

THE RELATION BETWEEN VITAMIN B₆ AND THE UNSATURATED FATTY ACID FACTOR*

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The work of Burr, Burr, and Miller (1-3) showed that rats fed on a diet totally devoid of fat suffered from a deficiency disease which was curable by the administration of fats containing highly unsaturated fatty acids or by pure methyl linolate. The disease was characterized by a scaly condition of the skin and tail, swollen, red paws, increased water consumption, and renal lesions which caused albumin or blood to be present in the urine.

Birch and György (4) found that rats suffering from a lack of vitamin B₆ often developed a scaliness of the tail and skin, while swollen, red paws were always seen in this deficiency. These authors also found that certain fats had what was termed a sparing action on vitamin B₆, and they suggested, on the basis of Burr and Burr's work, that this action was due to the linoleic acid present in the fat.

Hogan and Richardson (5) have described a dermatitis induced in the rat by feeding diets of low fat content supplemented by yeast irradiated with ultraviolet light. The description they give of this dermatitis corresponds with that given by György (6, 7) to the acrodynia-like dermatitis of vitamin B₆ deficiency. Hogan and Richardson (8) found that the symptoms could be cured by certain vegetable oils as well as by water extracts of yeast. Birch and György suggested that these observations might be explained by their finding that fats had a sparing action on vitamin B₆. Richardson and Hogan (9), however, main-

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tained that it was unlikely that linoleic acid was the curative factor, as corn oil, flaxseed oil, and walnut oil which are such rich sources of linoleic acid were relatively ineffective in healing the dermatitis. Moreover, the factor was not extracted from yeast by ether but was present in watery extracts.

The experiments presented in this paper were, therefore, undertaken to investigate the relationship between the antidermatitis factor present in oils or fats and the water-soluble vitamin B₆.

EXPERIMENTAL

This investigation has been pursued along two lines. Firstly, the influence of vitamin B₆ on the production of the fatty acid deficiency of Burr and Burr was studied. This was carried out by feeding rats on a fat-free diet supplemented by varying amounts of vitamin B₆. Secondly, a study has been made of the influence of fat in vitamin B₆-free diets on the development of the acrodynia-like dermatitis.

In order to carry out the first part of this investigation it was necessary to provide a source of vitamin B₆ free from all traces of fatty acids. For this purpose an extract of yeast was made.

Preparation of Yeast Extract—500 gm. of dried brewers' yeast were extracted with 1600 ml. of cold 60 per cent alcohol for 24 hours. It was then filtered and reextracted with a further 1600 ml. of 60 per cent alcohol. The combined extracts were then evaporated down to remove the alcohol, adjusted to pH 2 with hydrochloric acid, and shaken with petroleum ether three times to remove all neutral fat and fatty acids. The solution was then heated on a water bath to remove the petroleum ether and made up to 500 cc.

This extract was tested for vitamin B₆ activity by using Diet 8 as a basal vitamin B-free diet. It is similar to that used by György (7) with the exception that sucrose is substituted for

<i>Diet 8</i>	
<i>per cent</i>	<i>per cent</i>
Casein E.....	18
Sucrose.....	66
Butter fat.....	9
Cod liver oil.....	1
Salt Mixture 185*.....	4
Agar.....	2

* McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, **33**, 63 (1918).

rice starch. It was supplemented by 3 units of thiamine and 10 micrograms of lactoflavin per rat per day.

This diet has been used consistently for testing concentrates of vitamin B₆ and has proved quite as satisfactory as the diet containing starch; in fact the animals tend to develop the vitamin B₆ deficiency symptoms earlier on this diet than on the starch diet.

Fig. 1 shows the growth curves of three animals on Diet 8 and the effect is seen of administering 0.5 and 0.2 ml. of the yeast extract. The acrodynia was cured in 3 weeks with 0.2 ml. and slow growth resulted. With 0.5 ml. a similar cure was brought about but growth was more rapid.

It is evident, therefore, that 0.2 ml. of this yeast extract is sufficient to cure animals of the acrodynia-like dermatitis.

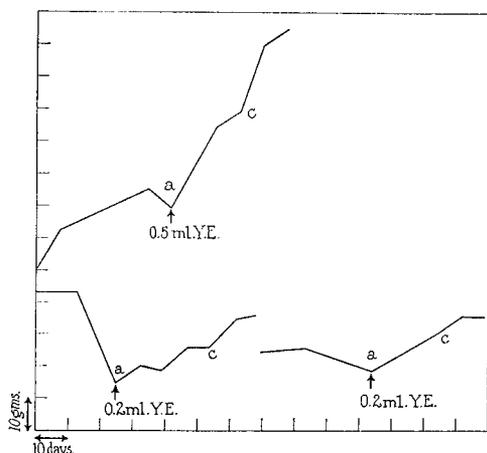


FIG. 1. Growth curves of rats on Diet 8. Yeast extract was fed at the point indicated by the arrow. *a* acrodynia, *c* cured.

