--------|------------------|----------------|------------------|----------------|------------------|----------------|----------------|
| R <sub>1</sub> | 280    | 24.6             | -              | 83.8             | 6.1            | 187.9            | 7.7            | 60             |
| R <sub>2</sub> | 290    | 22.7             | 6.9            | 106.7            | 7.8            | 184.2            | 10.8           | 70             |
| R <sub>3</sub> | 300    | 22.9             | 5.7            | 105.6            | 8.5            | 179.5            | 9.9            | 66             |
| R <sub>4</sub> | 320    | 25.5             | 4.5            | 100.2            | -              | 204.1            | 10.2           | 68             |
| R <sub>5</sub> | 265    | 24.4             | 4.6            | 99.1             | 12.7           | 212.2            | 12.9           | 56             |
| R <sub>6</sub> | 370    | 26.0             | 4.9            | 125.2            | 7.9            | 210.8            | 10.8           | 57             |
| R <sub>7</sub> | 380    | 30.0             | 4.5            | 121.2            | 7.4            | 209.2            | 9.7            | 68             |

b) Rats received 10 calories in 10 cc water (No testosterone)



The Effect of Cardiac Glycosides on Serum Potassium and Survival Time in Nephrectomized Rats

Series III

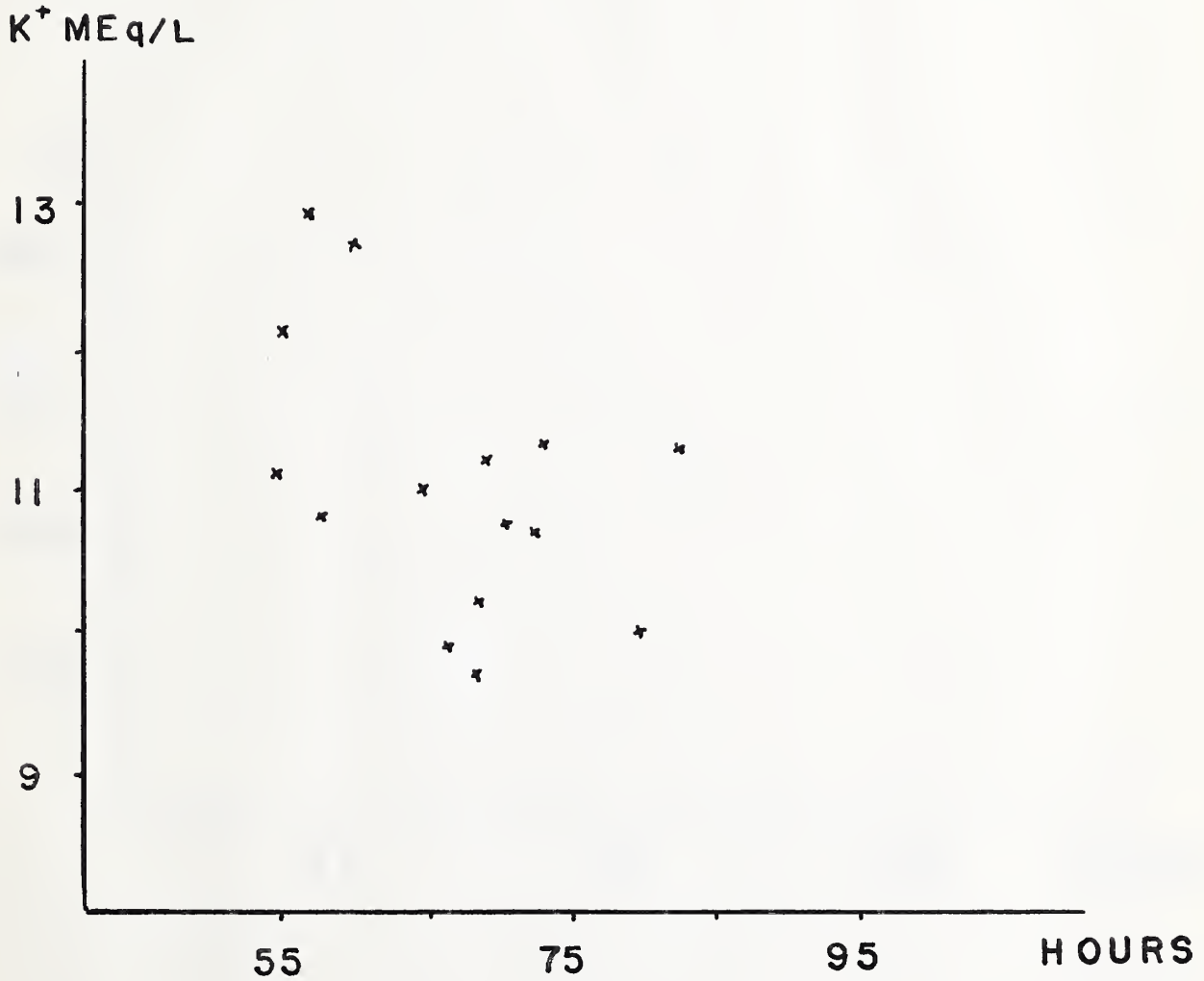
|   | Weight         | $K_0$ | $K_1$ | $K_2$ | Survival Hours |
|---|----------------|-------|-------|-------|----------------|
| a |                |       |       |       |                |
|   | R <sub>1</sub> | 310   | 5.5   | 11.0  | 64             |
|   | R <sub>2</sub> | 255   | 5.1   | 11.2  | 68             |
|   | R <sub>3</sub> | 280   | 5.3   | 11.1  | 54             |
|   | R <sub>4</sub> | 310   | 5.5   | 11.4  | 72             |
| b |                |       |       |       |                |
|   | R <sub>1</sub> | 330   | 4.6   | 11.5  | 64             |
|   | R <sub>2</sub> | 320   | 4.3   | 9.8   | 60             |
|   | R <sub>3</sub> | 325   | 4.3   | 9.0   | 60             |
|   | R <sub>4</sub> | 360   | 4.4   | 9.3   | 58             |
|   | R <sub>5</sub> | 340   | 4.4   | 9.1   | 70             |
|   | R <sub>6</sub> | 310   | 4.4   | 9.7   | 58             |
| c |                |       |       |       |                |
|   | R <sub>1</sub> | 315   | 4.8   | 9.4   | 68             |
|   | R <sub>2</sub> | 305   | 4.4   | 9.6   | 66             |
|   | R <sub>3</sub> | 350   | 4.7   | 8.7   | 56             |
|   | R <sub>4</sub> | 370   | 4.4   | 8.8   | 58             |
| d |                |       |       |       |                |
|   | R <sub>1</sub> | 300   | 5.1   | 10.2  | 56             |
|   | R <sub>2</sub> | 290   | 4.9   | 12.3  | 56             |
|   | R <sub>3</sub> | 300   | 5.0   | 12.4  | 66             |
|   | R <sub>4</sub> | 250   | 5.2   | 10.1  | 68             |

