ing the clotting of hemophilic blood.

It will be noted in Fig. 1 that the initial one-stage prothrombin values were lower than those obtained with the control plasma. Thus, in the normal human experiments (Fig. 1 B), the prothrombin time of the control plasma was 14.8 seconds (100%), while in the experimental series the plasma prothrombin time was 16.2 seconds (74%). The chief difference between these 2 plasmas was the age at the time of testing. The one-stage plasma determination in the experimental series was made within 5 minutes after venipuncture, while in the control plasma the test was not made until 40 minutes after venipuncture. Other studies have shown that during the first 30 to 45 minutes after blood is collected, the prothrombin time of citrated plasma gradually shortens. These findings are in accord with those of Owren(12), and indicate the importance of the time elapsing between collection of blood and performance of the one-stage test. While prothrombin times longer than 11 or 12 seconds on normal human plasma by the usual method are generally attributed to a relatively inactive thromboplastin, they may actually be due to the prompt testing of freshly collected plasma. Further studies are needed to determine whether the changes in plasma prothrombin time immediately after venipuncture are due to the same factor(s) that causes hyperactivity of serum in the one-stage test.

Summary. 1. A comparison was made of the changes in prothrombin during clotting, as indicated by the one- and 2-stage methods.

2. By the 2-stage method, progressive disappearance of prothrombin from serum was observed. Prothrombin utilization was slower in human blood than in dog blood, and was delayed greatly in canine hemophilic blood, platelet-poor human plasma, and in blood clotting in silicone-treated glassware.

3. By the one-stage method, an initial period of hypoactivity in the plasma was followed by a hyperactive phase in the serum. At the peak of hyperactivity, "prothrombin" values were about 180% of the control plasma. In slowly clotting bloods, the hyperactive phase developed less rapidly and persisted for a longer period than in normal blood. The abnormally high serum prothrombin values obtained by the one-stage test appear to be due to the evolution and persistence of the recently recognized serum factor which accelerates thrombin formation. Apparently this factor does not influence the 2-stage serum prothrombin values.

Received April 18, 1950. P.S.E.B.M., 1950, v74.

A Diabetes Insipidus-like Condition Produced in Dogs by a Potassium Deficient Diet. (17928)

SUSAN GOWER SMITH AND THOMAS E. LASATER
(Introduced by D. T. Smith)
From the Department of Neuropsychiatry, Duke University School of Medicine, Durham, N. C.

It is well known that administration of desoxycorticosterone acetate, an indirect means of reducing the serum potassium level, results in the production of a syndrome in dogs of polydipsia and polyuria resembling diabetes insipidus(1-3). To our knowledge the effect of direct dietary withdrawal of potassium on the fluid exchange has not been reported.

Twelve dogs housed in metabolism cages were placed on basic diets* containing approximately 0.01% potassium. The details

1. Kuhlmann, D., Ragan, C., Ferrebee, J. W., Atchley, D. W., and Loeb, B. F., *Science*, 1939, v90, 496.

2. Mulinos, M. G., Spingarn, C. L., and Lojkin, M. E., *Am. J. Physiol.*, 1941, v135, 102.

3. Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchley, D. W., and Loeb, R. F., *Am. J. Physiol.*, 1941, v135, 230.