Experiments on Fat-Free Diets—The composition of the fat-free diet (Diet 16) is shown in Table I. In addition to this diet all animals received 1 drop of halibut liver oil per week for vitamins A and D. Eighteen animals were used and were divided into three groups of six. Group I received 2 ml. of the yeast extract, Group II 1.0 ml., and Group III 0.5 ml. All groups were later given 5.0 international units of thiamine and 10 micrograms of lactoflavin per rat per day, in order to make sure that the animals on the lower doses of yeast extract were getting sufficient thiamine and lactoflavin.

Group I. 2 Ml. of Yeast Extract per Day—The animals in

this group grew well for about 5 weeks (Fig. 2). Growth then slackened and 8 to 9 weeks after the commencement of feeding the experimental diets a scaliness appeared around the eyes, nose, and feet. The animals remained in this condition for several weeks without growing. However, the skin and fur gradually became worse; first the skin became very scurfy and later the fur came out and sore denuded patches appeared on some of the animals. Death eventually occurred unless unsaturated fatty acids were fed. The typical acrodynia was not seen and the scaly tail described by Burr and Burr was seen in only one animal. When lard or a preparation of fatty acid from maize oil¹ was fed (Rats 1 and 2), resumption of growth occurred and an im-

TABLE I
Composition of Diets (Per Cent)

Each diet contained 5 per cent of McCollum's Salt Mixture 185.

Diet No.	Cane-sugar	Glaxo Casein E	Lard	Linseed oil
16	75	20		
14	70	25		
17	70	20	5	
18	65	20	10	
19		20	75	
15	55	20	20	
9	60	20		15
10	45	20		30

provement in the skin condition was seen. Rat 5 was given 10 drops of hardened cottonseed oil per day after being on the deficient diet 5½ months. A curious result was obtained. The hardened oil was fed by placing it on top of the diet. The animal being devoid of fat ate greedily but was apparently unable to digest the fat, for it died after a few days. On postmortem examination the stomach was found to be full of fat; some had been regurgitated and there was foam around the mouth. A similar result with hardened cottonseed oil was obtained with Rat 12 in Group II.

Group II. 1.0 Ml. of Yeast Extract per Day—The animals

¹ This preparation was kindly furnished by Dr. T. Moore.

in this group grew well for the first few weeks. The growth curves then flattened or showed a decline in weight (Fig. 3). The animals developed the typical acrodynia-like dermatitis within 10 weeks

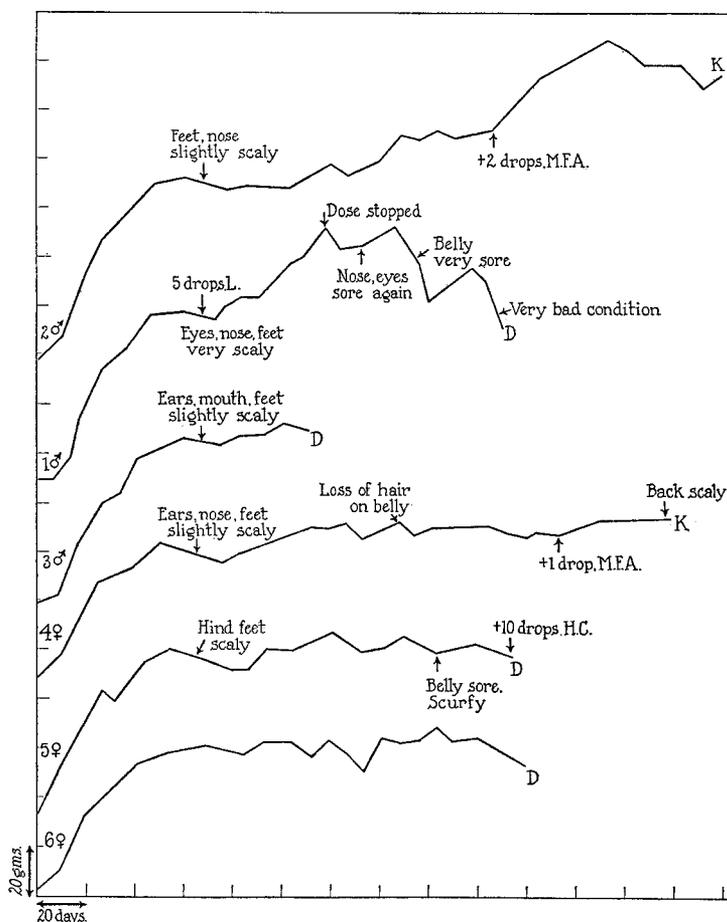


FIG. 2. Growth curves of rats on fat-free Diet 16, supplemented by 2.0 ml. of yeast extract per day. *D* died, *K* killed, *M.F.A.* fatty acids from maize oil, *L.* lard, *H.C.* hardened cottonseed oil.

from the commencement of feeding the experimental diets. Death shortly ensued unless the rats were treated with material containing unsaturated fatty acids. Feeding 5 to 10 drops of lard

per day brought about a rapid cure of the acrodynia and an increase in weight. When the lard was withheld, the weight remained steady and the animals later developed scurfy coats and sore patches appeared on the skin. When Rat 11 was given 3 drops of fatty acids from maize oil, resumption of growth occurred and new hair appeared on the back.

