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Vitamin B_6, Pantothenic Acid, and Unsaturated Fatty Acids as They Affect Dermatitis in Rats

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ABSTRACT

Pyridoxine and pantothenic acid together permanently healed rat dermatitis. If vitamins A and D were provided by including cod liver oil in the diet, which also supplied essential fatty acids, female rats attained weights of 175 to 180 grams. If the fatty acid content of the diet was reduced to a minimum by supplying vitamins A and D in a fat free form the rats developed a marked scaly condition on the tails and feet and succumbed except when linoleic or arachidonic acid was supplied. If either fatty acid was supplied in addition to the two vitamins, pyridoxine and pantothenic acid, the rats were normal and females attained weights of 175 to 195 grams.

Linoleic acid was more effective than methyl arachidonate in protecting against dermatitis but neither gave permanent protection. A combination of a fatty acid with only one of the two vitamins, pyridoxine or pantothenic acid, was equally ineffective.

The lesions characteristic of the dermatitis due to either pyridoxine or pantothenic acid deficiency were indistinguishable on gross examination.

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During their studies on dermatitis in rats Hogan and Richardson ('35a, '35b) observed that wheat germ oil and corn oil heal the disease, an observation that has been confirmed by several laboratories. According to Salmon ('38) corn oil, wheat germ oil, and the fatty acids from linseed oil and soybean oil are effective curative or preventive agents. Quackenbush, Platz, and Steenbock ('39) reported that this type of dermatitis is cured by peanut oil, wheat germ oil, corn oil, or Wesson oil in daily doses of one drop or less. The dermatitis is also healed by one-half drop per rat per day of the ethyl esters of the fatty acids prepared from wheat germ oil, or by one-half drop of ethyl linolate. In a later paper Schneider, Steenbock, and Platz ('40) reported that pyridoxine and a factor present in the filtrate after fuller's earth adsorption, prevent or permanently heal the disease but that one vitamin alone is ineffective. On the other hand ethyl linolate is effective in daily doses of 20 mg. and the action of the ethyl linolate is independent of the action of either pyridoxine or the filtrate factor. Birch ('38) states that two factors are essential for permanent healing of the dermatitis. One is the essential unsaturated fatty acid factor, the other is pyridoxine. According to Gyorgy and Eckhardt ('39) and Supplee, Bender, and Kahlenberg ('40) pyridoxine, and a factor present in the filtrate after fuller's earth adsorption, are both required to heal dermatitis permanently. Gyorgy, Poling and SubbaRow ('39) cured rats of skin lesions which had persisted after treatment with pyridoxine by the use of a purified, but still crude, zinc salt of pantothenic acid. Richardson and Hogan ('40) showed in a preliminary report that both pyridoxine and pantothenic acid are required to heal the dermatitis permanently.

The reports just cited suggest that three factors may be concerned in the prevention and cure of dermatitis in rats. One is pyridoxine, a second is pantothenic acid, and a third is the essential unsaturated fatty acid factor. The relation of these three factors to dermatitis will be described in this paper.

*A preliminary report was presented before the American Society of Biological Chemistry, Chicago, Ill., April 17-19, 1941.

EXPERIMENTAL

The basal ration was supplied to the mother and litter when the young were 15 days old, and the young were weaned and put in individual cages at 22 days of age. Two basal rations were used in these studies; one contains cod liver oil and the other is very low in lipids. The composition of these rations is given in Table 1. The supplements were supplied daily in small glass dishes.

TABLE 1.—COMPOSITION OF BASAL RATIONS

Constituents	Ration No. 1669	Ration No. 4784
Casein (acid washed)	20	20[1]
Sucrose	71	73
Salt Mixture (Hubbell et al. ('37))	4	4
Cellu-flour[4]	3	3[1]
Cod liver oil	2	
Daily Supplement		ug./rat
Thiamin[2]	20	20
Riboflavin[2]	20	20
Carotene		50
Calciferol[3]		0.25
		Mg./rat
Alpha-tocopherol[2]		0.25
Choline[2]		10

[1]Acid washed casein and celluflour were thoroughly extracted with boiling ethyl alcohol and with ether.
[2]Kindly supplied by Merck and Co., Rahway, N. J.
[3]Kindly supplied by the Winthrop Chemical Co., N. Y.
[4]Obtained from Chicago Dietetic Supply House, Inc., Chicago, Illinois.

