

factor in our work. The average weight of the females from normal mothers was 25% higher at 300 days of age than the average weight of the females from low manganese mothers. In the case of the males there was very little difference in the average weights.

Summary. We have not been able to demonstrate any abnormal tibia development in the albino rat raised on a diet low in manganese, provided the rat is normal at 21 days of age. In this respect the rat differs from the chick, at least in being much less sensitive to a low manganese diet. Only 2 cases of abnormal tibias have appeared in a total of 16 rats, born of females reared on a low manganese diet. Our limited data on rats from females reared on a low manganese diet suggest that the growth of the female is impaired, while that of the male is not.

12065

Relationship of Pantothenic Acid and Inositol to Alopecia in Mice.

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It has recently been shown in two laboratories^{1, 2} that mice become hairless over large areas of the body when they are maintained on a purified diet. It has also been shown^{3, 4} that inositol is a curative agent for the alopecia. With the isolation and identification of the anti-alopecia factor, it became of interest to study pantothenic acid deficiency in the presence of all other known essentials and also to determine if there were other unknown vitamins required by mice. It was observed that even in the presence of inositol, but in the absence of pantothenic acid, alopecia developed. The present report deals with alopecia which results from a lack of pantothenic acid and with that which results from a lack of inositol.

A highly purified basal diet which contained no unknown water-soluble constituents was prepared as previously described.⁵ Panto-

¹ Woolley, D. W., *J. Biol. Chem.*, 1940, **136**, 113.

² Norris, E. R., and Hausehildt, J., *Science*, 1940, **92**, 316.

³ Woolley, D. W., *Science*, 1940, **92**, 384.

⁴ Woolley, D. W., *J. Biol. Chem.*, in press.

⁵ Woolley, D. W., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 352.

thentic acid in the form of *d,l* sodium pantothenate⁶ and inositol were added in the amounts desired for various experiments. Since only one optical isomer of pantothenic acid is biologically active, the levels of racemic acid quoted throughout this paper must be divided by two in order to obtain the quantity of active material which the animals received. General care of the mice was similar to that previously described.¹

For the study of pantothenic acid deficiency the basal ration was supplemented with 100 mg of inositol per 100 g of ration. For the positive controls, it was further supplemented with 5 mg of pantothenic acid per 100 g. The first sign of pantothenic acid deficiency was retarded growth. After about 3 weeks on the experimental diet, the deficient animals became hyperirritable. A few days later they displayed difficulty in controlling their hind quarters. They seemed to be seized by periodic spasms of pain, for at intervals very violent and rapid movement would take place and the animals would squeal occasionally, as if in pain. The hind quarters sometimes exhibited convulsive, circular movements. After a few days the violent movements became subdued and a more or less complete paralysis of the hind legs ensued. In addition, the eyes of many of the animals were affected and in some cases they were unable to open them. A few days following the appearance of these symptoms, alopecia began to develop on the ventral surface of the body. The hair was lost first just behind the fore limbs on the chest. The denuded areas spread slowly over the entire ventral surface and then up the sides, and finally across the back. Some animals became completely naked except for the head. All of these symptoms were frequently not observed in the same animal. For example, the nervous and muscular manifestations were not invariably followed by alopecia, and alopecia sometimes developed without the appearance of the other symptoms. Occasionally animals died during the severe early manifestations and hence did not survive long enough to lose their hair.

When pantothenic acid was administered to animals at any stage of these conditions, a slow return to normality resulted. The violent movements could be eliminated within 1 or 2 days following the administration of 5 mg of pantothenic acid per 100 g of ration. The effect on alopecia was manifest much more slowly and it was usually necessary to continue treatment for about 3 weeks before restoration of hair began. Increasing the dosage of pantothenic acid up to 1 mg per day did not reduce this period. Eventually, however, the animals assumed a normal appearance.

⁶ Woolley, D. W., *J. Am. Soc.*, 1940, **62**, 2251.

The symptoms preceding alopecia suggested those observed in pigs⁷ and the syndrome of running fits in dogs.⁸ It will be of interest to test the effect of pantothenic acid in these conditions.

The amount of pantothenic acid which the mouse requires cannot be stated with accuracy. Some indication of the minimum level was found in a series of experiments in which graded doses were given. Definite symptoms of pantothenic acid deficiency (effects on hind quarters, ventral alopecia) were noted with 500 γ per 100 g of ration. A few cases were observed following administration of 1 mg per 100 g. With 2 mg or 5 mg levels, no symptoms were observed and growth was satisfactory.

In the presence of adequate pantothenic acid and in the absence of inositol, alopecia resulted. For these studies the basal ration was supplemented with 5 mg of pantothenic acid per 100 g. After mice had been fed this ration for about 6 weeks, many of them began to lose their hair. The first bare spots were usually seen on each thigh, well up towards the spine. The hairless areas spread rapidly up the back and over the sides. The ventral surface was not affected. Usually the last areas to become affected in pantothenic acid deficiency were the first to show a lack of inositol, and vice versa. The difference in affected areas was usually most strikingly apparent in the early stages of the disease.

Administration of inositol to such animals resulted in regeneration of the growth of hair, usually about 10 days after therapy had been started. Soon the animals presented a normal appearance. It was observed that when such hairless animals were continued without inositol many of them regained their hair; only a very few lost weight and eventually died. For example, in a group of 13 hairless mice, 5 regained their hair within 3 weeks after the onset of the disease, one died, and 7 remained hairless throughout a period of 6 weeks. Some of the animals which had presented spontaneous cure did not exhibit a normal appearance but rather appeared unkempt and somewhat greasy. Such animals could be restored rapidly to a normal appearance by the administration of inositol.