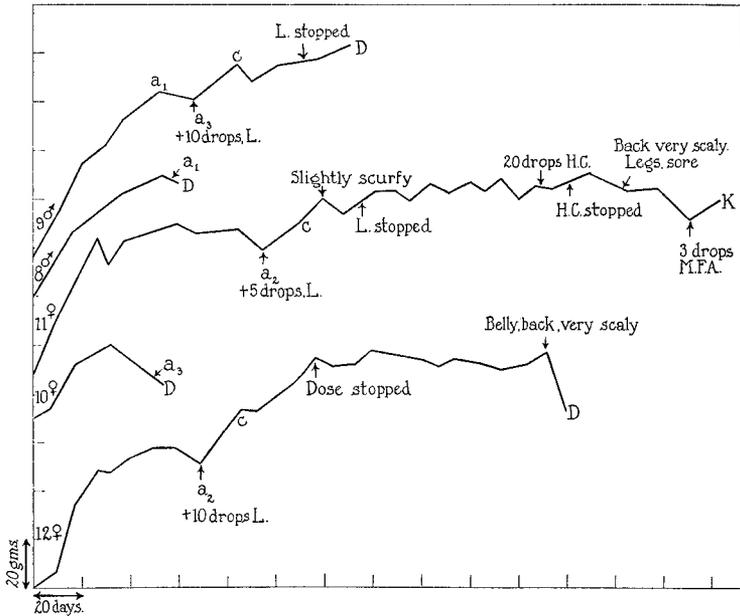


FIG. 3. Growth curves of rats on fat-free Diet 16, supplemented by 1.0 ml. of yeast extract. a_1 slight acrodynia, a_2 moderately severe acrodynia, a_3 very severe acrodynia, c cured of acrodynia. The other abbreviations are the same as in Fig. 2.

Group III. 0.5 Ml. of Yeast Extract per Day—All the animals in this group developed the acrodynia-like dermatitis in 4 to 8 weeks (Fig. 4). It was possible to cure this symptom by feeding either lard or fatty acids prepared from maize oil. When the supply of fatty acid was stopped, the animals developed either the acrodynia again or the Burr and Burr syndrome. Rat 15 which developed acrodynia in 5 weeks was cured by administering 20 drops of lard per day. When the lard was withheld, the animal

died 4 weeks later. The symptoms at death consisted of a slight dermatitis on nose and feet; the incisor teeth were also malformed and decayed.

These results show that rats may develop the acrodynia-like dermatitis if the diet is free from fat even when moderately large amounts of vitamin B₆ are given. The possibility that the disease observed by Burr and Burr (1) might be due to a low intake of

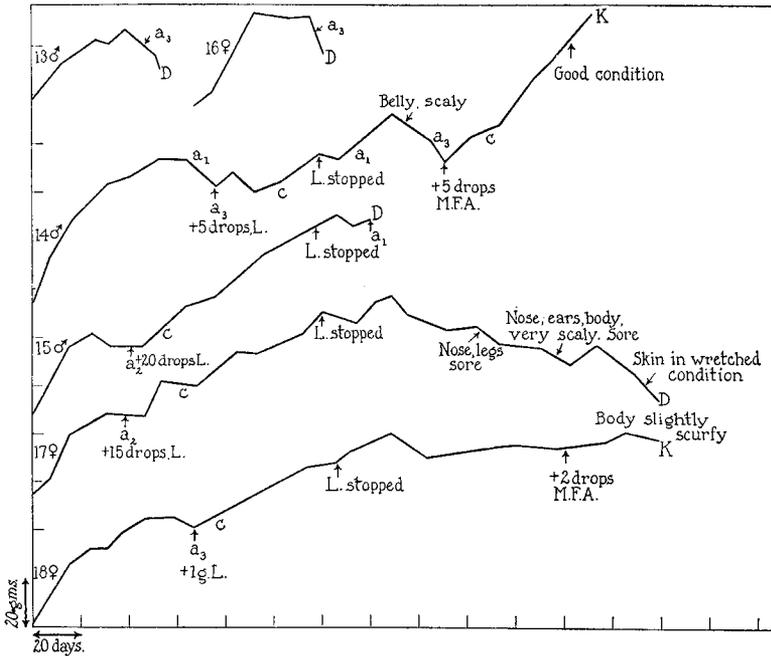


FIG. 4. Growth curves of rats on fat-free Diet 16, supplemented by 0.5 ml. of yeast extract per day. The abbreviations are the same as in Figs. 2 and 3.