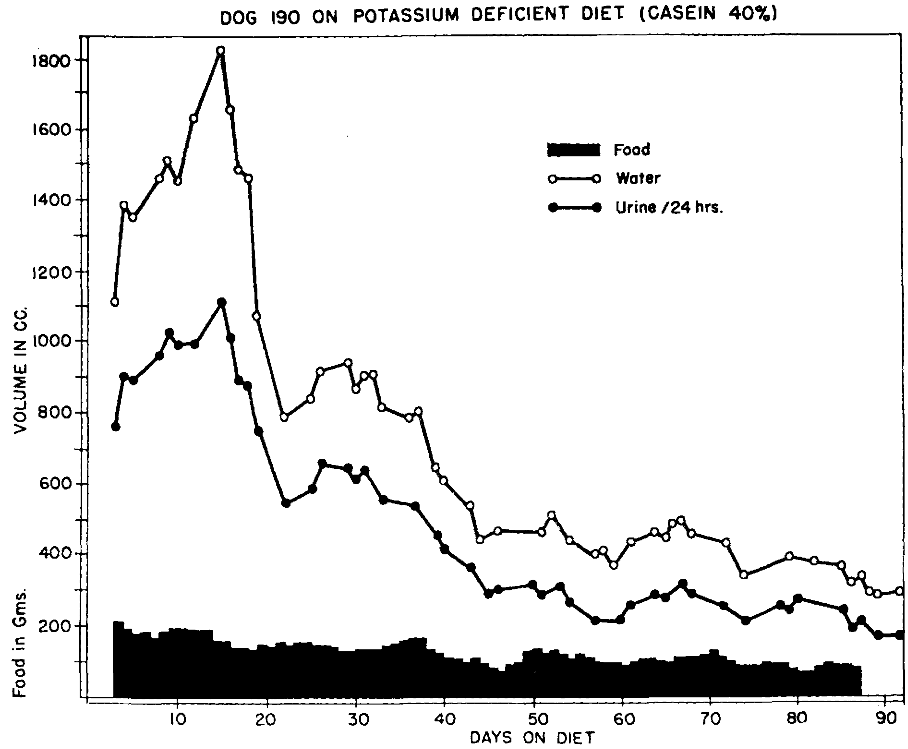

FIG. 1.
The fluid exchange (water intake and urine output in ml) of a dog on the 40% casein diet is expressed as a moving mean plotted against the time in days. The food is expressed as the actual value indicated on a given day.

of these diets* were given in a previous communication(4). Five additional dogs served as controls, 2 receiving potassium at a level of 0.24% as potassium chloride introduced into the salt mixture. The other 3 received potassium at approximately the same level but supplied in the form of brewer's yeast at a level of 10% replacing an equivalent amount of sucrose. The yeast also supplied any possible missing B-complex factors of the diet. As far as the fluid exchange was concerned there was no difference in the two control diets but the general well being was far superior in the yeast-fed dogs. Those receiving the yeast always lived throughout the experimental period and remained in excellent condition whereas the controls receiving the 8 synthetic B-complex factors plus potassium chloride usually were in poor condition and died during the experimental period, the average longevity of 6† dogs being 43 weeks. Two of the yeast control dogs have been allowed to continue on the experimental diet for periods of 5 and 6 years respectively. They are both living and in excellent condition.

When the 24-hour urine volumes (15 - 520 cc) of potassium depleted dogs after various periods on the diet were compared with those

* Composition of basic diets (1) high protein; casein 40%, sucrose 44, cotton seed oil 10; cod liver oil 2, salt mixture 4% (2) low protein: casein 20, sucrose 64, cotton seed oil 10, cod liver oil 2, salt mixture 4%. The fat soluble vitamins A, D, E, and K dissolved in oil; the B-complex factors, thiamin, riboflavin pyridoxine, nicotinic acid, pantothenic acid, para-aminobenzoic acid, inositol and choline dissolved in water were fed individually to each dog.

4. Smith, S. G., Black-Shaffer, B., and Lasater, T. E., *Arch. Path.*, 1950, v49, 185.

† Two of these dogs were used in this investigation and 4 in a previous study.

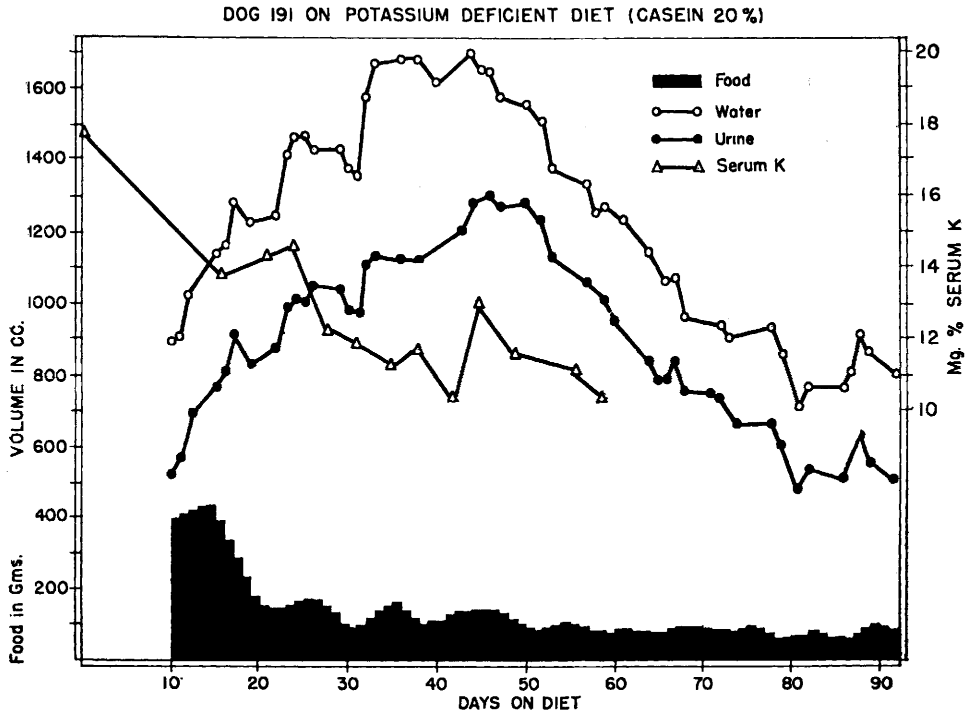

FIG. 2.
The fluid exchange of a dog on the 20% casein diet expressed as a moving mean against time in days. The food and serum potassium values are expressed as the actual value on a given day.