Dermatitis Produced on a Basal Ration Which Contained Cod Liver Oil

In the first series of experiments the rats received a basal ration which contains 2 per cent of cod liver oil as the only added lipid. After mild dermatitis had developed, the rats received curative supplements as follows: Group I, pyridoxine; Group II, pantothenic acid; Group III, pyridoxine and pantothenic acid; Group IV, linoleic acid; Group V, methyl arachidonate. These data are summarized in Fig. 1.

All the rats which received pyridoxine and pantothenic acid healed and grew at a rapid rate until the females had attained weights of 175 to 180 grams (Curves I and II). Every rat was in excellent condition at 20 weeks when the observations were discontinued.

Ten, 20, and 30 gamma of pyridoxine healed most of the rats temporarily, but every one that survived long enough had a recurrence of the dermatitis and eventually succumbed. The pyridoxine was increased to 100 gamma for two rats which had had a recurrence on 20 gamma, but this did not stop the decline. Four of 10 rats which received 70 gamma of calcium pantothenate per rat daily healed temporarily then had a recurrence,

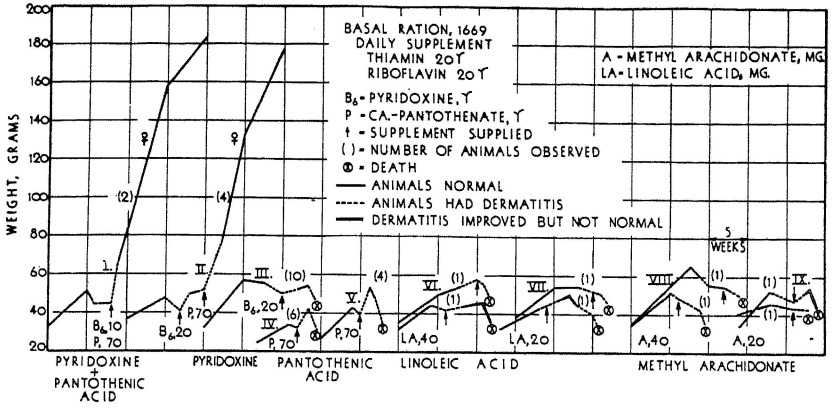

Fig. 1. Pyriodoxine and pantothenic acid together permanently healed dermatitis which developed in rats that received ration No. 1669 (I and II). The rats grew at a fairly rapid rate without any additional fatty acids. Either pyridoxine (III) or pantothenic acid (IV and V) when supplied singly, healed the dermatitis temporarily, but the lesions recurred in every case. Linoleic acid (VI and VIII) and methyl arachidonate (VII and IX) were ineffective.

(Curve V); the other six gained weight temporarily but the dermatitis did not improve (Curve IV).

The eyes of the animals which received only pyridoxine were more severely affected than of those which received only pantothenic acid. The other lesions characteristic of this type of dermatitis, if either pyridoxine or pantothenic acid is supplied singly, are indistinguishable on gross examination.

Linoleic acid[1] and methyl arachidonate[1] were tested for their curative action on dermatitis in daily doses of 20 and 40 mg. Two of 4 rats which received linoleic acid improved slightly but were never normal, and one of them had a recurrence and died with severe symptoms. The other two died without improving. Only one of 4 rats on methyl arachidonate improved and it died with mild dermatitis without becoming normal (Curves, VI, VII, VIII, and IX).

The Amount of Pantothenic Acid Required by the Rat

The amount of pantothenic acid required by the rat is indicated in Fig. 2. Ten gamma of calcium pantothenate per rat daily protected against dermatitis, but the amount required for optimum growth was 100 gamma. This agrees with the data reported by Unna ('40) who found that 80 gamma of pantothenic acid supported a maximum rate of growth. He did not investigate the amount required to prevent dermatitis.

[1]Kindly supplied by Dr. J. B. Brown.

