15034

Some Factors Affecting Duration of Life in Total Anuria.*

S. RODBARD.† (Introduced by L. N. Katz.)

From the Cardiovascular Department, Michael Reese Hospital, Chicago, Illinois.

In a series of studies planned to inquire into the pathological physiology of the anurias, we first attempted to determine the factors which might allow the longest life expectancy in the experimental animal after the induction of total anuria. Results obtained on 61 dogs following total nephrectomy are discussed in this report.

Three to 7 days appears to be the usual duration of life in dogs rendered totally anuric by binephrectomy.[1,2,3] Some animals have been kept alive for longer periods under special conditions.[3] Obstruction of the renal arteries or of the ureters[4,5,6,7] frequently leads not only to anuria but also to acute nephrogenic hypertension. Total obstruction of the renal veins often is followed by massive hemorrhage into the renal capsule with death sometimes occurring in a few hours.[8] Obstruction of the renal arteries and veins is equivalent to removal of both kidneys, but this method leaves a mass of necrotic tissue *in situ*. In the present study, binephrectomy was the method of choice to produce anuria. The kidneys were approached by lumbar incision and removed without entering the peritoneum. Ether or nembutal anesthesia was used.

The binephrectomized dog appears outwardly normal for 24 to 48 hours postoperatively. On the second or third day he loses his interest in food but usually begins to show a marked thirst. At this time the animal will drink large quantities of water, quickly vomit, and then repeat this cycle over and over again. If this process is not prevented by limiting the water intake, loss of chlorides will lead to hypochloremia and early death. Vomiting can usually be controlled by giving water in small amounts (25 cc) several times daily.

Results. Binephrectomy in a single operation. The post-operative duration of life ranged from 60 to 130 hours, averaging 85 hours in a series of 16 dogs subjected to binephrectomy in a single operation. The data on this group of animals and those subsequently discussed are given in Table I.

Binephrectomy in two stages. To reduce the trauma experienced by the animals at the

TABLE I.
Hours of Life After Bi-nephrectomy.

One-stage operation		Two-stage operation		
No DCA	DCA treated	No DCA	DCA treated	DCA pre-treated
60	76	58	110	132
60	108	60	115	165
60	110	72	123	170
65	110	92	196	206
72	144	100	223	230
73	145	110		235
77	146	115		
78	148	115		
80	168	120		
84	173	120		
85	185	130		
85	200	130		
97		130		
127		140		
130		178		
130		192		
Avg 85	143	116	153	190
Stand. Dev. ±23	±34.5	±35.4	±47	±37

* Aided by the A. D. Nast Fund for Cardiovascular Research. The Department is supported in part by the Michael Reese Research Foundation.

† Serving as a First Lieutenant in the Army Air Forces.

1 Hoff, H. E., Smith, P. K., and Winkler, A. W., *J. Clin. Invest.*, 1941, **20**, 607.

2 Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 92.

3 Winternitz, M. C., Mylon, E., Waters, L. L., and Katzenstein, R., *Yale J. Biol. and Med.*, 1940, **12**, 623.

4 Hartwich, A., *Ztschr. f. d. ges. exp. med.*, 1930, **69**, 462.

5 Katz, L. N., Landowne, M., and Friedberg, L., *Cardiologia*, 1941, **5**, 25.

6 Megibow, R. S., Katz, L. N., and Rodbard, S., *Am. J. Med. Sci.*, 1942, **204**, 340.

7 Taquini, A. C., *Am. Heart J.*, 1940, **19**, 513.

8 Friedberg, L., *Am. Heart J.*, 1944, **28**, 786.

time of the induction of the anuric state, 16 dogs were subjected to binephrectomy in two stages: uninephrectomy was performed 1 to 4 weeks before the remaining kidney was removed. After removal of the second kidney, these animals survived from 58 to 192 hours, averaging 116 hours.

These results suggest that the reduced trauma, due to the need for only unilateral surgery at the time of induction of the anuria, allowed a significant increase in the duration of life.

Binephrectomy in one operation and post-operative treatment with DCA. Reports that desoxycorticosterone acetate (DCA) increased the life expectancy in certain conditions associated with trauma[9,10] suggested that this substance might act in a similar fashion to reduce the apparent effect of trauma in our animals. A group of 12 dogs subjected to binephrectomy in one operation was given subcutaneously 10 to 50 mg‡ of DCA post-operatively and at intervals of 24 hours thereafter. These animals survived from 76 to 200 hours, averaging 143 hours.

These results suggested that the DCA favorably affected the survival time in these animals.

Binephrectomy in two stages and post-operative treatment with DCA. The above results suggested that perhaps a combination of reduced trauma attendant upon the two-stage operation, together with the use of DCA, might further increase the duration of life of the totally anuric animal.

A group of 5 dogs was uninephrectomized 2 weeks before removal of the second kidney. Twenty mg of DCA was administered subcutaneously post-operatively and at intervals of 24 hours thereafter. These animals survived from 110 to 223 hours, averaging 153 hours.