vitamin B₆ is ruled out by the finding that even with 10 times the minimum curative dose, unsaturated fatty acid is necessary for normal health and growth. The fatty acid factor is, therefore, an essential constituent of the diet for rats. The amount of vitamin B₆ ingested by the rat appears to determine the kind of dermatitis which is to develop. When ten minimum doses of vitamin B₆ are fed, the symptoms found are similar to those

TABLE II

*Symptoms Found on Vitamin B₆-Free Diets Other Than
Acrodynia-Like Dermatitis*

Rat No. and sex	Weight	Diet	Symptoms	Remarks
23 ♀	<i>gm.</i> 50	On Diet 8 for 6 mos.; cured once then given slightly active concentrate when symptoms had again developed	Scaly tail and acrodynia	Cured when linseed oil fed in addition to vitamin B ₆
27 ♂	67	5 mos. on Diet 8; cured once then given slightly active concentrate when symptoms again appeared	Necrotic tail, feet scaly	Cured by addition of linseed oil to vitamin B ₆ dose
21 ♀	35	5 mos. on Diet 8; cured once, then given slightly active concentrate	Acrodynia and necrotic tail	Cured with active concentrate but tail fell off
197 ♀	64	5 mos. on Diet 8, then given partly active concentrate	Acrodynia, tail necrotic, blood in urine	Not cured with maize oil
178 ♀	80	5 mos. on Diet 8, then given slightly active concentrate	Acrodynia, body sore and scurfy, urine bloody	Not cured with more active concentrate
223 ♀	75	2 mos. on Diet 8, then changed to Diet 15. Symptoms developed 1-2 mos. later	No acrodynia, albumin in urine several days before death; blood in urine at death; coat sparse and greasy, covered with dried blood	Died

TABLE II—*Concluded*

Rat No. and sex	Weight	Diet	Symptoms	Remarks
224 ♀	<i>gm.</i> 78	Same as for Rat 223	No acrodynia; coat sparse and greasy, covered with dried blood; hemorrhage from margin of ears; albumin in urine and slight amount of blood at death	Died
216 ♂		On Diet 8 for 5 mos.; cured once of acrodynia by vitamin B ₆ , then vitamin B ₆ dose stopped	Sparse fur, dried blood on coat; ureter blocked by calcium stone	"

described by Burr and Burr, notably, "The hair on the back of the body becomes filled with dandruff. There is a tendency to lose the hair. . . Sores often appear on the skin." When only 2 to 5 times the minimum dose of vitamin B₆ is fed, the acrodynia-like dermatitis appears. These results would indicate that the function of vitamin B₆ and the unsaturated fatty acid factor in the animal body are closely connected.

Symptoms Found on Vitamin B₆-Free Diets Other Than Acrodynia-Like Dermatitis—The scaly condition of the tail is sometimes observed when animals are kept on Diet 8 for long periods with only small amounts of vitamin B₆. Instances of this are given in Table II. The passing of albumin and blood in the urine has also been observed with rats on Diets 8 and 15 (see Table II). Burr and Burr (2) noted both these conditions in their fat-free animals and they postulated that hemoglobinuria was the immediate cause of death. The appearance of these symptoms on a vitamin B₆-free diet, containing appreciable amounts of fat, affords further evidence of a connection between vitamin B₆ and the fatty acid factor.

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Vitamin B₆-Free Diets Containing Varying Amounts of Fat—Groups of animals were fed on diets containing varying amounts of lard or linseed oil, and the time taken for them to develop the vitamin B₆ symptoms and the severity of the symptoms when developed were noted.

