Considerable evidence indicates that the toxic action of heavy metals may be due to combination with SH enzymes.[5,6] That at least some of the organic compounds mentioned above, specifically safranine, acriflavine and the styrol quinoline compound, may likewise act as inhibitors of enzyme systems and in this way exert their damaging action on the kidneys in a manner paralleling the action of the heavy metals biochemically as well as pathologically, is indicated by Dickens'[7] demonstration of a striking and selective inhibition of the normal aerobic inhibition of fermentation by extremely low concentrations of phenosafranine, the chemical structure of which is closely related to that of safranine, and he has demonstrated a like action of acriflavine as well as a quinoline relative of the styrol quinoline compound No. 90.

*Summary.* The intravenous administration of the dye, safranin O, to rabbits and dogs produces selective renal tubular necrosis, involving predominantly the proximal convoluted tubules. The histologic picture very closely resembles that observed in heavy metal poisoning.

Safranin O, in a dose of 10 mg per kg, produces minimal histologic injury in the kidney in rabbits, which is readily and rapidly repaired. The intravenous administration of 20 mg per kg is followed by more extensive and severe necrosis of the proximal convoluted cortical tubules, which is, in perhaps half the animals, reflected in elevation of the blood NPN. Increasing the dose to 40 mg per kg results in almost complete necrosis of the renal proximal convoluted tubular epithelium, as well as some damage to the distal tubules; this dose is probably uniformly fatal to rabbits. With all amounts of the substance, noteworthy lesions of other organs than the kidney, in the absence of examination of the brain, appear to be limited to a relatively mild degree of fatty degeneration and sinusoidal congestion of the liver.

The suggestion is made and evidence cited that the nephrotoxic action of safranin, as well as that of certain other nephrotoxic organic compounds, parallels the action of heavy metals biochemically as well as pathologically, and that these organic compounds exert their nephrotoxic effect by acting as inhibitors of vital enzyme systems.

[5] Peters, R. A., and Thompson, R. H. S., *Nature*, 1945, **156**, 616.

[6] Waters, L. L., and Stock, C., *Science*, 1945, **102**, 601.

[7] Dickens, F., *Biochem. J.*, 1936, **30**, 1233.

## 15285

## Potassium Deficiency in the Dog.*

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Recent reports by Lambooy and Nasset[1] and Smith[2,3,4] have indicated that factors, in addition to the better known nutrients, are necessary for optimum growth, prevention of paralysis, maintenance of a healthy skin and prevention of anemia in the dog. We have found a basal ration containing only the 6 water soluble vitamins (thiamin, riboflavin,

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We are indebted to Merck and Company, Rahway, N. J., for the synthetic vitamins and to Abbott Laboratories, North Chicago, Ill., for haliver oil.

[1] Lambooy, J. P., and Nasset, E. S., *J. Nutrition*, 1943, **26**, 293.

[2] Smith, S. G., *Science*, 1944, **100**, 389.

[3] Smith, S. G., *Am. J. Physiol.*, 1944, **142**, 476.

[4] Smith, S. G., *Am. J. Physiol.*, 1945, **144**, 175.

TABLE I.

| | Basal ration (Smith) | Basal ration (modified Street basal) |
|---|---|---|
| Sucrose | 36% | 66% |
| Casein | 40 | 19 |
| Cottonseed oil | 18 | 11 |
| Cod liver oil | 2 | 0 |
| Salt mixture | 4 | 4 |
| Thiamin | 1.4 mg/dog/day | 0.1 mg/kg/day |
| Riboflavin | 0.7 " " " | 0.1 " " " |
| Nicotinic acid | 6.0 " " " | 2.0 " " " |
| Ca-d-pantothenate | 6.0 " " " | 0.5 " " " |
| Pyridoxine | 6.0 " " " | 0.06 " " " |
| Choline chloride | 100.0 " " " | 50.0 " " " |
| Inositol | 100.0 " " " | 0 " " " |
| p-aminobenzoic acid | 6.0 " " " | 0 " " " |
| Vit. A | 1800 I. U. " " | 1800 I. U./dog/day |
| Vit. B | 500 " " " | 500 " " " |
| Vit. E | ¾ cc mixed tocopherols/week (Lederle) | 0 |

