linity of the specimen, producing alkaline hematin which does not respond; settling or entrainment of red corpuscles by the precipitating phosphates. 2. These factors may be controlled by pre-acidification and heating of the specimen, by storage for 12 to 24 hours at room temperature to increase hemolysis, or by centrifugation and making up to the original volume with distilled water to insure hemolysis. The latter treatment is recommended for urine samples which have been allowed to stand in the refrigerator.

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14046

Attempts to Produce Vitamin Deficiency Diseases by Feeding Compounds Related Structurally to Vitamins.

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It is now well known that it is possible to inhibit bacterial growth by addition to the medium of substances related structurally to certain vitamins. Thus sulfanilamide, which is the sulfur analog of the growth factor *p*-aminobenzoic acid,[1] pyridine-3-sulfonic acid, the analog of nicotinic acid,[2] and N-(β,β-dimethyl-α,γ-dihydroxybutyryl)-taurine, the sulfur analog of pantothenic acid,[3-5] have been shown to inhibit the growth of various bacteria. Since these inhibitions were overcome by supplying additional amounts of the vitamins concerned, it was considered that the inhibitions were due to deficiencies brought about in some way. We have attempted to produce vitamin deficiency diseases of animals by feeding the sulfur analogs of pantothenic acid and of nicotinic acid, and wish to report our observations since they differ from those observed with bacteria.

Symptoms of pantothenic acid deficiency of mice[6] were not observed when the animals were fed the sodium salt of N-(β,β-dimethyl-α,γ-dihydroxybutyryl)-taurine at a level 2000 times greater than the level of pantothenic acid in the ration. (For the sake of brevity, this substance is called sodium thiopanate.) Furthermore, symptoms of pantothenic acid deficiency were no more severe and appeared no sooner in mice fed a ration devoid of pantothenic acid and which contained thiopanate than in those fed the pantothenic acid deficient ration alone. Similarly, pantothenic acid deficiency symptoms were not observed in hamsters fed thiopanate. Both mice and hamsters require pantothenic acid for normal growth and health, and hence it had been expected that the analog which inhibited bacteria that require pantothenic acid would likewise cause disease in animals. However, this did not prove to be the case.

It was not possible to produce a disease in mice by the feeding of large amounts of pyridine-3-sulfonic acid. Since the mouse does not require an external source of nicotinic acid, the failure to cause symptoms of deficiency with this substance may be similar to the failure to inhibit bacteria which do not require the addition of nicotinic acid for growth.[2] With a species like the dog, which requires dietary nicotinic acid, the situation

1 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

2 McIlwain, H., *Brit. J. Exp. Path.*, 1940, **21**, 136.

3 Snell, E. E., *J. Biol. Chem.*, 1941, **139**, 975; **141**, 121.

4 Kuhn, R., Wieland, T., and Möller, E. T., *Ber. deutsch. chem. Ges.*, 1941, **74**, 1605.

5 McIlwain, H., *Biochem. J.*, 1942, **36**, 417.

6 Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 565.

TABLE I.
Effect of Pantothenic Acid, Nicotinic Acid, and Their Sulfur Analogs on Rate of Growth of Mice.

Calcium pantothenate, μg per 100 g ration	Sodium thiopanate, g per 100 g ration	Nicotinic acid, mg per 100 g ration	Pyridine-3-sulfonic acid, g per 100 g ration	Avg weekly gain, g
500	—	1	—	4
500	1	1	—	4
—	—	1	—	1.7
—	1	1	—	2.2
—	0.2	1	—	2.2
500	—	—	—	5
500	—	—	5	5
500	—	500	5	4

may be different, for Woolley *et al.*[7] have observed that dogs deficient in nicotinic acid were killed by pyridine-3-sulfonic acid, while normal dogs were apparently not harmed.

In the experiments with pantothenic acid, the following ration was used: sucrose 76 parts, vitamin-free casein (Labco) 18 parts, salts[8] 5 parts, fortified corn oil[9] 1 part. To each 100 g of ration were added thiamin 200 μg, riboflavin 500 μg, pyridoxin 200 μg, nicotinic acid 1 mg, choline 5 mg, inositol 100 mg, and calcium pantothenate 500 μg. The thiopanate ration was made by adding 1% of sodium thiopanate prepared from levo-β,β-dimethyl-α-hydroxybutyrolactone* according to Snell's directions.[3] Weanling male mice were fed these rations for 4 to 6 weeks and the rates of growth on the two rations were compared. Data from one experiment are shown in Table I. During the past 13 months, 402 mice fed the thiopanate ration have been observed. None of the characteristic symptoms of pantothenic acid deficiency in mice[6] were observed in these animals. When very young mice (14 to 16 days old) were fed the thiopanate ration, they did poorly for the first week, as evidenced by diminished rate of growth and rough fur. Occasionally a few such animals died. Following this period, normal growth was resumed. With mice older than 20 days, no difficulty was experienced. All of the thiopanate-fed mice showed diarrhea.

