effectiveness in hemophilia thus does not compare with transfusion of either fresh blood or plasma. Furthermore, the toxicity of trypsin is great and extreme caution is advised in administering it intravenously. However, further investigations may be able to make its use safer and more effective.

14694

Biotin-Pantothenic Acid Interrelationship in Rats Fed Succinylsulfathiazole.

GLADYS A. EMERSON AND ELIZABETH WURTZ.

From the Merck Institute for Therapeutic Research, Rahway, N.J.

The feeding of so-called "purified" rations containing sulfaguanidine or succinylsulfathiazole to rats has a marked depressing effect upon growth; an inhibition which can be counteracted by the feeding of liver extracts.[1,2] Daft and co-workers[3] reported that this deficiency syndrome was ascribable in part to a lack of biotin. The biotin deficiency was not progressive, due in all probability to the coexisting lack of folic acid. These findings were confirmed by Nielsen and Elvehjem[4] and by Martin.[5] The only other means of producing biotin deficiency in the rat is either by the feeding of a purified diet containing egg white or a purified ration with casein as the source of protein but supplemented with an avidin concentrate: the deficiency state thus produced progresses and leads to the ultimate death of the animal.

Wright and Welch[6,7] have reported that folic acid and biotin are necessary for the utilization of pantothenic acid by rats receiving purified diets containing succinylsulfathiazole. Animals fed this type of ration gave evidence of pantothenic acid deficiency despite the inclusion of adequate amounts of this vitamin in their diet. Even parenteral administration of pantothenic acid failed to restore the levels of this vitamin in the liver to the normal. West *et al.*[8] observed changes usually associated with a lack of pantothenic acid in rats maintained on a sulfapyridine containing low protein diet. However, the feeding of excess pantothenic acid cured these deficiency manifestations.

In the course of an investigation dealing with the influence of succinylsulfathiazole upon the utilization of pantothenic acid it was noted that signs of biotin depletion were much more marked in rats receiving the pantothenic acid deficient diet than in controls supplied with this vitamin. At a time when the rats receiving pantothenic acid showed only incipient manifestations of biotin deficiency, namely, spectacled eyes and sparse mole-like fur, the rats fed the pantothenic acid deficient diet showed a more advanced state of biotin depletion such as sore mouth, ventral alopecia and encrustations. These animals also had the porphyrin caked whiskers and pattern greying associated with a lack of pantothenic acid. Curative therapy with biotin was initiated at a time when the rats were failing rapidly from a lack of folic acid. Although some improvement was seen, weight losses were continuous and only incipient

1 Black, S., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 308.

2 Welch, A. D., *Fed. Proc.*, 1942, **1**, 171.

3 Daft, F. A., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

4 Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **145**, 713.

5 Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 353.

6 Wright, L. D., and Welch, A. D., *Science*, 1943, **97**, 426.

7 Wright, L. D., and Welch, A. D., *J. Nutrition*, 1944, **27**, 55.

8 West, H. D., Jefferson, W. C., and Rivers, R. E., *J. Nutrition*, 1943, **25**, 471.

TABLE I.

Group	No. rats	Avg gain in wt, g		No. survivors,	Deficiency signs		
					Biotin		Pantothenic acid
		30 days	42 days	42 days	First observed days on test	Severity attained	First observed days on test
Diet H5 (Pantothenic acid deficient)	10	60	70	All	—	—	29
Diet H5S (Pantothenic acid deficient + 0.5% succinylsulfathiazole)	10	32	42	3	16	Marked	24
Diet H5S + 5 μg biotin	8	50	53	All	—	—	30
Diet H5S + 500 μg Ca pantothenate	8	91	97	All	28	Mild	—
Diet H5S + 5 μg biotin and 500 μg Ca pantothenate	10	118	135	All	—	—	—
Diet H5 + 500 μg Ca pantothenate	10	148	202	All	—	—	--

changes were noted in the pelage during the short survival period. Accordingly a prophylactic experiment was designed in order to show the effect of biotin, pantothenic acid, and the combination of both upon the production of the two deficient states.

Methods and Results. Weanling male rats were segregated into 5 dietary groups, each being represented by 8 to 10 animals as indicated in Table I. The pantothenic acid free ration (H5) had the following composition: vitamin free casein (Labco), 24; sucrose, 60; salts (U.S.P. No. 1), 4; hydrogenated vegetable oil, 10; cod liver oil, 2. The diet was supplemented with 1 mg each of thiamin and pyridoxin, 2 mg of riboflavin, 10 mg of inositol and 100 mg of choline per 100 g. In the diet containing succinylsulfathiazole (H5S) 0.5% of the drug replaced an equivalent amount of sucrose. Groups receiving biotin and calcium pantothenate were fed these vitamins daily by stomach tube.