| Smith salt mixture | | Salt mixture (Salts IV—Wis.) | |
|---|---|---|---|
| Bone meal, steamed | 57.8% | $CaCO_3$ | 32.34% |
| Sodium chloride | 24.4 | $K_2HPO_4$ | 34.73 |
| Lime stone | 12.2 | $CaHPO_4$ | 5.35 |
| Iron sulfate | 3.7 | $MgSO_4$ | 6.67 |
| Magnesium oxide | 1.2 | NaCl | 18.04 |
| Copper sulfate | 0.3 | $Fe(C_6H_5O_7)_2$ | 2.37 |
| Manganese sulfate | 0.1 | KI | 0.08 |
| Zinc oxide | 0.1 | $MnSO_4$ | 0.36 |
| Cobalt carbonate | 0.1 | $ZnCl_2$ | 0.03 |
| Potassium iodide | 0.1 | $CuSO_4$ | 0.02 |

nicotinic acid, pyridoxine, pantothenic acid and choline) together with vitamins A and D to be remarkably satisfactory for growing dogs.[5] In fact, excellent growth and blood regeneration have been obtained even when this ration contained levels of succinylsulfathiazol as high as 4%.[6] Previous results[5] have indicated that these differences are not due to variations in the carbohydrate, fat and protein content of the rations nor to the addition of p-aminobenzoic acid and inositol. However, the low choline level in Smith's ration, even in the presence of high protein, might be a limiting factor. In recent work, we[7] have observed a mortality of 75% in dogs receiving low levels of vitamins in which the choline level was comparable to that fed by Smith (10 mg/kg body weight/day) and autopsy revealed a fatty infiltration of the liver. When the choline level was raised to 50 mg/kg body weight/day, the animals grew normally and remained in good health.

With this information at hand, an experiment was undertaken in which Smith's basal ration (Table I) was duplicated as nearly as possible and fed to a litter of 6 8-week-old pups. For purposes of comparison the composition of the basal ration used in our previous work is also given in Table I. Two of these animals (dogs 1 and 2) received added amounts of choline (50 mg/kg body weight/day) and one dog (dog 6) was fed 7 μg of biotin/kg body weight/day. All animals were fed ad libitum and received an aqueous suspension of the B vitamins twice a week administered by pipette. Three ml blood samples were obtained from the radial vein on the same day every week before the morning feeding, and the hemoglobin

5 Ruegamer, W. R., Michaud, L., Elvehjem, C. A., and Hart, E. B., *Am. J. Physiol.*, 1945, **145**, 23.

6 Michaud, L., Maas, A. R., Ruegamer, W. R., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 148.

7 Unpublished data.

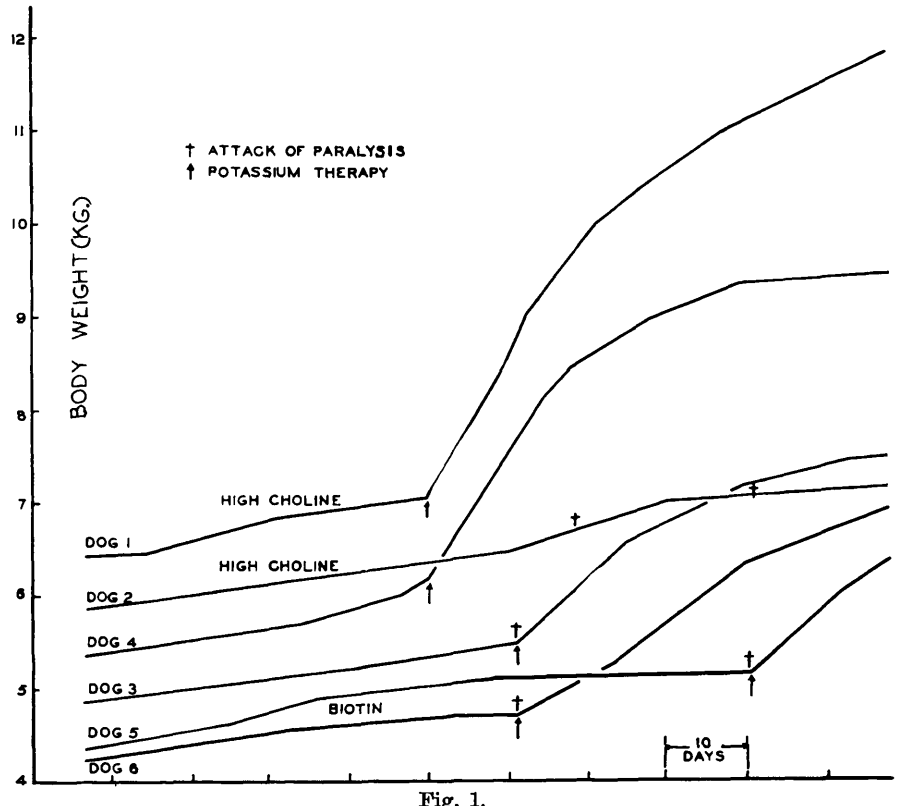


**Fig. 1.**
The growth response of dogs on the Smith basal ration with and without added potassium.

level determined by the method previously used.[6]