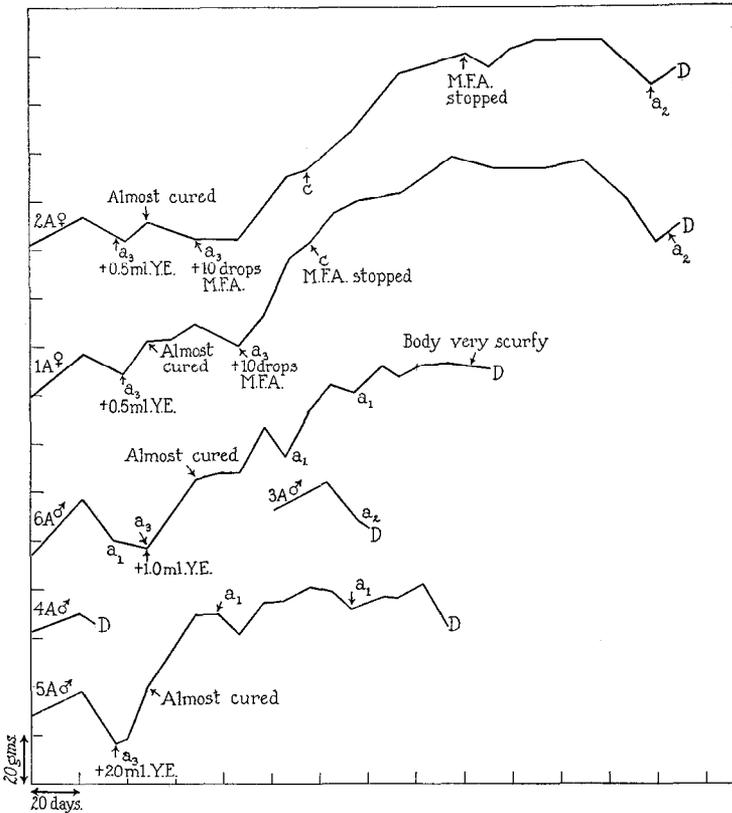


FIG. 5. Growth curves of animals on a vitamin B₆-free diet containing no fat. *Y.E.* yeast extract. The other abbreviations are the same as in Figs. 2 and 3.

The diets used are seen in Table I. All groups were given 10 micrograms of purified lactoflavin and 5 international units of thiamine per day, and 1 drop of halibut liver oil per week.

Diet 16. No Fat—From a group of six animals placed on this diet five developed severe acrodynia in 3 to 4 weeks (Fig. 5).

Rats 1A and 2A were given 0.5 ml. of yeast extract when the acrodynia had developed. Some improvement was noted almost immediately and the animals were almost cured in 2 weeks. The improvement was not, however, maintained and during the next few weeks they declined in weight and the acrodynia became much worse. The animals were then treated with 10 drops of fatty acids from maize oil. Rat 1A responded immediately by a rapid gain in weight and the acrodynia-like symptoms cleared up in 3 weeks. Rat 2A responded more slowly, owing to its not taking the maize oil acids readily. Later, when the fatty acids were withheld, the animal declined in weight, developed acrodynia, and died. Rat 6A was given 1.0 ml. of yeast extract when the acrodynia had developed. There was an immediate resumption of growth and the symptoms cleared up to a great extent. The animal continued to grow somewhat irregularly but the acrodynia was never completely cured; slight dermatitis on nose and feet remained all the time. Finally the weight remained steady, and the animal became very weak and died; the skin was at this time very scurfy. Rat 5A was given 2 ml. of yeast extract and behaved in a similar manner to Rat 6A.

It is evident, therefore, that when rats are fed on this fat-free diet without any additional vitamin B₆, severe acrodynia develops very rapidly. Feeding vitamin B₆ may relieve the symptoms to some extent but for complete cure it is necessary also to administer the unsaturated fatty acids.

Diet 17. 5 Per Cent Lard—Fig. 6 shows the results obtained with five rats fed on this diet. The onset of the dermatitis is observed usually about the 6th or 7th week. It may be as late as the 8th or 9th week before the symptoms become at all marked. Each of Rats 11A and 12A was given 0.5 ml. of yeast extract. The acrodynia was quickly cured and growth restored. When the dose was stopped, the animals declined in weight and died after several weeks. No marked external symptoms were visible at death. It is evident that this amount of lard in the diet does not prevent the acrodynia-like dermatitis from developing but does delay the onset of the symptoms to some extent; moreover, the dermatitis is usually not so severe.

Diet 18. 10 Per Cent Lard (Fig. 7)—Results obtained with this diet were similar to those reported for Diet 17 except that

the onset of the acrodynia was still further delayed. Five animals out of six developed acrodynia, two during the 8th and 9th weeks and three during the 12th to 14th weeks. Cures were obtained with 0.25 and 1.0 ml. of yeast extract.

Diet 15. 20 Per Cent Lard—From a group of ten animals, five showed slight acrodynia and two had moderately severe symptoms (Fig. 8). Some of the animals on this diet instead of showing the typical acrodynia developed hemorrhage from the nose and

