in estimating the extent of atrophy it is evident that there is a greater net loss of creatine than can be explained simply on the basis of weight loss. Both weight loss and creatine loss are progressive with time up to 14 days after denervation.

15594

Potassium vs. Biotin in the Treatment of Progressive Paralysis in Dogs.

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In earlier reports from this laboratory[1,2] a progressive paralysis was described in dogs subsisting on a diet lacking some members of the B complex and very low in potassium, 0.006%. This paralysis was invariably fatal if untreated and ran its course in 12-24 hours after the progressive stage was established. The mineral lack was suspected as a contributing cause, but since the first dog treated with potassium chloride, 700 mg total dose (55 mg/kg), failed to improve and later responded to 1 mg total dose (80 μg/kg) of synthetic biotin, we then centered our attention on the significance of biotin.

While this work was in progress, Elvehjem and his colleagues[3] succeeded in curing with much larger doses of potassium several dogs that had become paralyzed while subsisting on the same deficient diet. Consequently, the next 3 dogs to become paralyzed in this laboratory were treated orally with potassium chloride in much larger doses, 2-5 g total dose (200-500 mg/kg) as compared with the 0.7 g (55 mg/kg) used in the first unsuccessful treatment. This dose level proved entirely adequate, resulting in prompt and complete reversal of the paralytic process.

There is a marked difference in the therapeutic effects of potassium and biotin on the paralysis even in the same animal (Fig. 1). In making therapeutic tests, only animals that had reached the progressive stage of the paralysis were used. In our experience, animals with neck symptoms only are subject to spontaneous recovery, and, therefore, are not reliable for such tests. The paralyzed animal is usually somewhat emaciated, markedly dehydrated, anuric and suffers from complete anorexia during the attack. After treatment with either biotin or potassium chloride and about the time there is evidence of clinical improvement, copious urination occurs.

In the potassium-treated dogs the reversal of the paralysis was very rapid after it was once initiated. There was usually a delay of 3-6 hours before any clinical improvement occurred in our potassium-treated dogs with a dosing schedule of 1 g of potassium chloride in water solution given orally at 30-60 minute intervals. It was rather dramatic to see a dog sprawled on the floor, apparently functionless in neck and all 4 extremities, suddenly stagger to his feet and in a matter of a few minutes regain function to the extent of being able to walk in an almost normal manner. Without further treatment there is a prompt return of appetite, usually some gain in weight and return to normal function while still subsisting on the deficient diet. A much smaller total dose of potassium is required to overcome anorexia than to overcome the paralysis (Fig. 1). A single adequate potassium treatment affords protection from paralysis for 6-10 weeks. The smallest dose curative of paralysis was 200 mg of potassium chloride per kg of body weight. One hundred mg per kg failed to cure, and intermediate amounts have not yet been tried.

In contrast to this, the biotin-treated dogs

1 Smith, S. G., *Science*, 1944, **100**, 389.

2 Smith, S. G., *Am. J. Physiol.*, 1945, **144**, 175.

3 Elvehjem, C. A., personal communication, December, 1945.

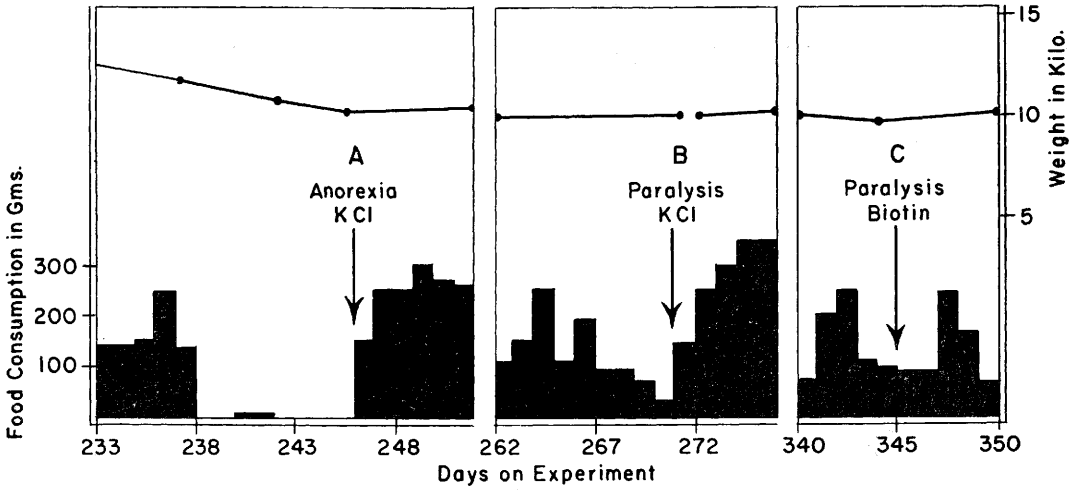

FIG. 1.