*Results.* All animals showed poor growth and a plateau in the growth curves was soon evident (Fig. 1). After a few weeks the animals became restless and began to develop symptoms very similar to those described by Smith. At this time, the ration was checked and found to be very low in potassium. In spite of the fact that Smith mentions the administration of potassium chloride to one animal in 100 mg doses every hour for 7 hours without apparent effect, it was decided to try potassium in the case of two of the animals. A single dose of potassium chloride (3 g) was given by capsule to dogs 1 and 4, and the animals continued on the Smith ration but the salt mixture was changed to contain 160 g of KCl/1000 g of salt mixture. An immediate growth response occurred which continued for 30-40 days. One of the animals (dog 1), which received the potassium supplement had been on the high choline level from the start of the experiment and had exhibited no growth until potassium therapy was started.

The remainder of the animals were kept on the Smith basal ration until dog 6, receiving supplements of crystalline biotin, developed a moderate attack of paralysis. The paralysis first started in the muscles of the neck and the animal developed what might be termed a "stiff neck" and was unable to hold its head erect. Several hours later, the animal was given 3 g of potassium chloride by capsule and was placed on the potassium supplemented ration, as in the case of dogs 1 and 4. This animal likewise showed a very marked growth response of approximately 200 g per day, and the paralytic symptoms disappeared completely.

Two days later, dog 3 developed a neck paralysis which grew progressively worse until the animal became severely paralyzed

about 10 hours later. The rear legs were completely paralyzed and the front legs became so weak that the dog was unable to raise itself from the bottom of the cage. At this point, 3 g of potassium chloride was given by capsule and one hour later the animal was able to stand and walk around. This animal was also placed on the potassium supplemented Smith ration and failed to develop any recurrent attacks of paralysis.

Dog 2 (high choline ration) showed 2 attacks of paralysis which were relatively mild and involved only the neck region. The first attack suffered by dog 5 (low choline ration) involved only the neck, but the second attack was quite severe. Three g of potassium chloride was given by capsule and the dog was placed on the potassium supplemented ration. This animal responded well to the potassium therapy and experienced no further transient attacks.

Hemoglobin values ranged between 15 and 16 g per 100 cc of blood just before the animals developed paralysis. Since these animals were only 3½-4 months old at this time, it is possible that a hemo concentration existed as a result of a salt imbalance. After the administration of potassium, the hemoglobin levels dropped to 13-14 g per 100 cc.

As the experiment progressed and before potassium supplements were given, the teeth of all dogs became discolored and the enamel appeared to be eroded. With potassium therapy this condition appeared to improve somewhat but recovery was not complete.

*Discussion.* When Smith's ration was duplicated as nearly as possible and fed to littermate dogs, all animals ceased to grow and eventually developed paralysis. When potassium therapy was started all dogs demonstrated a rapid growth response of 200-250 g per day and the paralysis disappeared.

It is interesting to note that choline appears to have no effect on the deficiency syndrome observed, since added amounts of choline failed to stimulate growth and prevent the onset of paralysis. It should be pointed out that even though dog 6 received 7 μg of crystalline biotin per kg body weight per day throughout the experiment, this animal suffered attacks of paralysis and failed to grow until potassium therapy was started.

From these data, it would appear improbable that a multiple deficiency developed since the administration of potassium alone brought about an immediate growth response of 200-250 g per day. If there had been a multiple deficiency, it would seem that there should have been an initial response to potassium and that further deficiency would have prevented the animals from making such dramatic weight gains over such an extended period.

