CURE OF PARALYSIS IN RATS WITH BIOTIN CONCENTRATES AND CRYSTALLINE BIOTIN*

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In a previous paper (1) we showed that a "spectacle eye condition" in rats, produced by the addition of 10 per cent egg white to a purified ration containing synthetic B vitamins, could be prevented and cured by the addition of biotin. If the rats were continued on the egg white ration for 6 to 8 weeks after the "spectacle eye" symptom appeared, a typical paralysis or spasticity of the hind legs developed. Other workers (2–5), using various types of egg white diets, have reported varying degrees of paralysis. We wish to report in this paper that this syndrome also responds to biotin therapy. A definite increase in muscle creatine is associated with this biotin deficiency. Since Mannering et al. (6) reported a paralysis which is very similar to that seen in a biotin-deficient rat on a diet low in riboflavin and high in fat, the effect of varying the fat, riboflavin, and pyridoxine will also be reported.

EXPERIMENTAL

The basal ration had the following percentage composition: sucrose 73, Labco casein 8, commercial egg white 10, Salts 4 (7) 4, and corn oil 5. Choline hydrochloride was added to this basal ration at 1 gm. per kilo of ration. The B complex vitamins were fed daily in supplement dishes at the following levels: thiamine hydrochloride 20, riboflavin 20, pyridoxine 25, and calcium pantothenate 100 γ. 2 drops of haliver oil were given each rat per week.

At present we have produced this paralysis in thirty rats maintained on the ration cited. Since a biotin deficiency was indicated in these animals, a biotin concentrate† was injected at a level to supply 1 γ of biotin per rat.

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1 Biotin concentrate No. 5000 was obtained from the Research Laboratories, S. M. A. Corporation, Chagrin Falls, Ohio.
CURE OF PARALYSIS WITH BIOTIN

We have cured twelve rats of this paralysis or spasticity over a 2 to 3 week period of therapy. After these results were confirmed, it seemed desirable to investigate the effect of crystalline biotin. We had a group of four rats which showed severe paralysis. Two rats were given the methyl ester of biotin at a level of 1 γ per day for 10 days and the other two rats were given biotin (acid) at the same level for 10 days. The four rats were then observed over a period of 3 to 4 weeks. In all the animals, a complete cure was noted at the end of this period. In order to give some idea of the responses observed, Fig. 1 was taken at the end of the 14th week on a high fat ration (weight 138 gm.); Fig. 2 portrays the same rat after it had been given 1 γ of biotin for 10 days and then allowed an additional 16 days to recover (weight 190 gm.). The response to the free biotin seemed to be more rapid than that observed with the methyl ester of biotin. Since we did not feed limiting levels and since the methyl ester contains about 10 per cent of its total activity as free biotin, it is difficult to draw a definite conclusion, but it would appear that the rat does not utilize the ester as efficiently as the free biotin.

It was possible to maintain rats for 25 weeks on the ration described if at the 14th week 1 γ of biotin per day was administered for a 2 week period. At autopsy such animals at the 25th week showed no pathological changes other than those characteristic of a mild biotin deficiency. It would thus appear that biotin was the only limiting factor in this diet.

A fat-free ration was devised by replacing the corn oil in our ration with sucrose. Four rats were placed on this ration and the typical paralysis was noted in all the animals at the end of 9 weeks. Throughout these
studies we have had thirty rats on the ration described and the onset of this symptom appears in from 9 to 12 weeks. The level of fat in the ration was studied, since high levels of fat in a riboflavin-low ration increased this type of paralysis (6). The high fat ration employed had the following percentage composition: sucrose 28, casein 11, commercial egg white 14, Salts 4 (7) 6, and lard 41. Choline hydrochloride was added at 3 gm. per kilo of ration. This ration contains 41 per cent of fat and the sucrose was replaced isocalorically by lard. Six rats were maintained on this ration while the riboflavin supplementation was increased to 100 γ per day. The

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Period on ration</th>
<th>Final weight</th>
<th>Degree of paralysis</th>
<th>Creatine</th>
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<tr>
<td></td>
<td>wks.</td>
<td>gm.</td>
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<td>1</td>
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<td>3</td>
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<td>120</td>
<td>&quot;</td>
<td>4.61</td>
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<tr>
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<td>13</td>
<td>120</td>
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<td>140</td>
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<td>9</td>
<td>72</td>
<td>&quot;</td>
<td>4.44</td>
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</table>

* Basal ration; cured with biotin over a 3 week period.
† Basal ration with 1 γ of biotin given orally as a daily supplement.
‡ Basal ration with egg white replaced by casein.
§ High fat ration.
¶ Fat-free ration; cured with biotin.

onset of spasticity was materially lengthened, usually occurring in from 12 to 14 weeks. Three additional animals were maintained on the high fat ration but received a normal level of riboflavin. The onset of spasticity was 12 to 14 weeks. From these studies it appears that fat has a slight sparing action, in contrast to the results obtained in rats fed a riboflavin-low ration. Albino rats develop a biotin deficiency sooner than piebald rats, but piebald rats were used in all the studies reported in this paper.