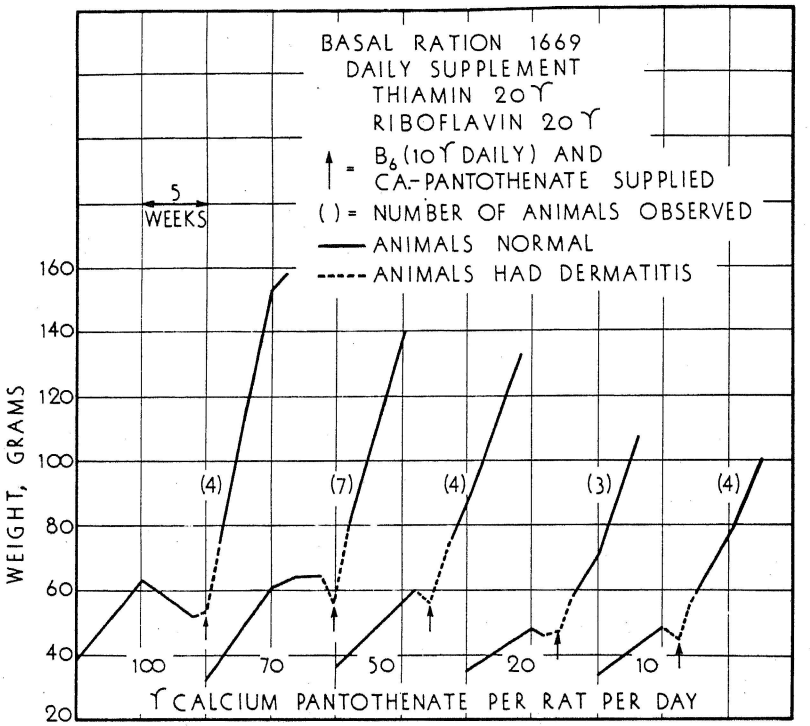

Fig. 2. Five series of rats which had developed dermatitis on Ration 1669 were given 10 gamma of pyridoxine and 10, 20, 50, 70, or 100 gamma respectively of calcium pantothenate per rat daily. The dermatitis was healed in every group, but 100 gamma of the pantothenate promoted the most rapid rate of growth.

Dermatitis Produced on a Basal Ration Which Contained no Added Lipids

Since the essential unsaturated fatty acids did not heal dermatitis when the basal ration contained 2 per cent of cod liver oil and was deficient in both pyridoxine and pantothenic acid, it was decided to study the relation of these three factors on a basal ration, No. 4784, which is low in lipids.

The acid washed casein and the celluflour were extracted thoroughly with boiling ethyl alcohol and with ether to reduce the lipid content of this basal ration to a minimum. They were then analyzed separately for lipids by the method described by Shaw ('20). The other components of the basal ration were assumed to be lipid-free. The non-vitamin portion of the basal ration contained 0.025 per cent of lipids as calculated from analysis of the casein and celluflour. The vitamins were supplied daily in pure form.

The results of feeding essential unsaturated fatty acids alone are summarized in Fig. 3, Series A; pantothenic acid and fatty acids in Series B; and pyridoxine and fatty acids in Series C. Groups I, II, and III of each series received linoleic acid and Groups IV, V, and VI methyl arachidonate. Groups I and IV received the supplements as a prophylactic while the other groups received them as a curative treatment.

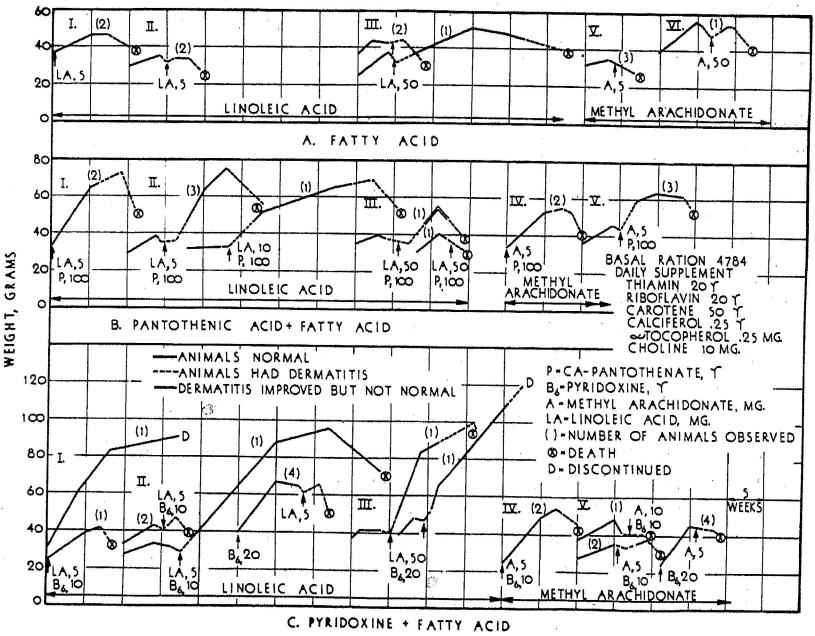

Fig. 3. All rats received the basal ration, No. 4784. Neither linoleic acid nor methyl arachidonate heal dermatitis permanently (A). Neither pantothenic acid (B), nor pyridoxine (C) alone, even if combined with one of the essential fatty acids, provides permanent protection. The rats which received methyl arachidonate with either pantothenic acid or pyridoxine did not gain as rapidly as those which received linoleic acid.