The cardiac failure observed so frequently by Smith is another point in favor of an existing potassium deficiency since cardiovascular lesions have frequently been found in rats fed diets deficient in potassium.[8]

In the light of this work, it is difficult to explain the results obtained by Smith with biotin. However in all cases, the biotin seemed to have only a temporary beneficial effect, and the animals soon relapsed into a state of paralysis. Only when the animals were placed on a ration containing 10% yeast which would contain ample amounts of potassium, did the animals recover completely from the paralysis and return to a normal state of health. It is possible, though unlikely, that the administration of potassium increases the intestinal synthesis of biotin, which is actually the curative agent and that our biotin dog did not receive sufficient amounts of crystalline biotin to prevent paralysis.

If the deficiency syndrome observed is due to a potassium deficiency, the paralysis might be explained on the basis of a breakdown in certain enzyme systems. Nachmansohn *et al.*[9] have suggested that potassium seems to be concerned with *in vivo* enzyme systems responsible for phosphorylation. This theory was further substantiated by the work of Boyer *et al.*[10] who found that potassium markedly accelerates the transfer of phosphate from 3-phosphoglycerate to creatine in

[8] McCollum, E. J., Orent-Keiles, E., and Day, H. G., *The Newer Knowledge of Nutrition*, 5th ed., The Macmillan Company, New York, 1939, 203.

[9] Nachmansohn, D., and John, H. M., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 361.

[10] Boyer, P. D., Lardy, H. A., and Phillips, P. H., *J. Biol. Chem.*, 1943, **149**, 529.

homogenized fresh muscle, and that potassium is necessary for the phosphorylation of creatine to accompany pyruvate oxidation by animal tissues. Thus the paralysis may be due to a breakdown in the enzyme system responsible for muscular contraction.

Potassium therapy has been of some value in clinical investigations of certain types of paralysis. Aycock[11] has recently reported a case of familial paralysis in which the administration of potassium brought about a prompt recovery from the paralysis. Likewise, potassium chloride has been found to have a mild effect on the symptoms of myasthenia gravis. Several dog breeders and veterinarians have called our attention to the frequent existence of a paralysis in animals maintained on commercial dog foods. The possibility of a potassium deficiency existing in these rations is being studied at the present time.

*Summary.* In order to study the paralysis described in dogs by Smith, a ration similar to that described in her work was prepared and fed to growing dogs. When placed on this ration, our animals failed to grow and developed a paralysis which was curable with potassium. Even though Smith has obtained cures with the administration of biotin, one of our animals receiving biotin became paralyzed and responded to potassium therapy.

[11] Aycock, W. L., and Foley, G. E., *Am. J. Med. Sc.*, 1945, **210**, 397.

## 15286 P

## Antigenic Relationship of *Shigella dispar*, Types I and II, to *Shigella paradysenteriae*, Boyd Type P143.

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Attempts to classify certain paradysentery-like bacteria have indicated a possible antigenic relationship between *Shigella dispar* and *Shigella paradysenteriae*, type P143.[1] In particular, a culture (No. 2-193) isolated by Major William H. Ewing, Sanitary Corps, United States Army, has been classified on various grounds as *Sh. paradysenteriae*, type P143,[2] *Sh. alkalescens*,[3,4] and *Sh. dispar*.[2] The present investigation was undertaken to attempt to clarify the relationship between *Sh. dispar* and type P143.

Four serotypes and several subtypes of *Sh. dispar* were previously reported.[5] Representative strains of these were employed. Type P143 was secured through the courtesy of Dr. K. M. Wheeler of the Connecticut Public Health Laboratories as his culture No. 573.[6] Antiserums were prepared by immunizing rabbits with living vaccines of this and of the type and subtype strains of *Sh. dispar*.

Cross agglutination tests indicated the existence of antigens common to type P143 and *Sh. dispar*, types I and II. This was confirmed by reciprocal adsorption of the cross-reacting serums, as shown in Table I. From these data were derived the partial antigenic formulae listed in Table II. The relationship between *Sh. dispar*, types I and II, and *Sh. paradysenteriae*, type P143, is apparent. While antigens *B*, *C* and *D* are apparently minor factors in P143, nevertheless, they appear to account for the strong agglutination (to titers of 1280 or 2560) of types I and II in antiserum P143.

Culture 2-193 is biochemically like *Sh.*

[1] Boyd, J. S. K., *J. Hyg.*, 1938, **38**, 477.

[2] Ewing, W. H., personal communication.

[3] Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 200.

[4] Neter, E., *J. Imm.*, 1945, **51**, 151.

[5] Carpenter, P. L., *J. Bact.*, 1944, **47**, 419.

[6] Wheeler, K. M., *J. Imm.*, 1944, **48**, 87.