(10-1665) of controls receiving potassium, the former were usually much greater, but because of the tremendous variation in output the differences were often not statistically significant. It seemed likely, however, that the urine excretion was following a pattern that might be consistent if discernible. We, therefore, used a moving mean‡ to smooth out the curve and thus show the pattern. The first diet used in these experiments contained 40% casein, which was later reduced to 20% at the expense of the carbohydrate (sugar) component. On both diets there was a sharp rise in volume beginning often within 24 hours after the animal was placed on the deficient diet. After the peak was reached the volume of output decreased just as rapidly as it had risen. Eleven of the dogs were studied in great detail, 3 on the high protein (40% casein) diet, 3 on the normal protein (20% casein) diet and 5 on the control diets. This is not a large series but the consistency of the results is rather impressive. The high (40%) casein diet showed the expected sharp rise with a peak at 13, 16 and 31 days respectively in these 3 dogs (Fig. 1). The normal protein of 20% casein resulted in a shift of the peak to the right at 37, 46 and 50 days respectively (Fig. 2). The control dogs showed a fairly smooth curve with a daily urine output averaging less than 300 cc while one potassium deficient dog went as high as 1710 cc (Fig. 3).

The urine volume excreted by the different dogs is about what would be expected if the pattern described is followed consistently. The

‡ The moving mean as applied in this study consisted of the averaging of the first 5 values for urine output, etc. The mean obtained from the sum of these figures would then correspond with the 3rd figure. The 1st figure is then dropped, the 6th added and a mean obtained which in turn corresponds with the 4th figure and so on through the data. In this way (using 5 figures) 4 points are sacrificed, two at the beginning and 2 at the end of the curve but it is smoother and reflects the trend more clearly.

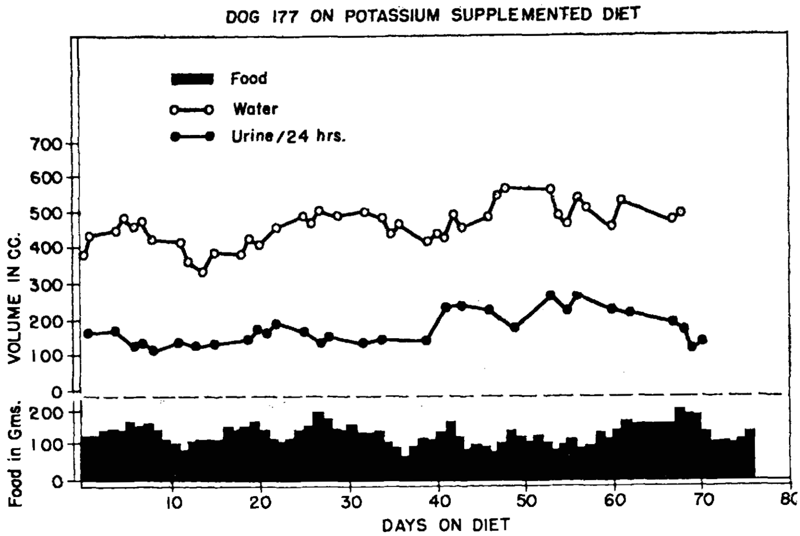

FIG. 3.
The fluid exchange of a potassium control dog represented as a moving mean against time expressed in days. Food is expressed as the actual value observed on a given day.

performance of Dog 6 is interesting in this respect. Eleven 24-hour urine volumes taken after 9 weeks on the deficient diet averaged 327 cc. The dog later became paralyzed, was placed on the potassium control diet for 6 days during which time the following consecutive daily volumes were recorded, 120, 535, 770 and 900 cc per day. The dog was then switched back to the potassium deficient diet and the next eleven consecutive volumes recorded averaged 1049 cc per day.