Every rat which received 5 milligrams of linoleic acid, either by the prophylactic, Curve I, or by the curative procedure, Curve II, eventually died with severe dermatitis. Two rats which received linoleic acid as a curative agent healed temporarily, but both of them had a recurrence before death. The linoleic acid was then increased to 50 milligrams per rat daily for three rats, Curve III. Two died without healing after they had survived for a total of 8 weeks. The other healed and remained normal for 20 weeks, and during this period it gained 20 grams. It then had a recurrence and died with severe dermatitis after it had survived for 34 weeks.

Five milligrams of methyl arachidonate were ineffective in every case but 50 milligrams healed one rat temporarily (Curves V and VI).

The results obtained when the fatty acids were supplemented with pantothenic acid as 100 gamma of calcium pantothenate are summarized in Series B, Fig. 3. The rats gained more and survived longer when they received the pantothenic acid in addition to the fatty acids but every one of them died with severe dermatitis. Fifty milligrams of linoleic acid daily was no more effective than five milligrams.

Supplementing the linoleic acid with 10 to 20 gamma of pyridoxine produced larger gains, longer survival periods, and milder symptoms (Series C, Fig. 3). Individual animals, however, varied markedly. One rat which received 5 milligrams of linoleic acid and 10 gamma of pyridoxine, as a curative substance healed and survived for 34 weeks without a recurrence. Two others received the same supplements for 3 weeks but they survived only 8 weeks and did not heal. Two rats received the same supplements by the prophylactic method. On was normal at the end of 17 weeks when the observations were discontinued and the other died with mild symptoms at 8 weeks.

In all, nine rats received 5 milligrams of linoleic acid and 10 to 20 gamma of pyridoxine. Seven of them had dermatitis at death but only one had severe symptoms. Two rats which received 50 milligrams of linoleic acid and 20 gamma of pyridoxine daily healed rapidly, but the symptoms gradually reappeared until both rats had fairly severe dermatitis at the end of 17 weeks, when it became necessary to discontinue the observations.

In all, seven rats received 50 milligrams of linoleic acid daily. Two received an additional supplement of pyridoxine, and two received an additional supplement of 100 gamma of calcium pantothenate. Six died with severe dermatitis and the other had mild dermatitis when the linoleic acid was discontinued.

The results obtained when all three factors were supplied together are summarized in Series A, Fig. 4. When 5 or 10 milligrams of linoleic acid or methyl arachidonate were fed along with 10 or 20 gamma of pyridoxine and 100 gamma of calcium pantothenate, the rats grew at a fairly rapid rate until the females attained weights of 175 to 195 grams. Every animal was normal when the observations were discontinued and some of them were observed for 32 weeks.

Methyl arachidonate was ineffective in preventing dermatitis or promoting growth when fed alone, or when combined with either pyridoxine or pantothenic acid. However, when

supplied with the two vitamins it is just as effective as linoleic acid. Five milligrams of either fatty acid were as effective in promoting growth as 10 milligrams, and 5 milligrams of both fatty acids were no more effective than 5 milligrams of either one alone.