- a) Rats injected with no cardiac glycosides
- b) Rats injected with 0.25 mg. Ouabain, followed by 0.125 mg. daily
- c) Rats injected with 0.05 mg. Digitoxin, followed by 0.01 mg. daily
- d) Rats injected with 0.1 mg. Digitoxin, followed by 0.02 mg. daily





FIGURE 1



SURVIVAL RELATED TO SERUM POTASSIUM (48 HRS)







## DISCUSSION

In selecting and preparing rats for experimental uremia, an effort was made to employ the healthiest animals (as inferred by previous weight gain and normal blood urea nitrogen and serum potassium concentration) under optimal conditions. As pointed out by Peters (27) there is an increased breakdown of protein following severe disease and trauma which is self-limiting and followed by an anabolic phase during which time protein is stored. Brown and associates (28, 29) have found that during this anabolic phase renewed injury caused relatively less waste of protein and that there was an earlier anabolic response to administration of high caloric diets. On these grounds, it was decided to perform nephrectomy utilizing the two-stage procedure in the expectation that the rats would be placed in a metabolically more favorable position regarding protein economy.

In starvation experiments, it will be recalled that Gamble (9) demonstrated that 100 grams of glucose per day as the sole source of calories will, in the normal 70 Kg human, maximally spare endogenous protein for energy requirements. This conservation is equivalent to one half the amount of body protein utilized for energy by the fasting individual and is not materially exceeded by increase in the glucose administration even up to caloric requirements. Under the circumstances of a sub-energy maintenance intake, ingested protein is oxidized in support of the energy metabolism and will not contribute to the sparing of body protein until caloric requirements are met.



Butler, et al. (30) showed a similar pattern of protein sparing in normal humans administered only glucose, but treated with testosterone propionate. The administration of 50 grams/day of glucose resulted in a moderate decrease in nitrogen excretion while the administration of 100 grams prompted as great a decrease in the urinary nitrogen as did 300 grams.

