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.

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

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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.

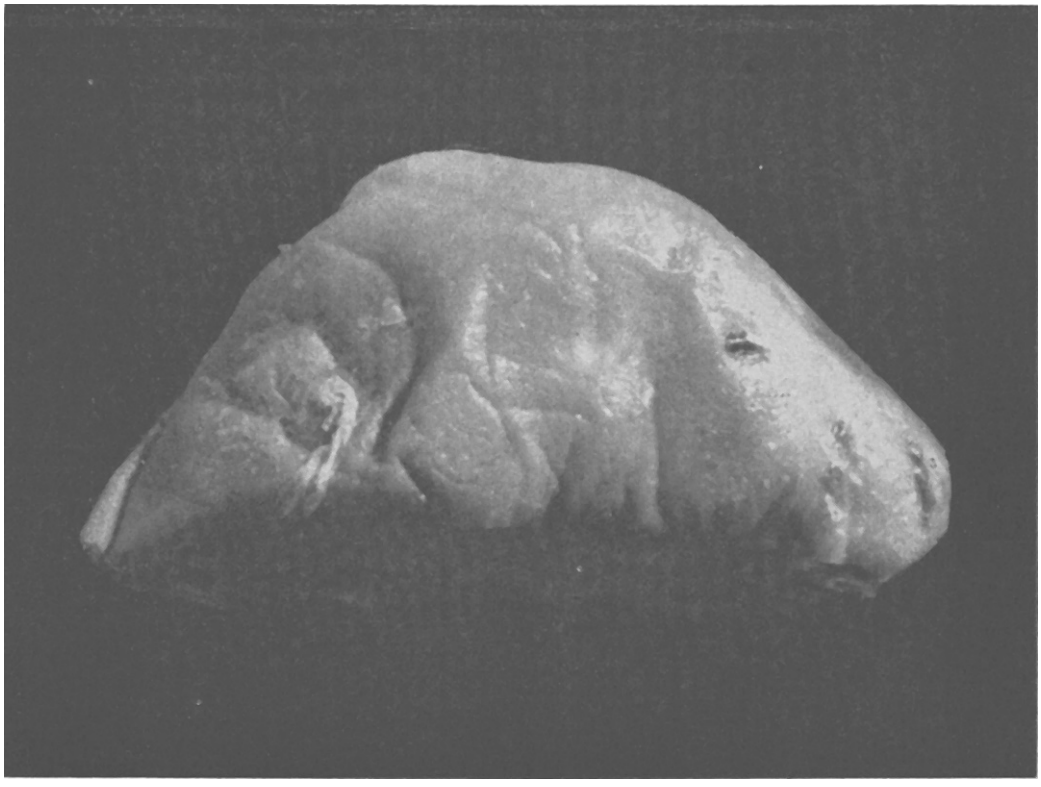

FIG. 1.
Showing abnormal "open eye" in newborn rat.

of the vitamin. If the growth curves of the rats became flat during this preparatory period, which happened about 3 or 4 times, each rat was given 5 g of horse muscle.

The purified diet (Diet W) which was completely free of vitamin A had the following percentage composition: Sucrose, 68; vitamin test casein, 18; vegetable oil, 10; salt mixture,[5] 4. One hundred grams of this diet were supplemented by 0.8 mg thiamine hydrochloride, 0.8 mg pyridoxine hydrochloride, 0.8 mg riboflavin, 1 mg calcium pantothenate, 10 mg niacinamide, and 100 mg choline chloride. This diet was supplemented every tenth day by vitamin D (1 drop of a 1:4 dilution with olive oil of Drisdol), vitamin E (1 drop of a solution of 5 g alpha-tocopherol in 100 ml of olive oil), and vitamin K (1 drop of a solution of 1 mg of 2-methylnaphthoquinone in 5 ml of olive oil).

[5] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutr.*, 1937, **14**, 273.