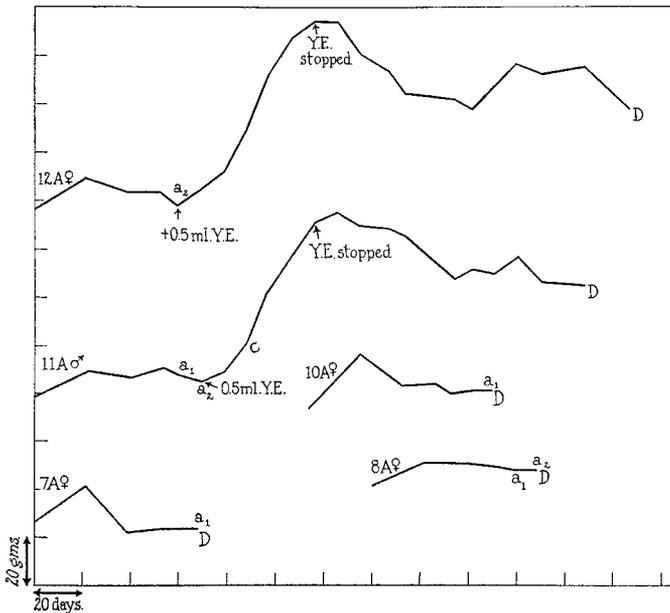


FIG. 6. Growth curves of rats on a vitamin B₆-free diet containing 5 per cent lard. The abbreviations are the same as in Figs. 3 and 5.

edges of the ears. Blood could also be found in the urine (see Table II) and the rats exhibited a sparse, greasy fur which became covered with dried blood. Whether these symptoms are due to lack of the factor which is responsible for the cure of the acrodynia-like dermatitis has not been determined but cures have been obtained with yeast extract.

Diet 19. 75 Per Cent Lard (Fig. 9)—Four animals out of six showed slight acrodynia. The onset of the symptoms was late,

usually about the 13th week. Rat 21A was cured by administering 0.5 ml. of yeast extract but Rat 22A did not respond to 0.25 ml. Rat 20A had blood in its urine at death.

Diets 9 and 10 Containing 15 and 30 Per Cent Linseed Oil—The results with linseed oil (Diets 9 and 10) are not shown in the figures but were similar to those found with the lard diets. On Diet 9 three rats out of five developed acrodynia in about 7 weeks,

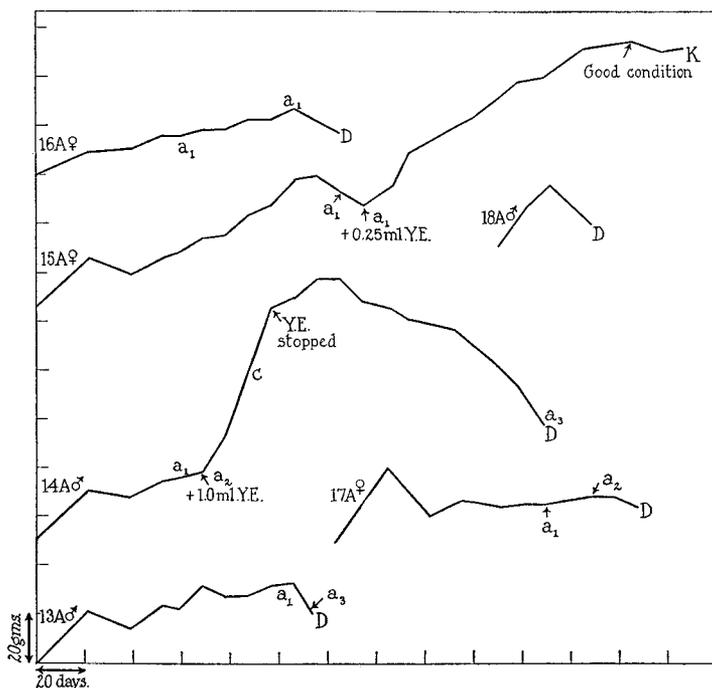


FIG. 7. Growth curves of animals on a vitamin B₆-free diet containing 10 per cent lard. The abbreviations are the same as in Figs. 2, 3, and 5.

while three out of five showed acrodynia on Diet 10 in about the same time. The other two animals showed only slight signs of dermatitis at death.

These experiments show that the inclusion of fats such as lard or linseed oil in the diet of vitamin B₆-deficient rats tends to ward off the onset of the acrodynia-like dermatitis. The symptoms, when they are observed, are usually not so severe as those seen with animals on Diet 8.

