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Failure of Succinylsulfathiazole to Aggravate Egg White Induced Biotin Deficiency in Rats.

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Manifestations of deficiency disease may be induced in the rat by the feeding of sulfonamide-containing purified rations.^{1,2} Daft and co-workers³ demonstrated that the syndrome was ascribable, in part, to a lack of biotin. Nielsen and Elvehjem⁴ confirmed this finding and, in addition, reported that a "folic acid" concentrate had an even more marked effect in reversing the action of the sulfa drug than did biotin. Martin⁵ and Welch and Wright⁶ likewise observed the counteracting effect of these vitamins upon the action of the sulfonamide.

Biotin deficiency in the rat can also be induced by the feeding of an egg white containing purified diet. The depletion signs thus produced may be prevented or overcome by biotin thus demonstrating the existence of a single deficiency. Untreated animals decline in weight, show a progressive alopecia and dermatitis and death finally ensues. On the other hand, the sulfonamide induced syndrome is complicated by a co-existing "folic acid" deficiency. Only the initial signs of biotin depletion (mole-like pelage and spectacled eye) are apparent; general alopecia and exudative dermatitis are not seen. The animals succumb from the dyscrasia^{7,8}

resulting from a lack of "folic acid".⁹

The experiments herein described were designed to demonstrate the influence of succinyl-sulfathiazole upon the progress of biotin depletion in rats receiving egg white containing diets with and without a source of "folic acid."

Experimental and Results. Two diets, employed routinely in the production of biotin deficiency, have been used in these studies. The rations were identical except that one contained 2% of dried beef liver replacing an equivalent amount of sucrose. This dried liver contained 2.4 μ g of biotin and 31 μ g of "folic acid" per gram.* Succinylsulfathiazole was incorporated in the rations as indicated at a level of 0.75%.

The composition of the diets is as follows:

	23	23S	24	24S
Dried egg white	15	15	15	15
Casein (Labco)	15	15	15	15
Sucrose	52	51.25	54	53.25
Salts (U.S.P. No. 1)	4	4	4	4
Hydrogenated veg. oil	10	10	10	10
Cod liver oil	2	2	2	2
Dried beef liver	2	2	—	—
Succinylsulfathiazole	—	0.75	—	0.75

All of the rations were supplemented with 0.8 mg each of thiamine and pyridoxine, 1.6 mg of riboflavin, 5 mg of Ca pantothenate, 10 mg of nicotinamide and 100 mg of choline chloride per 100 g.

Male rats, placed at weaning on the several diets, were observed for a period of 42 days. As can be seen from Table I the feeding of the sulfonamide in conjunction with the egg white liver containing ration (23S) did not shorten the depletion period required for the production of the syndrome ascribable to a

⁹ Daft, F. S., and Sebrell, W. H., *Public Health Rep.*, 1943, **58**, 1542.

* The "folic acid" determination was carried out with *Lactobacillus casei* and unitage was based on a "potency of 40,000."¹⁰

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. S., 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 Welch, A. D., and Wright, L. D., *J. Nutr.*, 1943, **25**, 555.

⁷ Spicer, S. S., Daft, F. S., Sebrell, W. H., and Ashburn, L. L., *Public Health Rep.*, 1942, **57**, 1559.

⁸ Kornberg, A., Daft, F. S., and Sebrell, W. H., *Science*, 1943, **98**, 20.

TABLE I.
Biotin Deficiency in Rats Fed Egg White-Succinylsulfathiazole Rations.

Diets (Groups of 5 male rats each)	Gain in wt, 42 days, g	No. survivors	Biotin deficiency first observed days
Diet 23	91	3	24
Diet 23 + 5 μ g biotin daily	238	5	—
Diet 23S	114	5	26
Diet 23S + 5 μ g biotin daily	229	5	—
Diet 24	87	5	24
Diet 24 + 5 μ g biotin daily	203	5	—
Diet 24S	72	4	24
Diet 24S + 5 μ g biotin daily	132	5	—
Diet 24S + 25 μ g "folic acid"	103	5	28
Diet 24S + 5 μ g biotin + 25 μ g "folic acid"	206	5	—

deficiency of biotin: furthermore the average weight increment was augmented slightly by the feeding of the drug. This "growth stimulating" effect of succinylsulfathiazole has been observed routinely in this laboratory with rats receiving purified diets containing liver. Alopecia and depression of growth were prevented by the prophylactic oral administration of 5 micrograms of biotin daily. Thus, it appears that succinylsulfathiazole, at the level fed, produced no untoward effects in rats receiving the egg white liver containing ration providing that biotin was supplied.

The sulfonamide had a profound inhibiting effect upon growth when fed in conjunction with the egg white containing ration in which the dried whole liver was replaced by sucrose (24S). The depletion period, however, was not shortened nor were the lesions more marked. The daily feeding of 5 micrograms of biotin prevented the appearance of the dermatitis ascribable to a lack of the vitamin but did not evoke a growth response comparable with that observed in the absence of the sulfonamide. The feeding of a "folic acid" concentrate, containing 0.20 μ g of biotin and 25 μ g of "folic acid"¹⁰ by microbiological assay, likewise produced only an incomplete growth response. However, when

5 μ g of biotin was fed daily in conjunction with this concentrate the growth response equalled that observed in the absence of the sulfonamide. The rate of growth was somewhat less than that noted with the dried liver containing diet (23) supplemented with biotin. However, the intakes of both "folic acid" and biotin were in excess of the amounts of these vitamins supplied in the 2% liver present in diet 23.

Summary. The feeding of succinylsulfathiazole in conjunction with an egg white containing diet with or without liver, did not aggravate the signs of biotin depletion in the rat nor was the period required to produce such deficiency manifestations shortened.

When biotin was administered in conjunction with the sulfonamide ration with liver, normal growth resulted. Biotin and a "folic acid" concentrate, when fed separately as adjuncts to the diet without liver, gave only minimal growth responses. The feeding of both vitamins produced a growth response that almost equalled that observed with rats receiving the liver containing ration supplemented with biotin.

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¹⁰ Mitchell, H. K., and Snell, E. E., *Univ. of Texas Publication*, 1941, **41**:37, 36.