As can be seen from Table I the feeding of the ration deficient in pantothenic acid which contained sulfathiazole had a marked effect in depressing growth. After 6 weeks 7 of an original 10 animals had succumbed. Signs of a mild degree of biotin deficiency, namely, spectacled eyes and sparse mole-like fur, were apparent in this group after only 16 days on test. This condition progressed rapidly to involve the ventral aspects of the head and trunk. Hopping gait was observed in one animal. Signs usually associated with a lack of pantothenic acid, namely, porphyrin deposits on the whiskers and pattern greying, were first observed after 24 days on the regimen. This is at a somewhat earlier period than with the pantothenic acid deficient diet without the sulfonamide.

The daily feeding of 5 μg of biotin evoked only a slight growth response; all animals, however, survived the 42-day test period. The rats were completely free from the syndrome associated with a deficiency of biotin. Moreover, the feeding of biotin not only prevented manifestations of biotin deficiency but also tended to delay the onset of pantothenic acid deficiency. Although achromotrichia was present porphyrin caked whiskers were seen in only one rat. It is of interest to note that Unna[9] did not observe porphyrin caked whiskers in rats receiving 40 μg of calcium pantothenate although greying was not prevented.[10]

When pantothenic acid was fed, changes ascribable to a deficiency of pantothenic acid were not observed. In addition the feeding of the vitamin had a marked growth stimulating effect. This observation indicates that calcium pantothenate can be utilized to some extent, a finding in variance with that reported by Wright and Welch.[6,7] However, in the present experiments succinylsulfathiazole composed 0.5% of the diet whereas in the ration of Wright and Welch, a higher level of the sulfonamide, namely 2%, was employed.

[9] Unna, K., *J. Nutrition*, 1940, **20**, 565.

[10] Unna, K., Richards, G. V., and Sampson, W. L., *J. Nutrition*, 1941, **22**, 553.

The feeding of calcium pantothenate alone had an attenuating effect upon biotin deficiency as reflected both in the time of onset of changes ascribable to biotin deficiency and in the severity of the lesions.

Rats receiving both biotin and calcium pantothenate were, at the termination of the experiment, completely free from the skin and pelage changes associated with biotin and pantothenic acid deficiencies. The fact that the growth did not equal that observed in the absence of the sulfonamide, could, in all probability, be ascribed to a lack of folic acid and other factors, since it has been shown that growth is restored to the normal by the feeding of folic acid concentrates.

Summary. Biotin deficiency induced in weanling rats by the feeding of a succinylsulfathiazole containing purified ration may be aggravated by superimposing a deficiency of pantothenic acid. The feeding of biotin protected against these changes and, in addition, appeared to lessen the severity of the syndrome associated with a lack of pantothenic acid. Calcium pantothenate, fed prophylactically, was completely protective against deficiency signs usually associated with the absence of pantothenic acid. Furthermore, signs of a mild degree only of biotin depletion were observed and the period required to produce such changes was extended beyond that observed in the absence of pantothenic acid. Rats receiving both biotin and calcium pantothenate were free from signs of both deficiency states.

14695 P

Congenital Malformations of the Eyes Induced in Rats by Maternal Vitamin A Deficiency.*

JOSEF WARKANY AND ELIZABETH SCHRAFFENBERGER.

From the Children's Hospital Research Foundation, Cincinnati, Ohio.

It has been repeatedly suggested that congenital blindness in animals may be caused by a maternal nutritional deficiency. Blindness in cattle associated with a constriction of the optic nerve of probable nutritional origin was reported by Moore *et al.*[1] who also reviewed the literature concerning this subject. Hale [2] reported the occurrence of anophthalmos and microphthalmos in pigs whose mothers had been fed a diet deficient in vitamin A. No corresponding findings have been reported in rats. Mason[3] who studied reproduction of vitamin A deficient rats described foetal death, prolonged gestation and difficult parturition but not congenital malformations of the offspring. Cannon[4] also reported failure to produce congenital anomalies in the young of rats whose mothers were depleted of vitamin A.

We have recently observed congenital defects of the eyes in the offspring of rats raised on a diet containing very small amounts of carotene and bred on a diet entirely free of carotene and vitamin A. The preparatory diet (Diet U) fed for approximately 3 months after weaning had the following percentage composition: Ground whole wheat, 74; crude casein (Borden), 15; brewer's yeast (Mead Johnson), 10; sodium chloride C.P., 1. This diet was supplemented by 60 I.U. of vitamin D (Drisdol, Winthrop) and 25 μg of carotene (SMACO) in cottonseed oil every tenth day. This small amount of carotene made possible slow growth and maturation but no storage

* This work was aided in part by a grant from the Nutrition Foundation, Inc., New York, N.Y.

1 Moore, L. A., Huffman, C. F., and Duncan, C. W., *J. Nutr.*, 1935, **9**, 533.

2 Hale, F., *J. Hered.*, 1933, **24**, 105.

3 Mason, K. E., *Am. J. Anat.*, 1935, **57**, 303.

4 Cannon, M. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 129.