The minimum requirement of 100 grams/day of glucose for the 70 Kg man corresponds to 0.5 grams/day for the 350 gram rat. The results obtained after administration of 1 gram and 2.5 grams to the rats in the present study showed a leveling off similar to that noted by Gamble and Butler in the protein-sparing ability of increasing amounts of glucose and again demonstrated that a salutary diminution of protein breakdown with prolongation of life may be gained far short of caloric requirements. This has been previously observed in nephrectomized rats by Bergman and Drury (31) who administered 1 gram/day of glucose to their animals. Masson (12), utilizing a more complex diet of carbohydrates, giving each animal 50 calories per day, reported a greater difference in blood urea nitrogen and survival between these animals and the controls than we were able to demonstrate. Since he did not compare diets with varying caloric values and gives no indication of the state of hydration of his control group, it is not feasible to evaluate his data quantitatively in the context of our comparative results.

Although a significant reduction was demonstrated in the daily rise of blood urea nitrogen in glucose fed, nephrectomized rats treated





with testosterone propionate, no influence of the hormone was observed on serum potassium concentration. A suggestive, but not statistically significant beneficial effect on survival time was also noted in the experimental group. Inasmuch as it has been well established that testosterone acts as a powerful stimulus to retention of nitrogen, potassium, and phosphorus, presumably in the formation of new tissue, it is interesting to speculate why a more marked suppression of the accumulation of protein breakdown products was not seen.

In metabolic balance studies, testosterone given intramuscularly in doses of 25 mg/day to normal young men produces a gradual fall in the excretion of nitrogen which, after 4-8 days reaches a peak (13). West and Tyler (32) administered the same total dose of testosterone propionate (162-200 mg) dissolved in human serum albumin, intravenously in a single dose to normal men and demonstrated an equivalent maximum retention of nitrogen only 24 hours after this dose. Since it was noted that smaller doses similarly given produced only slight retention and that 40 per cent of the large effective dose could be accounted for in the urine as 17-ketosteroids in 2 hours, 70 to 80 per cent recoverable in 24 hours, it was concluded that the effectiveness of the large intravenous dose could not be attributed to a sustained high level of hormone in the blood, but that a minimum high initial blood level was required for prompt penetration into cells and rapid action. Other routes of administration cannot provide as prompt a tissue distribution.

In the present analysis it becomes important to know whether testosterone may exert an anti-catabolic action as well as an anabolic



effect. The work of Perlman and Cassidy (33) demonstrates in dogs that at minimal balance levels of protein, constituting 11.8 per cent of the dietary intake, no nitrogen retention could be induced by testosterone propionate. From this and earlier work by Kochakian and Van der Mark (34), it may be interpreted that protein administration must contribute between 12 per cent and 18 per cent of the diet in order for testosterone to produce a retention of nitrogen. Studies by Kenyon and Knowlton (35) demonstrated a similar tendency in a normal human adult. If testosterone had an appreciable anti-catabolic action, one would expect to observe a decrease in the urinary nitrogen excretion in animals maintained on minimal protein intake.

To return to the role of testosterone in the therapy of experimental acute renal failure, it is proposed that effective protection against cellular breakdown cannot be expected in this context. Firstly, utilizing an intramuscular or intraperitoneal administration of the poorly soluble depot preparation, it is possible that maximal tissue levels are not realized before the death of the animals, roughly 72 hours after nephrectomy and 96 hours after the first injection. A more important factor limiting demonstration of the anabolic effect of testosterone is that the animals, of necessity, are maintained on a diet devoid of protein, the building block of new tissue. Furthermore, if we assume from the inferences of experiments on other animals that testosterone has no appreciable anti-catabolic action, then there is no difficulty in understanding why the hormone does not reproduce the effects seen in many of the



earlier studies on normal individuals, receiving adequately balanced diets.

The experimental data show a slight, although unquestionable conservation of protein, as reflected by the lower blood urea nitrogen in the treated animals. Evaluated in the light of the principles evolved above, it is proposed that although maximal end-organ concentration of testosterone may not have been achieved, there was some effective tissue penetration in the lifetime of the animals. Minimal anabolic effect was observed, and in the absence of exogenous sources, must be attributed to action on stored protein as the substrate.