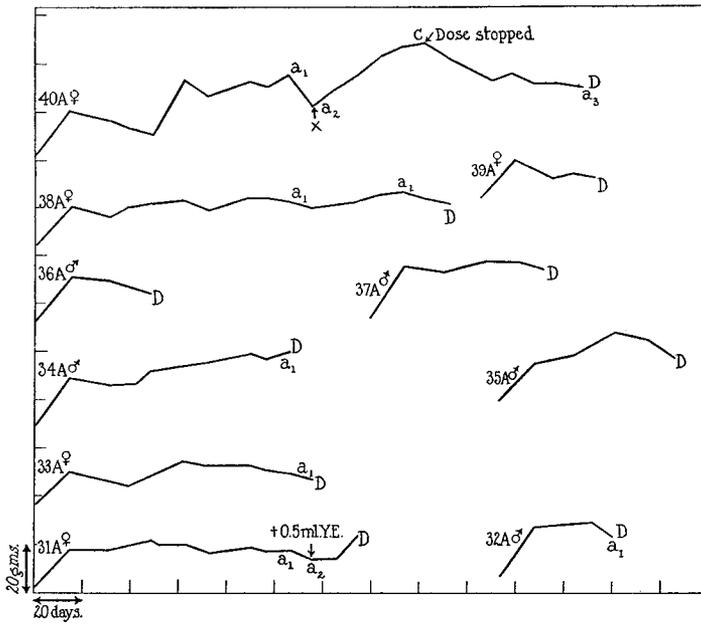


FIG. 8. Growth curves of animals on a vitamin B₆-free diet containing 20 per cent lard. *x* signifies that purified vitamin B₆ concentrate was fed. The other abbreviations are the same as in Figs. 3 and 5.

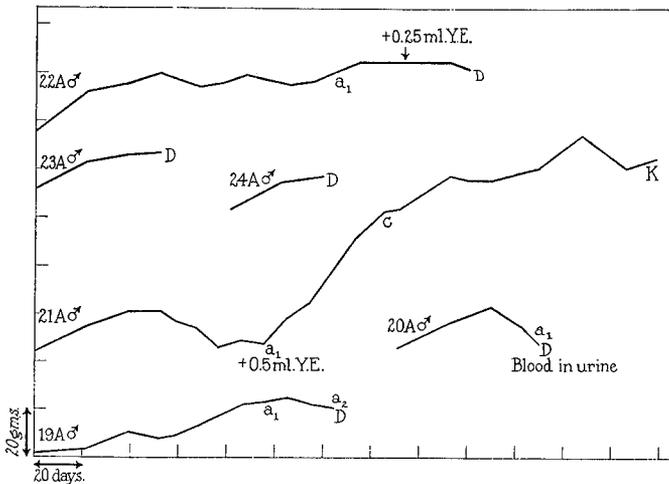


FIG. 9. Growth curves of animals on a vitamin B₆-free diet containing 75 per cent lard. The abbreviations are the same as in Figs. 3 and 5.

DISCUSSION

It is evident from the experiments recorded above that two factors are concerned in the cure of the acrodynia-like dermatitis. One is the water-soluble factor vitamin B₆ which is present in water extracts of yeast and wheat germ, while the other is present in the fatty acid fraction of certain oils and fats and appears to be similar to the "linoleic acid" of Burr and Burr (1, 2).

In György's (6, 7) experiments sufficient unsaturated fatty acids were present in his diets (contained in the starch, 9 per cent butter fat, 1.0 per cent cod liver oil) so that cures were obtained with only the water-soluble factor vitamin B₆. In Burr and Burr's experiments sufficient vitamin B₆ was provided by feeding 0.7 gm. of whole yeast powder so that cures were obtained with only unsaturated fatty acid. These latter authors state (1) that the symptoms described by them are to some extent similar to the pellagra symptoms in the rat described by Goldberger and Lillie (10). They concluded, however, that the symptoms were not related, because of the different methods of producing them and because no necrosis of the tail was described by Goldberger and Lillie.

This conclusion has not been substantiated, as it is seen that the exact type of symptom curable by unsaturated fatty acid is influenced by the amount of vitamin B₆ in the diet. Furthermore, caudal necrosis is often observed when animals are fed on diets which contain appreciable amounts of unsaturated fatty acid but which are devoid of vitamin B₆. Richardson and Hogan's (9) experiments, which showed that maize oil was relatively ineffective in curing the rat dermatitis, are difficult to correlate with those recorded here. One might assume that irradiation of the yeast in their experiments did not destroy all the vitamin B₆ present, but in the absence of sufficient fat (2 per cent cod liver oil) the animals developed the typical acrodynia-like dermatitis. The feeding of extra fat would, therefore, cause a remission of the acrodynia. Similarly feeding extra vitamin B₆ provided by a water extract of yeast would also cure the symptoms, as their diets were not completely devoid of fat. These authors were, therefore, possibly working on the border line of deficiency of both factors, so that feeding of either one produced a cure of the dermatitis. It must also be remembered that they did not find

maize oil absolutely inactive; it was merely less potent than wheat germ oil or an alcohol extract of corn-starch.

It may be suggested in explanation of this observation, that it is a particular isomer of linoleic acid, or a closely related substance, which is the active principle and that the amount of this substance in oils and fats does not correspond exactly to their linoleic acid content. Recent work by Turpeinen (11) has indicated that arachidonic acid is 3 times more effective in curing the unsaturated fatty acid deficiency than is linoleic acid. Burr, Burr, and Miller (3) found that methyl arachidonate had a "slight unexplained depressing effect" on the curative action of linoleic acid but lard and liver fat which contain arachidonic acid were very effective in curing the deficiency.