Sodium thiopanate was well tolerated intraperitoneally for, when a 50% solution was injected into 20 g animals, 8 of 8 survived after single doses of 0.2 cc, and 2 of 2 after 0.1 cc. Four of 4 mice which received 0.5 cc died within an hour following the injections, apparently due to the large dose of hypertonic salt solution. One hundred and thirty-seven weanling hamsters in a series of twelve experiments were fed the thiopanate ration, and the same number received the basal ration. Over a period of 6 weeks, no symptoms of pantothenic acid deficiency were seen in the thiopanate-fed animals.

To test the effect of sodium thiopanate on mice fed a ration deficient in pantothenic acid, the above basal ration minus calcium pantothenate was fed to a group of 6 weanling mice. A second group of 6 similar mice was fed the same ration, and in addition was given 25 mg per day of sodium thiopanate orally. A third group of 3 mice ate the pantothenic acid-free ration and were given 5 mg per day of thiopanate. The average rates of growth of the mice in each group are indicated in the table from which it may be seen that, as judged by this criterion, thiopanate did not prove harmful. The first characteristic symptom of pantothenic acid deficiency of mice is the extreme hyperirritability and violent jumping of the animals.[6] These symptoms appeared in the basal group on the 22nd day, in the group getting 25 mg of thiopanate on the 28th day, and in the group getting 5 mg of thiopanate

[7] Woolley, D. W., Strong, F. M., Elvehjem, C. A., and Madden, R. J., *J. Biol. Chem.*, 1938, **124**, 715.

[8] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

[9] Woolley, D. W., *J. Biol. Chem.*, 1942, **143**, 679.

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on the 28th day.

The above experiment with pantothenic acid-deficient rations was repeated with a group of 24 mice with similar results.

For the experiments with pyridine-3-sulfonic acid, a group of 12 weanling mice was fed the basal ration described at the beginning of this paper, minus nicotinic acid. Four mice received in addition 5% of the ration as pyridine-3-sulfonic acid, and 2.5% as $NaHCO_3$. Four were fed the ration plus 5% sulfonic acid, 2.5% $NaHCO_3$, and 0.5% nicotinic acid. As may be seen from the data in the table, those mice getting pyridine-3-sulfonic acid grew as well as did the controls. Furthermore, during the 4 weeks that the rations were fed, no symptoms of disease were seen in any of the animals.

Summary. In contrast to the findings with bacteria, pantothenic acid deficiency of mice and of hamsters was not produced by the feeding of the sulfur analog of pantothenic acid (N-(β,β-dimethyl-α,γ-dihydroxybutyryl)-taurine). Similarly, the feeding of pyridine-3-sulfonic acid did not produce symptoms of nicotinic acid deficiency.

14047

Effect of Posterior Pituitary Extract on Water Up-Take in Frogs After Hypophysectomy and Infundibular Lesions.*†

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It has been known for the last 20 years that injection of the posterior pituitary extract causes a weight increase in frogs due to the up-take of water.[1] However, very little is understood in regard to the pharmacological mechanism involved in this action. The experiments to be reported were performed in order to shed some light on the problem.

Material and Method. Male frogs weighing between 30 to 40 g were used. The procedure for total hypophysectomy has been previously described.[2] For partial hypophysectomy, the cartilage lining the base of the skull was incised along the border of the gland, leaving part of it intact so that it may be placed back in its original position following the removal of the gland. The anterior lobe was removed with forceps after it had been freed from the neighboring structures with a fine dental hook. For removal of the neural and intermediate lobes, suction was applied, the anterior lobe being kept away from the suction with the tip of a retractor.

The infundibular lesions were made in two ways: (1) a small puncture in the midline of the base of the third ventricle and (2) a wide incision made at the base of the third ventricle between the pituitary body and the tuber cinerium. After the removal of the anterior pituitary or with a wide lesion at the base of the third ventricle, the frogs became permanently dark and lost their ability of adaptation to light and darkness.

The posterior pituitary extracts were prepared according to the directions given in U.S.P. XI. They contain 2 or 4 units of the international standard powder per cc. The amount of extract given was in proportion to the body weight; each frog received not more than 0.5 cc. The solution was injected into the ventral lymph sac through the mouth.

The frogs were kept in separate cages in running water at a temperature between 15°C

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† A preliminary report of this work was given at the 1941 meetings of the Federation of American Societies for Experimental Biology.

1 Brunn, F., *Z. ges. exp. Med.*, 1921, **25**, 170.

2 Hogben, L. T., *Quart. J. Exp. Physiol.*, 1922, **13**, 177.