Twenty female rats of the Sprague-Dawley strain were raised on diet U until they reached a weight of 150-160 g and regular oestrus cycles were established. Then they were placed on the purified diet W and bred to males of the same strain. These males had been fed an adequate diet. Twelve of the females bred as was confirmed by the finding of sperm in the vaginal smears. Within a week the females fed diet W showed marked signs of nutritional deficiency and only three mothers carried their litters to or near term, while nine females resorbed their embryos. The three litters obtained by caesarean sections consisted of 19 dead young, all of which had abnormal eyes.

In 14 of these young the abnormality could be recognized by external examination. Instead of the closed eyes seen in normal newborn rats "open eyes" of a reddish color were seen (Fig. 1). These eyes as well as the closed eyes of 5 of the young were abnormal on histological examination.

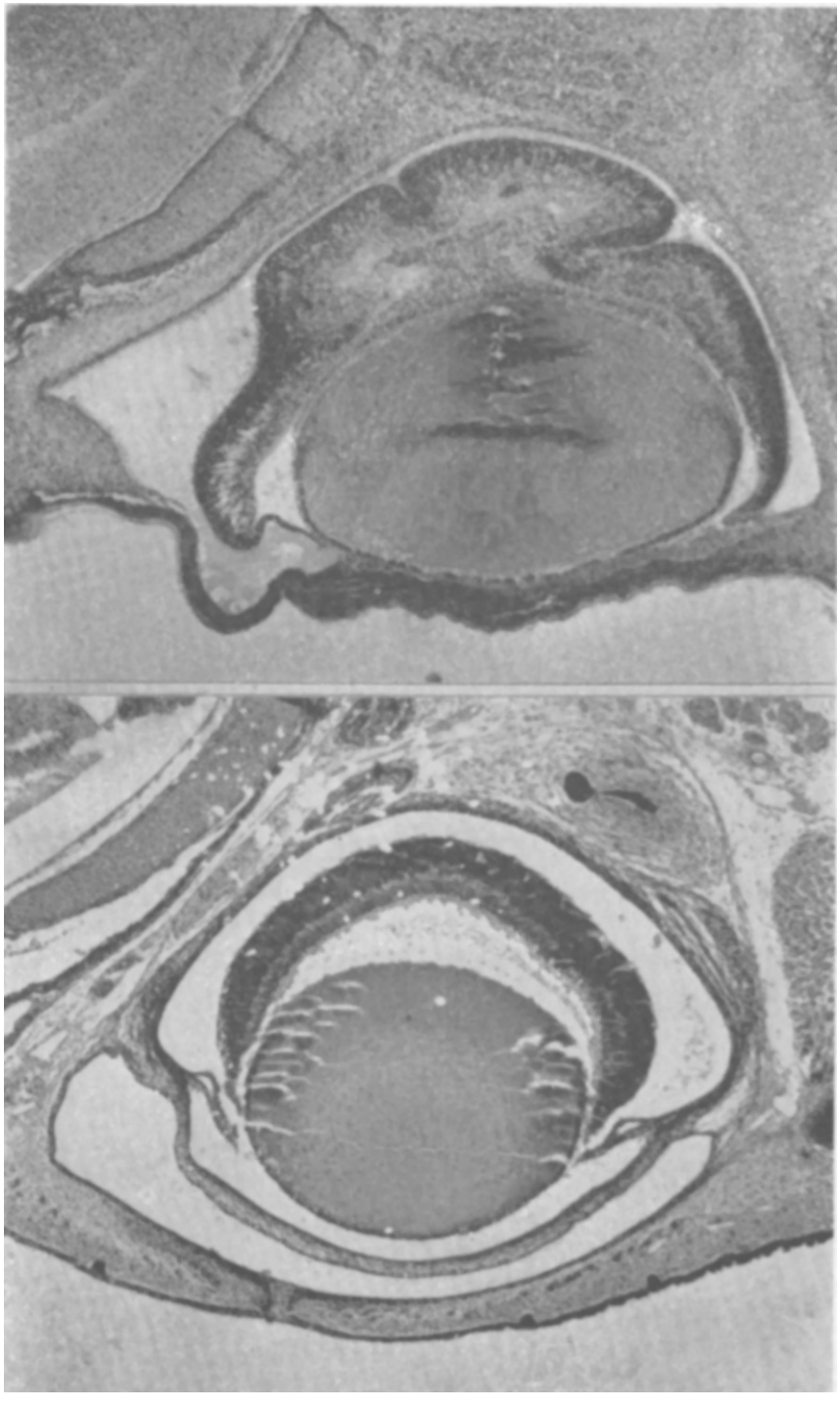

FIG. 2B.
Section of an abnormal "open eye." (× 70.)

FIG. 2A.
Section of the eye of a normal 19-day rat fetus. (× 70.)

Fig. 2A represents the eye of a normal 19-day rat fetus and Fig. 2B an "open eye." In the normal eye the lids, the conjunctival sac, the cornea, the anterior chamber and the iris appear as well defined separate structures and spaces. In the abnormal eye there is no

clear differentiation of the lids and the cornea; the anterior chamber is present in a rudimentary form only. In the normal eye the vitreous can be seen between the lens and the retina, while the same space in the abnormal eye is filled with connective tissue. The retina of the abnormal eye is folded and disorganized. In the closed abnormal eyes the lids are fused with the cornea and the anterior chamber appears as a linear space between the thick membrane thus formed and the lens. In several specimens a cleft can be seen in the inferior part of the retina and a strand of connective tissue which penetrates this cleft, spreads out in the space between the lens and the retina. This cleft represents a coloboma of the retina.

As controls 62 females of the same strain were fed diet W supplemented with vitamin A. They had 410 young, none of which showed abnormal "open eyes."

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Effect of Dietary Hepatic Injury on Inactivation of Estrone.