Showing the response of Dog No. 168 (weight, 10 kilo) when treated in consecutive attacks of paralysis with KCl and biotin respectively. A. After 9 days of almost total anorexia but no paralysis, 100 mg KCl total dose (10 mg/kilo) given subcutaneously resulted in prompt return of appetite. B. The first attack of paralysis treated with 5 g KCl total dose (500 mg/kilo) given orally, 1 g every ½ hour, resulted in complete reversal of the process 3 hours after treatment was initiated. C. The second attack of paralysis treated with a total dose of 2.4 mg biotin (240 μg/kilo) subcutaneously, 400 γ every hour, resulted in gradual reversal of process over a period of 13 hours. The animal was then maintained with biotin, 800 mg per day.

responded very gradually. There was usually a lag of 8 hours before improvement started even though the material was given parenterally, and during this time the symptoms actually became worse. There was then a gradual return of function over a period of 8-10 additional hours. The appetite returned from the state of complete anorexia during the attack to the low level of intake observed during the period preceding the attack. If no further treatment is given, the dog quickly relapses in from 4 to 48 hours, depending apparently on the vitality and general condition of the dog at the time of the attack.

During an attack of paralysis the serum potassium is definitely lowered. In 2 dogs with paralysis it was found to be 3.5 m eq per liter as compared with 5.36 in a control dog. The sodium, on the other hand, is slightly elevated, being 176.8 in one paralyzed animal as compared with 167.9 m eq per liter in the control.[4] Ferrebee *et al.*[5] have shown that in cases of familial periodic paralysis, a possible human analogue of this syndrome, the serum potassium is lowered during an attack and returns to normal after recovery.

Since the potassium therapy has proved so consistently effective in the treatment of this condition, we wanted to know whether there was any possible contamination with potassium of the biotin solutions used in the treatments reported earlier.[1,2] Fortunately, it was possible to assay the same solutions that were used in these treatments. They were found to be essentially negative although the reagent grade sodium chloride, employed for making the physiological saline (0.85%) used as solvent, according to analysis contained 0.01% potassium. On the basis of this, the saline solutions contained 0.85 μg potassium per cc. The largest total volume used was 50 cc, containing 42.5 μg of potassium. This amount obviously is insignificant as shown by the fact that solutions of biotin containing 4.3 μg of potassium were quite as effective in reversing the paralytic process as this one with 10 times the potassium content. More recently we have

[4] Smith, S. G., reported at meeting of Fed. Biol. Soc., Atlantic City, March 11-15, 1946.

[5] Ferrebee, J. W., Gerity, M. K., Atchley, D. W., and Loeb, R. F., *Arch. Neurol. and Psychiat.*, 1940, **44**, 830.

obtained positive results with biotin dissolved in distilled water and have failed to produce cures with as much as a gram (100 mg/kg) of potassium chloride given orally.

The Wisconsin investigators failed to get any protection against the paralysis by feeding biotin at a level of 7 μg per kg.[6] We found 50 μg per day completely inadequate to maintain a dog weighing approximately 7 kg.[2] When this amount was doubled, the dog was maintained satisfactorily for 2 weeks.

We have continued to get satisfactory therapeutic responses with biotin as far as the reversal of the neuropathological process is concerned. There is, however, little or no effect on the general condition of the dog, whereas the potassium not only reverses the process in a very short time but has a marked effect on the appetite and general condition. Potassium will maintain these dogs for a long time, possibly indefinitely. One dog has been maintained for 50 weeks and looks entirely normal.

It would appear then that potassium is the primary deficiency and that biotin works indirectly, possibly in mobilizing potassium temporarily or having a synergistic effect when the potassium reserves are too low to operate alone; or, as Ruegamer *et al.* suggests,[6] potassium may increase the intestinal synthesis of biotin. A relationship between biotin and potassium is an interesting possibility. Data are being collected to determine this.

[6] Ruegamer, W. R., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 234.

15595

Effect of Ascorbic Acid on Diabetogenic Action of Alloxan.

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Since it is known that alloxan will react with many substances, and because preliminary unpublished work in this laboratory suggested that ascorbic acid potentiated the action of alloxan, the effect of ascorbic acid on the diabetogenic action of alloxan was investigated.

Experimental. Hooded rats weighing approximately 250 g were used in this study. These animals were fed Purina Dog Chow during the course of the investigation. The animals were fasted 24 hours before the experiment in order to obtain blood samples.

Ascorbic acid was administered intravenously. It consisted of a solution of the free acid made up in distilled water so that 1 cc of solution contained 100 mg of the acid. Most of the animals which received the ascorbic acid, were given a 50 mg dose, while a few received 100 mg. *d*-Isoascorbic acid,* which was used as a control, was given at the same level as ascorbic acid. In all cases the intravenous injections were made in the tail of the rat. Following the immersion of the tail in warm water to dilate the veins, injection was easily accomplished using a 26-gauge needle.

Alloxan was administered intraperitoneally as a 5% aqueous solution in dosage of 100 mg per kg body weight. Previous trials had shown that this amount of alloxan is just below the level necessary to produce diabetes in the test animals.

Mapharsen (3-amino-4-hydroxyphenyl arsinoxide hydrochloride) was administered intravenously to a few rats at a dose level of 2 mg per rat.

* The *d*-isoascorbic acid used in these experiments was supplied, through the courtesy of the Charles Pfizer and Company, Brooklyn, N.Y.