Albright (36) states that the formation of tissue results in the accompaniment of each gram of nitrogen by 3 mEq of potassium. The data herein presented neither confirm nor contradict this statement. If it is assumed that production of tissue accounts for the difference observed between the blood urea nitrogen of the testosterone treated animals and the untreated animals at 48 hours after nephrectomy (Series II), this difference of 36 mg per cent would theoretically, in the 300 gram rat, produce a difference of approximately 1 mEq/L in the concentration of potassium between the two groups. This assumes a uniform distribution of <sup>added</sup> potassium in body water (65 per cent of body weight). It will be noted that the mean values of serum potassium determined at that time are virtually identical. However, the standard deviations from the mean of the experimental and control groups are quite wide, each more than 1 mEq/L.

In the last series of experiments, there was no protection against early death of the nephrectomized rats with administration of cardiac glycosides. It will be recalled that Zwemer and Lowen-

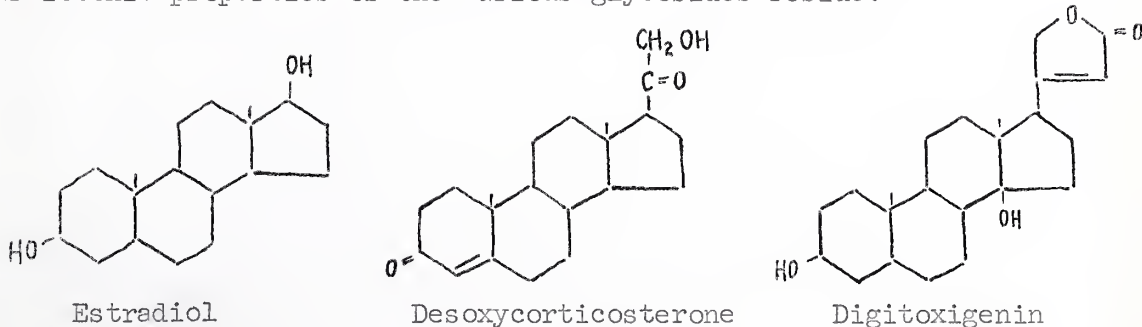




stein (25), using intact animals, protected with strophanthin against infused, lethal amounts of KCl.

To explain this paradox, it is important to consider first of all that death of the nephrectomized animal is due to more than a simple elevation of potassium in the blood. That the mortality is associated with a more complex picture and possibly the presence of other metabolites and imbalances is illustrated by the fact that there was no constant inverse correlation between concentration of serum potassium and length of survival after nephrectomy (Figure 1). This observation is in keeping with the well-known fact that there is seldom a predictable relation between measured serum potassium and signs of toxicity in the human patient in uremia (21).

To account for the protecting influence of digitalis in simple hyperkalemia, it is noted that the cardiotoxic glycosides all contain the cyclopentanoperhydrophenanthrene nucleus in common with the steroid hormones, cholesterol and the Vitamins D. At the C17 position is attached an unsaturated butyrolactone ring in which the cardiotoxic properties of the various glycosides reside.



The ability of the adrenocortical hormones to produce potassium diuresis and depletion is common knowledge. It is not difficult to imagine that the cardiac glycosides, in ample dose, or their breakdown products, may have a similarity in action to DCA to explain the





protection by digitalis observed by Zwemer against administered potassium which might have been produced by an increased excretion of potassium in the animals so treated. Obviously, nephrectomized animals are unable to respond in this way. These authors pointed out the similarity of their results to those reported earlier by Zwemer and Truszkowski (37) of the protection against potassium poisoning with adrenocortical hormone.

Lending some support to a steroidal activity of digitalis, there have been published several accounts of an estrogen-like effect producing gynecomastia, occasionally seen in men chronically treated with the drug (38, 39). However, there are no reports available of observations of an adrenocortical activity of digitalis. Further inquiry into this interesting possibility must await proper balance studies.



### SUMMARY

From results of studies on a small series of nephrectomized rats, several conclusions were reached.

The rat like the human, can be induced to spare endogenous protein by the administration of carbohydrate, in amounts much less than actual caloric requirements.

In experimental acute uremia, only minimal anabolic activity can be demonstrated by testosterone propionate. The absence of dietary protein mitigates against influences ordinarily producing new tissue formation or protein storage.

Digitalis was shown to have no effect on survival or serum potassium in anuric rats maintained on a minimum oral fluid intake.

The relation between serum potassium concentration and its toxicity in anuria is considered. In light of previous experimental data showing protection against uncomplicated potassium toxicity by digitalis, a mineralocorticoid activity of the cardiac glycosides is suggested.



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