Binephrectomy in two stages with DCA pre-treatment. Previous reports suggested that a contributing cause of death in anuric animals surviving more than 3 days might be the accumulation of potassium in the plasma until its concentration was toxic for the heart.[11] Chemical and electrocardiographic studies on our dogs confirmed this finding. The plasma potassium level in the normal dog, using a modified chloroplatinate method, averaged 18 mg %. After binephrectomy this increased at about 4 mg % per day until the plasma level was about 40 to 50 mg %, a concentration which causes increased irritability of the heart and may lead to ventricular fibrillation.[1]

It was felt that the duration of life after total nephrectomy might be further increased by the use of DCA pre-operatively, since it has been shown[12] that DCA given to normal dogs causes an increased excretion of potassium, and when continued for a period of 10 days may lower the plasma potassium to about half its normal level. Starting with a lowered initial level of plasma potassium, it was thought that a longer survival in anuria might occur before a toxic concentration of plasma potassium would be reached.

Six uninephrectomized dogs were given 20 mg of DCA intramuscularly daily for a period of 10 days, with lowering of the plasma potassium from 18 mg % to an average of 11 mg %. After removal of the second kidney these animals survived from 132 to 235 hours, averaging 190 hours. Before these animals died they showed marked electrocardiographic changes and toxic plasma potassium levels. Three of these animals received 20 mg DCA subcutaneously daily post-operatively, while 3 did not. There was no difference in the duration of life between these two sub-groups.

It was of interest to note that DCA lowered the plasma K in dogs with renal excretion, but did not affect the rate of accumulation of potassium in the plasma of binephrectomized dogs. This suggests that DCA operates via the kidney in lowering the plasma K in normal

9 Perlow, S., Killian, S. T., Katz, L. N., and Asher, R., *Am. J. Physiol.*, 1941, **134**, 755.

10 Katz, L. N., Killian, S. T., Asher, R., and Perlow, S., *Am. J. Physiol.*, 1942, **137**, 79.

‡ There was no correlation, with the doses used, between the amount of DCA given and life expectancy.

11 Winkler, A. W., Hoff, H. E., and Smith, P. K., *J. Clin. Invest.*, 1941, **20**, 119.

12 Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atcheley, D. W., and Loeb, R. F., *Am. J. Physiol.*, 1941, **135**, 230.

dogs, rather than acting on the extra-renal tissues. These results are in agreement with the studies of Durlacher and Darrow.[13]

Binephrectomy in two stages with fluid administration. The clinical condition of the animals in the foregoing experiments indicated that dehydration might be a contributory lethal factor. After removal of the second kidney, the effect of 50 cc daily of intravenous isotonic saline, 25% glucose in 50 cc isotonic saline, or 25% glucose in distilled water was tested on 2 dogs each. The average duration of life for the 3 series was 120, 83, and 154 hours respectively.

Blood Changes in Total Anuria. Aside from the changes in plasma potassium discussed above, certain other changes were observed. The plasma protein concentration, as indicated by the specific gravity of the plasma,[14] showed increases from the normal of 6 g % to as much as 10.4 g % on the sixth anuric day, thus giving confirmatory evidence of dehydration. The blood smear showed no significant changes.

The rate of accumulation of excretory products other than potassium was essentially the same in all groups of anuric dogs. The blood NPN (see [15] for method), averaging 35 mg % at the time of induction of anuria, increased 30 to 50 mg % per day.[16] Serum inorganic phosphorus (see [17] for method) was frequently found to increase from its normal of 4 mg to values as high as 18 mg % on the fourth anuric day.

Other constituents of the blood not measured by us, which have recently been reported to increase progressively after ablation of the kidneys, include total fatty acids, free and esterified cholesterol, phospholipids, free fat of serum,[18] phenol-like compounds,[19] and guanidine (Mason, quoted in [20]).

Cardiovascular changes. The heart rate did not change appreciably except terminally, when there was usually an increase in rate. Blood pressure changes were inconstant.

Limb lead electrocardiogram showed S-T depressions in leads I and II within 36 to 48 hours after induction of anuria. The T waves in all the leads flattened and became inverted or diphasic within 48 to 72 hours. After this the magnitude of the inverted T waves increased progressively and became notched. Among the other cardiac disturbances which developed progressively were auricular standstill or auricular fibrillation, intraventricular block and finally irregular ventricular tachycardia and ventricular fibrillation. These changes in the electrocardiogram are consistent with those seen in animals with progressive artificial increase in plasma potassium.[1] However, this does not necessarily exclude the participation of other mechanisms for their occurrence in anuria.