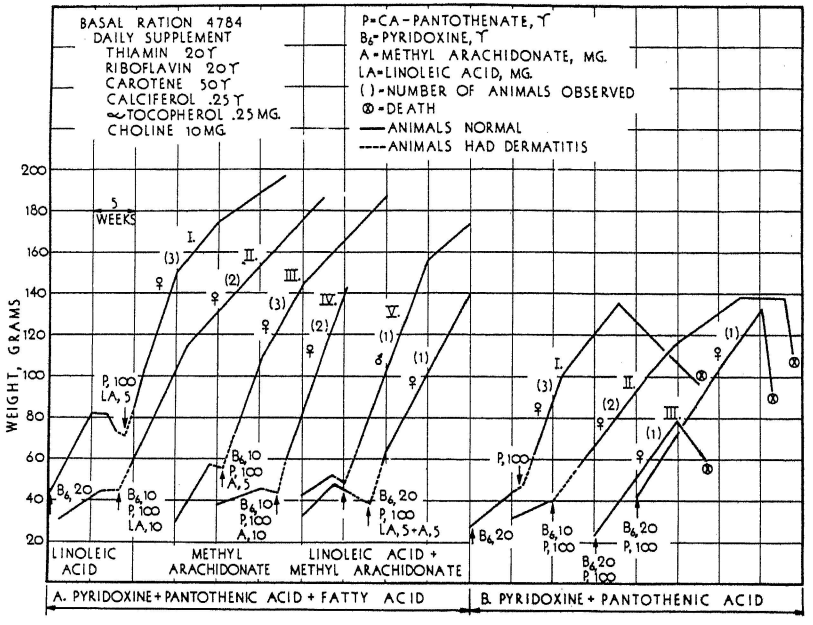

Fig. 4. All rats received the basal ration, No. 4784. Pyridoxine and pantothenic acid with either linoleic acid or methyl arachidonate protect against dermatitis and rats grow at a rapid rate (Series A). Methyl arachidonate (III and IV) is just as effective in promoting growth as is linoleic acid (I and II). Linoleic acid and arachidonic acid together (V) are no more effective than a single fatty acid. Pyridoxine and pantothenic acid protect against the characteristic dermatitis but every rat eventually succumbs to a fatty acid deficiency (Series B).

The effect of feeding pyridoxine and pantothenic acid together without fatty acids is summarized in Series B, Fig. 4. These two vitamins permanently healed or prevented the dermatitis but every rat, after survival periods of 13 to 34 weeks, succumbed with fatty acid deficiency. The symptoms most characteristic of the fatty acid deficieny were unkempt fur and marked scaliness of feet and tails. Two of the seven animals on pyridoxine and pantothenic acid had a spectacled appearance around the eyes and their paws were reddened, but the fissure type of lesions characteristic of pyridoxine or pantothenic acid deficiency was absent.

DISCUSSION

Linoleic acid gave some protection against dermatitis and it was more effective than arachidonic acid, but neither gave permanent protection under these experimental conditions. It is our experience that rats which receive both pyridoxine and pantothenic acid develop a severe scaly condition on the tails and feet but they do not develop typical dermatitis. When the rats receive an unsaturated fatty acid in addition to the two vitamins they are normal. Salmon ('41), and Quackenbush, Kummerow and Steenbock ('41) have also observed that an acrodynia-like dermatitis and scaly tails do not develop in rats when they receive essential unsaturated fatty acids in addition to pyridoxine and pantothenic acid. However, the data presented in this paper do not agree completely with the observations of Quackenbush et al. ('41) that linoleic acid protects against dermatitis permanently. We are unable to explain this discrepancy.

Unpublished observations in this laboratory have demonstrated that rats develop dermatitis when they receive daily doses of 50 or 100 Mg. of wheat germ oil alone, or when combined with either one of the two vitamins, pyridoxine or pantothenic acid. Larger doses of oil decrease both the incidence and the severity of the dermatitis.

In earlier publications, from this and other laboratories Salmon ('38) Quackenbush et al. ('39), it was asserted that wheat germ oil alone heals rat dermatitis. Since our present position is both pyridoxine and pantothenic acid are required, our previous conclusion as to the efficacy of wheat germ oil calls for some explanation. It was stated in our early reports that dermatitis develops in rats that receive an irradiated water extract of yeast as the source of water soluble vitamins. It seems clear now that the irradiation procedure destroyed most of the pyridoxine, which explains the development of dermatitis. According to our hypothesis wheat germ oil must contain a small amount of pyridoxine, enough to prevent dermatitis though not enough to sustain any marked increase in weight.

SUMMARY

Pyridoxine and pantothenic acid were both required for permanent healing of dermatitis. When both vitamins were supplied in a ration that contained cod liver oil, females attained weights of 175 to 180 grams even though they did not receive any additional fatty acids. Either pyridoxine or pantothenic acid temporarily healed the dermatitis in most of the rats, but it recurred in every one that survived long enough.

The addition of pyridoxine and pantothenic acid to a low-fat basal ration prevented or healed the characteristic dermatitis

but after a prolonged survival period every rat died of a fatty acid deficiency. If linoleic acid or methyl arachidonate was supplied in addition to the two vitamins the rats grew fairly rapidly and females attained weights of 175 to 195 grams.

Neither linoleic acid nor methyl arachidonate protected permanently against dermatitis. A combination of the fatty acids with only one of the two vitamins, pyridoxine or pantothenic acid, was equally ineffective.

The lesions characteristic of the dermatitis due to pyridoxine or pantothenic acid deficiency were indistinguishable on gross examination.

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