The effect of riboflavin and pyridoxine was studied in the following manner. A group of four rats was placed on the basal ration with the
riboflavin supplementation increased to 100 \( \gamma \) per day. All the animals developed the paralysis in from 10 to 12 weeks. Another group of four animals was given 100 \( \gamma \) of riboflavin per day by intraperitoneal injection; again the onset of paralysis was not changed. Four additional rats were maintained on the basal ration, the riboflavin was increased to 120 \( \gamma \) (orally) per day, and pyridoxine was increased to 75 \( \gamma \) per day with no noticeable effect. In another group of four rats the pyridoxine supplementation was increased to 100 \( \gamma \) per day and the paralysis developed over a period of 8 to 11 weeks. Judging from the results of these sixteen animals it appears that riboflavin, pyridoxine, or the combination of the two vitamins had no effect on this paralysis.

Muscle creatine was studied in the following manner. The animals were killed by stunning and samples of muscle taken from the thigh of the hind leg. Creatine (total creatine) was determined by the method of Rose, Helmer, and Chanutin (8), with the following modification. A 1 ml. aliquot of the clear filtrate was diluted with distilled water to 10 ml. in an Evelyn tube, the alkaline picrate added, and the color read in the colorimeter with a No. 520 filter. This method is not specific for creatine but Baker and Miller (9) have shown that the error is probably less than 10 per cent in the analysis of skeletal muscle. The creatine values are given in Table I. The rats which were biotin-deficient gave higher creatine values. The animals which were partially cured by means of biotin administration gave values which were approaching the normal values obtained. The musculature of the biotin-deficient animals had a sinewy appearance, but did not have a lower water content.

**DISCUSSION**

A typical paralysis or spasticity of the hind legs develops in biotin-deficient rats. This paralysis has been produced very consistently by the inclusion of egg white in a synthetic diet which allows good growth in the absence of egg white. A similar paralysis has been produced in rats maintained on a riboflavin-low ration by Mannering, Lipton, and Elvehjem (6). Under their conditions high levels of fat in the diet exaggerated the paralysis. High levels of fat in our ration did not hasten the onset of this spasticity but had a slight protective action. The paralysis reported in this paper was not corrected by riboflavin. It appears that riboflavin is necessary for the optimum synthesis of biotin in the intestinal tract of the rat (unpublished data). Since riboflavin appears to be involved in the synthesis of biotin, the riboflavin-low ration may be a means of producing an imbalance or a mild biotin deficiency. Biotin-deficient rats are very sensitive to handling in the lumbar area of the spine and this is also characteristic of the riboflavin-deficient animals.
The muscle creatine of biotin-deficient rats is abnormally high. The administration of biotin decreases the muscle creatine. The paralysis observed is not due to low creatine levels, an abnormality found in muscle dystrophy of vitamin E-low rats investigated by Knowlton and Hines (10) and Telford, Emerson, and Evans (11).

Findlay and Stern (3) in studies with rats maintained on an egg white ration have reported a lesion in the spinal cord and slight but definite changes in the peripheral nerves. These investigators used a ration which was low in riboflavin and may have observed the lesions found by Shaw and Phillips (12) as being characteristic of a riboflavin deficiency. Our animals did not show any gross pathology of the nerves as is the case with riboflavin-deficient rats. The tissues of the biotin-deficient animals have been given to Dr. P. H. Phillips and the histological studies will be reported in a separate paper.

SUMMARY

1. A paralysis is produced in rats by the inclusion of low levels of egg white in a synthetic ration. The administration of biotin concentrates or biotin is specific for this syndrome.
2. High levels of fat in the diet slightly prolong the onset of paralysis.
3. Riboflavin, pyridoxine, or the combination of the two vitamins was without effect.
4. High creatine levels were observed in the leg muscle of paralytic rats.

BIBLIOGRAPHY

CURE OF PARALYSIS IN RATS WITH BIOTIN CONCENTRATES AND CRYSTALLINE BIOTIN
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