Animals which received both inositol and pantothenic acid grew more rapidly than did those which received only pantothenic acid. With large doses (5 mg per 100 g of ration) of pantothenic acid the increment of growth produced by inositol was not large, but repeated trials have demonstrated that it was actual. For example,

⁷ Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J., *Biochem. J.*, 1938, **32**, 2207.

⁸ Arnold, A., and Elvehjem, C. A., *J. Am. Vet. Med. Assn.*, 1939, **95**, 303.

in 3 trials in which a total of 40 animals were employed, the average weights after 7 weeks with no inositol were 27 g, 33 g, and 24 g, whereas with inositol the weights were 26 g, 39 g, and 31 g. The average starting weights were the same.

It was evident that both pantothenic acid and inositol influenced the growth of hair in mice. From the description given by Norris and Hauschildt,² it appears that their animals were suffering from a multiple deficiency. The effect on growth of pantothenic acid deficiency was much more pronounced than was that of inositol deficiency. (Fig. 1.) Deaths were much more common in the absence of pantothenic acid than in the absence of inositol. The fact that hairless animals receiving pantothenic acid but no inositol in many cases eventually regained their hair raises the question as to what should be called the "anti-alopecia factor". In the presence of inositol, no alopecia developed whereas in its absence hair was lost. Whether or not the effect of inositol was merely to increase the food consumption of the animals cannot be decided because of the impossibility of determining accurately the food consumption of mice.

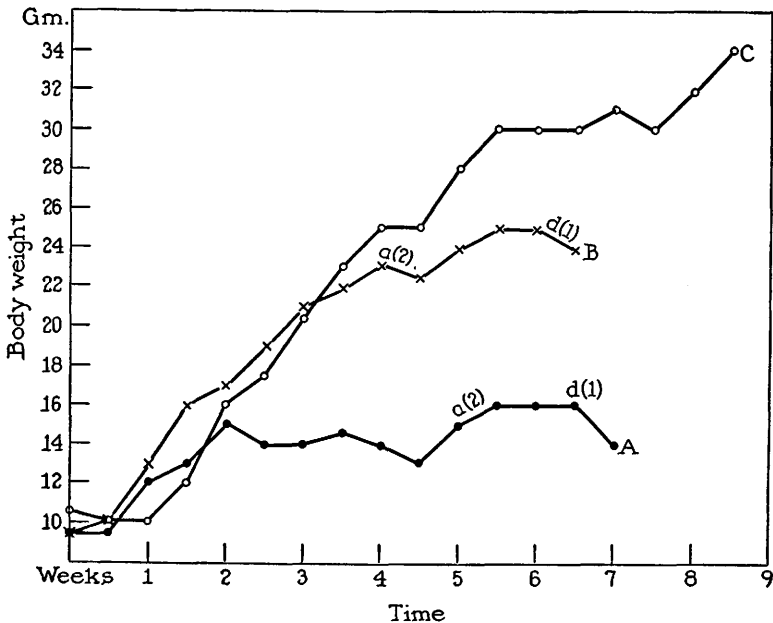


FIG. 1.

Growth responses to pantothenic acid, inositol, and pantothenic acid and inositol. Curve A represents the average response of 3 mice receiving inositol; Curve B represents the average response of 5 mice receiving pantothenic acid; Curve C represents the average response of 3 mice receiving both inositol and pantothenic acid. a = alopecia; d = dead; numbers in parentheses represent the number of animals affected.

TABLE I.
Incidence of Alopecia on Various Purified Rations.

Addition to 100 g of basal	Total No. of animals	No. of cases of alopecia
100 mg inositol	9	6
5 mg <i>d,l</i> pantothenate	39	9
5 mg <i>d,l</i> pantothenate and 100 mg of inositol	12	0
5 mg <i>d,l</i> pantothenate following a 2-week depletion period	35	19

It seems, however, that the inositol effect was not a mere reflection of pantothenic acid effect because of the difference in the early picture of alopecia in the two deficiencies.

The fact that mice may be raised satisfactorily and rapidly to maturity on a highly purified diet is noteworthy. The only constituents of the ration about which there can be uncertainty as to chemical composition are the oils and the casein; all the other constituents were pure compounds. It will be of interest to determine whether the oils and casein can be replaced by pure fat-soluble vitamins and pure amino acids. Whether or not natural foodstuffs contain materials which increase slightly the rate of growth obtained with the purified diet has not been determined.

12066

Rôle of the Intestinal Bacteria in the Decomposition of Pectin.

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Previous observations^{1, 2} have shown that orally administered pectin is decomposed in the human and dog gastrointestinal tract, that the decomposition occurs in the colon, and that it is probably brought about by bacterial action. A thorough study of the part played by intestinal bacteria in pectin decomposition has not as yet been made. Schneider,³ however, found that mixtures of intestinal bacteria destroy the hemocellulose of apple marc. He also observed that reducing sugars did not constitute part of the end products.

¹ Werch, S. C., and Ivy, A. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **44**, 336; *Am. J. Dig. Dis.*, in press.

² Kertesz, Z. I., *J. Nutrition*, 1940, **20**, 289.

³ Schneider, E. C., *Am. J. Physiol.*, 1912, **30**, 258.