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Golden and Sevringhaus[1] observed in the rat that when a functioning ovary was transplanted into the mesentery, estrus did not occur. They concluded that estrogen which was thus routed through the portal circulation had been inactivated by the liver. Failure of the liver to inactivate estrogen has been demonstrated by Biskind and Biskind[2] in rats maintained on a vitamin-deficient diet. These workers found that ovariectomized rats receiving a normal diet showed no vaginal cornification after estrone pellets were implanted in the spleen, while animals on a diet deficient in the vitamin B complex went into estrus within 1 to 3 weeks. The occurrence of estrus was interpreted as an indication of impaired liver function. Estrus could subsequently be made to disappear by the addition of yeast to the diet.

In the liver disorder produced by Biskind and Biskind, no anatomical changes in the organ were observed. The experiments were therefore concerned with functional changes resulting from the avitaminosis. It is easily possible to produce severe hepatic necrosis and cirrhosis by the administration of diets low in protein and high in fat.[3] The following experiments were performed in order to determine whether the hepatic lesions so produced would result in failure of estrogen inactivation and whether evidence of repair could be detected by the disappearance of estrus during treatment. In an additional series of rats, the experiments of Biskind and Biskind with B-complex-free diets were repeated.

Methods. Rats used in the cirrhosis studies were of Sprague-Dawley strain. Those that received the B-deficient diet were of 3 separate strains. The animals weighed approximately 200 g. About 10 days after ovariectomy, 2 γ of estrone were injected in order to test the reactivity of the animal. If reactive, a 5 mg pellet of estrone was implanted in the spleen.* More than half the rats went into estrus immediately thereafter but, in most cases, this lasted only from 2 to 4 days.

[1] Golden, J. B., and Sevringhaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

[2] Biskind, M. S., and Biskind, G. R., *Endocrinol.*, 1942, **31**, 109.

[3] György, Paul, and Goldblatt, Harry, PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 492; Webster, Graham, *J. Clin. Invest.*, 1941, **20**, 440; Blumberg, Harold, and McCollum, E. V., *Science*, 1941, **93**, 598; Lillie, R. D., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1941, **56**, 1255.

* We are indebted to Dr. D. K. Kitchen of Parke, Davis and Co. for the supply of Estrone (Theelin).