Pathological Changes. In 12 of the 59 animals studied, a striking lesion was noted in the ventral portion of the pulmonary artery in the region of the ductus arteriosus. The early stages of this lesion showed hemorrhagic areas in the adventitia. In more advanced cases there was involvement of the media with fibrinoid degeneration. The intima, while intact, frequently had a washboard appearance because of shrinkage of the subjacent media. Lesions similar to these have been described by Winternitz[21] following both binephrectomy and renal artery constriction.

Summary. 1. Technics for prolonging the life of the binephrectomized dog are given. 2. Some chemical, physiological, and anatomic changes seen in these anuric animals are described.

I am indebted to Dr. L. N. Katz, under whose guidance this study was carried out, for his con-

[13] Durlacher, S. H., and Darrow, D. C., *Am. J. Physiol.*, 1942, **136**, 577.

[14] Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, 1926, **69**, 625.

[15] Peters, J. P., and Van Slyke, D. D., *Quantitative Chemical Chemistry*, Baltimore, 1932, Williams and Wilkins Co., Vol. II, p. 525.

[16] Bergman, H. C., and Drury, D. R., *J. Clin. Invest.*, 1939, **18**, 777.

[17] Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 197.

[18] Winkler, A. W., Durlacher, S. H., Hoff, H. E., and Man, E. B., *J. Exp. Med.*, 1943, **77**, 473.

[19] Roen, P. R., *J. Urol.*, 1944, **51**, 110.

[20] Harrison, T. R., and Mason, M. F., *Medicine*, 1937, **16**, 1.

[21] Winternitz, M. C., and Waters, L. L., *Yale J. Biol. and Med.*, 1940, **12**, 451.

tinuous interest and advice. I also wish to express my gratitude to Dr. O. Saphir of the Department of Pathology for preparing and interpreting microscopic sections and to Dr. D. J. Cohn of the Department of Chemistry for some of the chemical determinations.

15035

Nitrogen and Sulfur Content of Liver Tissue in Relation to Carbon Tetrachloride Exposure.

C. BOYD SHAFFER AND FRANCES H. CRITCHFIELD. (Introduced by W. S. McEllroy.)

From the Multiple Industrial Fellowship on Chemical Hygiene, Mellon Institute, Pittsburgh, Pa.

Present interest in the use of methionine in the therapy of acute chlorinated solvent poisoning[1, 2] stems to a large extent from the work of Miller and Whipple,[3] who demonstrated that this amino acid, given either shortly before or within 4 hours after the period of inhalation, would protect protein-depleted dogs against the otherwise fatal effects of a chloroform anaesthesia. Coincident with these results were the findings that protein-depleted dogs have livers which are deficient in both nitrogen and sulfur. Sulfur, however, is depleted more than is nitrogen, and therefore the N/S ratio rises. The ratio of nitrogen to sulfur in the liver tissue of their normal adult dogs was 11 or 12 to 1. The protein-depleted dogs which were so susceptible to liver injury showed a nitrogen to sulfur ratio of 14 or 15 to 1, which presumably means a lower content of sulfur-containing amino acids.

That an animal may be rendered inordinately susceptible to the vapors of chlorinated solvents or to hepatotoxic agents in general by manipulation of certain constituents of his diet is unquestioned. The principal point at issue in this investigation is whether inhalation of a chlorinated solvent produces any specific demand for sulfur-containing amino acids upon the animal already receiving an adequate protein intake. If such is the case, one might expect to find a significant depletion of liver sulfur reserves during the course of repeated exposures to the solvent, similar to that found over a period of maintenance on a protein-deficient diet. The effect of repeated inhalation of carbon tetrachloride vapor on the nitrogen and sulfur content of rat liver has been studied with the results presented below.

Experimental. The subjects of this investigation were 40 albino rats averaging about 200 g in weight. They were fed the basal diet described by White and Jackson.[4] The content of sulfur-containing amino acids in this diet is purposely limited; nevertheless, according to these authors, the quantity and nutritional quality of the protein are sufficient to support good growth for relatively long periods of time. After an initial 2-week period on this diet, one-half of the animals were subjected to 14 successive daily exposures, 6 hrs. per day, to 1000 p.p.m. of carbon tetrachloride vapor. The remaining half (controls) were unexposed. As the exposures caused some reduction in food intake, the controls were limited to the average food consumption of the exposed animals of the preceding day.

At the conclusion of the 2-week period of exposures all animals were sacrificed. The livers were removed, blotted free of excess blood, and dried for several days at 76°-78°C to constant weight. The dried livers were crushed to a fine powder, weighed into tared paper thimbles and extracted continuously with ethyl ether for 12 hours in a Bailey-

[1] Beattie, J., Herbert, P. H., Wechtel, C., and Steele, C. W., *Brit. Med. J.*, 1944, No. 4336, 209.

[2] Eddy, J. H., Jr., *Ind. Med.*, 1945, **14**, 283.

[3] Miller, L. L., and Whipple, G. H., *J. Exp. Med.*, 1942, **76**, 421.

[4] White, A., and Jackson, R. W., *J. Biol. Chem.*, 1935, **111**, 507.