Turpeinen (11) suggests that arachidonic acid is the essential acid and that linoleic acid acts by being converted to arachidonic acid in the animal body. If this suggestion were correct, it would account for the curative action of fats not running strictly parallel to their linoleic acid content.

When these considerations are taken into account, it seems reasonable to conclude that the fatty acid factor described by Hogan and Richardson and the one described here are essentially similar to that of Burr and Burr, but whether it is actually linoleic acid, arachidonic acid, or a particular isomer of either of these substances it is impossible to decide at present.

A similar conclusion has been reached recently by Salmon (12) who finds that rats develop a severe form of erythematous dermatitis when fed on a fat-free diet supplemented with carotene, vitamin D, thiamine, riboflavin, and a limited amount of aqueous extract of brewers' yeast which has been subjected to dry heat treatment (24 hours at 120–130°) before extraction. The further addition of corn oil, linseed oil, wheat germ oil, or the fatty acids of linseed or soy bean oil cures or prevents the dermatitis. If the heated yeast extract is omitted, however, the oils do not cure or prevent the condition. Quackenbush and Steenbock (13) also find that rats develop acrodynia on a fat-free diet supplemented by carotene, calciferol, thiamine, and riboflavin. This symptom was healed completely by the daily administration of 10 mg. of wheat germ oil, corn oil, or Wesson oil, 200 mg. of coconut oil, or 500 mg. of butter fat, without further addition of yeast extract.

The curative factor in these fats was traced to the unsaturated fatty acid fraction.

The close relationship between the amount of unsaturated fatty acid in the diet and the severity of the acrodynia-like dermatitis would indicate that vitamin B₆ is connected in some way with the metabolism of the unsaturated fatty acids. The sparing action which fats have upon thiamine (Evans and Lepkovsky (14)) was used as an argument in favor of the assumption that thiamine was concerned in carbohydrate metabolism. This sparing action, however, could only be demonstrated by feeding 25 to 50 per cent of natural fat in the diet and seemed to be due rather to the exclusion of carbohydrate than to a specific action of the fat. A more recent observation by Salmon and Goodman (15) shows that 5 per cent glyceryl caprylate exerts a sparing action on thiamine and that the rat with beriberi may be cured by feeding the pure glyceride. If this observation is correct, a different interpretation of the thiamine-sparing action of fat must be considered. With vitamin B₆ an effect can be demonstrated by feeding only a few drops of maize oil and so is certainly not due to the exclusion of carbohydrate from the diet and must be put down to some specific action of the unsaturated fatty acids.

No information has been obtained concerning the exact biological relationship between vitamin B₆ and the unsaturated fatty acids. The only conclusion one can draw from these experiments is that in the absence of an adequate supply of vitamin B₆ the animal is unable to make proper use of the unsaturated fatty acids or alternately in the absence of adequate amounts of unsaturated fatty acid the animal is unable to utilize its vitamin B₆. Whether vitamin B₆ is concerned in the mobilization or transport of fat or whether it is concerned in oxidation has yet to be determined. The possibility also remains that vitamin B₆ merely combines with the unsaturated fatty acids to form some essential constituent of the cell, similar to lecithin or its allied substances. This latter possibility seemed likely, as vitamin B₆ appears to have similar properties to choline (Birch and György (4)). These authors suggested that part of the vitamin B₆ present in tissue might exist combined with the protein as a prosthetic group, for it was found that the vitamin could be extracted quantitatively only after autolysis of the tissue.

If, therefore, vitamin B₆ exists in the living organism combined with fatty acid, it must be firmly bound to the tissue, as it is not extracted in such a form by lipid solvents and after autolysis it exists as a water-soluble basic substance.

SUMMARY

Evidence is presented which shows that two factors are concerned in the production and cure of the acrodynia-like dermatitis of rats. One is the water-soluble basic substance vitamin B₆; the other is fat-soluble and is present in the fatty acid fraction of maize oil. The evidence indicates that the fat-soluble factor is similar to the "fatty acid factor" of Burr and Burr and to the fat-soluble antidermatitis factor of Hogan and Richardson.

It is suggested that the physiological function of vitamin B₆ is connected with the utilization of the unsaturated fatty acids.

I have pleasure in expressing my thanks to Dr. L. J. Harris of the Nutritional Laboratory, Cambridge, for his hospitality during the time this work was in progress there and to Dr. J. T. Wearn for the facilities afforded at Cleveland. I am also indebted to the I. G. Farbenindustrie of Germany and the S.M.A. Corporation of Cleveland for gifts of thiamine and lactoflavin. The work carried out in Cleveland was financed by the Bourne Fund in the Department of Medicine of Lakeside Hospital.

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