The urine excretion of another dog recorded during the 24 hours preceding a paralytic attack was 960 cc. At the time of the attack, May 11, the dog was treated with biotin (400 mg every hour for 9 hours), then maintained on biotin (800 mg per day given subcutaneously in 2 - 400 mg doses) for a period of 10 days. The next 5 days on which urine was measured (May 14 - 18 inclusive) the volumes excreted were 555, 590, 645, 570 and 500. Then the volume began to increase and rose to 1105 on May 21st. During that day the dog experienced another paralytic attack which was treated with potassium chloride (5 g orally). Urine volumes recorded from May 23 - June 4 were as follows: 970, 1320, 1740, 1520, 1490, 840, 1725, 1235, and 1140 cc/24 hr.

In our earlier studies it was observed that the dogs usually became paralyzed well after the tendency to polydipsia and polyuria had spent itself and the curve of urine output had reached a plateau within or very near the normal level. Biotin was found to reverse the paralytic process though less satisfactorily than potassium. The dog cannot be maintained on biotin free from paralytic attacks for more than 2 weeks. To determine the effect of biotin on the fluid exchange, 4 dogs were treated with 800 mg per day subcutaneously for 2 weeks. In each case the average urine volume dropped 51.7, 39, 33 and 69% respectively for the first week but for the second week the average was as high or higher than the predosing level. In the 2 control dogs treated with biotin there was no consistent change.

As the urine output and water consumption increases the food consumption and the level of serum potassium decreases. The serum potassium falls gradually from a normal value of 17 - 20 mg % to 10 - 12 mg % where it remains for the most part until the dog becomes paralyzed. The paralysis in most cases starts a new cycle. The period of depletion is so long that no dog has been followed metabolically for the entire time but isolated instances indicate that the polydipsia and polyuria start up again and are unchecked by the potassium therapy which so effectively controls the paralytic episodes. This was also true of the diabetes insipidus and paralysis produced in dogs receiving desoxycorticosterone acetate(3).

The pH of the urine remained the same in a given dog, on all diets and during the successive changes in fluid exchange varying from 8.2 - 9.2.§ These values were obtained daily on 3 animals for 10 days on the yeast control period—then for a similar period after being placed on the potassium deficient diet—and later once a week.

Discussion. An unusual pattern of polydipsia and polyuria has been observed in dogs following the withdrawal of potassium from a synthetic diet. The onset of this increased fluid exchange begins rather precipitously after placing the dog on the deficient diet. The peak of the water intake and urine output occurs usually in 3 - 7 weeks while a gradually progressive dehydration takes place during the entire period of potassium depletion. The polydipsia and polyuria seems to bear no relation to the blood potassium level. Nor does this phenomenon parallel the usual clinical symptoms or signs of potassium deficiency as previously described(4). The acute spasticity and progressive paralysis observed usually occur long after this heightened fluid exchange has subsided. No adequate explanation for this abnormal fluid exchange has yet been found. It could be related to an electrolyte readjustment scheme initiated by the organism for the purpose of preserving normal osmotic relationships(5). The transient nature of this phenomenon during the continued course of potassium depletion suggests the possibility of mediation through the adrenal cortex(6,7). Should the process depend upon a hormone of the cortex the cessation could be explained by the depletion of some essential component necessary for the elaboration of this hormone. More data are needed to test these hypotheses and experiments are now in progress to this end.

§ We are grateful to Dr. Hilda Pope for having these determinations made in her laboratory.

Conclusions. Dogs depleted of potassium show a marked increase in fluid exchange. The increase begins usually within 24 hours after being placed on the potassium deficient diet provided the dog is healthy at the start of the experiment, a condition we have assured in our dogs by placing them on a yeast control diet for 3 weeks preexperimental period. A peak in the curve is reached after 3 - 7 weeks and then there is a gradual decline back to nearly normal values. Factors responsible for this phenomenon are still undetermined.

We gratefully acknowledge that: The inositol, choline, menadione, vi-delta liquid concentrates of vitamins A and D and the material used for immunizing the dogs against distemper were furnished by the Lederle Laboratories through the courtesy of Drs. J. H. Jukes and Charles R. Schroeder.

The brewer's yeast was supplied by Standard Brands, Inc., through the courtesy of Dr. Charles N. Frey.

5. Gamble, J. L., Chemical Anatomy, physiology and pathology of extra-cellular fluid, a lecture syllabus, Harvard University Press, Cambridge, Mass., 1949.

6. Selye, H., *J. Clin. Endocrinology*, 1946, v6, 117.

7. Thorn, G. W., The diagnosis and treatment of adrenal insufficiency, page 6, Charles C. Thomas, 1949.

Received May 9, 1950. P.S.E.B.M., 1950, v74.