/THE METABOLISM OF LINOLENIC ACID/

by

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INTRODUCTION

Burr and Burr (5) showed that rigid dietary restriction of fat produced a typical dermatitis and retarded growth. As the addition of either linoleic or linolenic acids to the diet alleviated these symptoms, they concluded that some unsaturated fatty acids were essential and must be supplied in the diet. Furthermore, the animal was apparently unable to synthesize these acids.

The work of Burr and Burr (5) contradicted the idea of Leathes and Wedell (9) that the animal body is capable of desaturating fatty acids. The oleic acid present in all animal fats would undoubtedly have been desaturated to linoleic acid and thus prevented the development of the deficiency symptoms had the animal possessed this power. However, Stetten and Schoenheimer (18) have shown by the use of deuterium that the animal can desaturate palmitic and stearic acids to palmitoleic and oleic acids, respectively. There has been no evidence to prove that the desaturation can progress further than oleic acid as no deuterium labeled linoleic acid was found. Thus, further evidence was presented which showed that the latter could not be synthesized in the animal body.

The relative potencies of the fatty acids in the cure of deficiency diseases have been studied by various workers. Hume et al. (7) found that linoleic acid was six times more

potent than linolenic acid in curing dermatitis and promoting
growth. Burr (4) has stated generally that both are very ef-
fective curative agents. Quackenbush, Steenbock, Kummerow and
Platz (15), on the other hand, found linolenic acid to be non-
functional in reproduction.

Some of the B-complex vitamins have also been shown to
be functional in fat metabolism (11). The effect of pyridoxine
and pantothenic acid seems of special importance. Birch (1)
concluded that pyridoxine was in some way connected with the
unsaturated fatty acid factor of Burr and Burr (5) and there-
fore was important in fat metabolism. Furthermore, pyridoxine
was discovered by Birch, Gyorgy and Harris (2) to be effective
in protecting the rat from acrodynia. Schneider et al. (17)
have suggested that pyridoxine and the essential fatty acids
are interchangeable. However, Quackenbush et al. (15) have
shown these conclusions to be invalid. Umbriet and Gunsalus
(19) have suggested that pyridoxine is also concerned in amino
acid metabolism.

Lippman (10) has recently shown that pantothenic acid can
be added to the group of B-complex vitamins which function as
coenzymes. For some time it has been realized that pantothenic
acid protects the chick from a characteristic dermatitis in
much the same manner as pyridoxine protects the rat. On the
other hand, Quackenbush et al. (15) found that both pyridoxine
and pantothenic acid were necessary for proper function of the
unsaturated fatty acid factor. Their work indicated that the

two vitamins were functional, but they were unable to deter-
mine how these factors were involved in fatty acid metabolism.

Kummerow et al. (8) have found that the onset of rancid-
ity was dependent on the presence of highly unsaturated fatty
acids in the depot fats of turkeys and chickens held in cold
storage for an extended period of time. Ethanolamine seems
to be concerned in the overall metabolism; i.e., when ethanol-
amine was present the birds seemed to deposit less fat. The
removal of linolenic acid seemed of particular value in the
prevention of rancidity. Therefore, it would appear that
ethanolamine, as well as the B-complex vitamins, may be con-
cerned with fat metabolism.

Since some question exists regarding the biological ef-
fectiveness of linolenic acid, the present experiment was
undertaken in an attempt to study the factors involved in the
basic metabolism of the acid. Because rancidity of cold
storage pountry is a great problem in the frozen food industry,
a possible alteration of the deposited body fat is important.
Linolenic acid metabolism is significant since it is conducive
to rancidity development. This experiment was undertaken to
clarify problems which are of interest to the frozen food in-
dustry and to nutritionists.

EXPERIMENTAL

Rats of the Sprague-Dawley strain, three weeks of age
and weighing from 40 to 50 g, were used for the experiment.
Each rat was weighed, transferred to an individual wire cage
which was numbered, and given water and diet V ad libitum.
The animals were kept in a constant temperature room at 28° C.

Diet V (Table 1) of Quackenbush, Platz and Steenbock (13),
which is free of pyridoxine, pantothenic acid and fat, was
prepared as follows: 200 mg of thiamin hydrochloride and 400
mg of riboflavin were dissolved in N/50 acetic acid, mixed
with 18 pounds of casein, dried and then mixed with 78 pounds
of cerelose and 4 pounds of Wesson salts in a standard feed
mixer. The diet of the animals was supplemented once a week
with one drop of fish liver oil containing 250 IU of vitamin
A and 100 IU of vitamin D_3 per drop.

Animals kept on diet V developed severe dermal symptoms
of fat deficiency or acrodynia in 7 to 8 weeks. The various
stages of the dermal condition of each animal was recorded
weekly by a modified method of Quackenbush et al. (13) as shown
in Table 2. The dermal index was a numerical expression of
the skin condition and was an indication of the condition of
the eyes, ears, lips, fore paws, hind paws and tail. These
parts of the body were the most acutely affected. Dermal in-
dex number increased with severity of the acrodynia.

The rats were divided into groups of three to six animals each and fed the supplements (Table 3) by calibrated medicine dropper. The ethyl linolenate was prepared from hexabromide according to the method of Rollet (16). The resulting product was distilled under high vacuum and kept under an atmosphere of oxygen at -13° C. Water soluble supplements were prepared by dissolving the synthetic vitamins in water. Ethanolamine, neutralized with concentrated hydrochloric acid and diluted with water, was used. After three weeks of supplementation, the animals were killed and extracted according to the method of Quackenbush and Steenbock (14). Iodine values of the resulting fatty acids were determined according to the method described by Yasuda (20). Composition of the mixed fatty acids was determined spectrophotometrically according to Brice et al. (3). All methods are outlined under experimental procedures.

Percentages of fatty acids as calculated from spectrophotometric analysis were converted to mg per 100 g. By considering the mean weight of fatty acid per 100 g, the resulting calculations reflected the difference more accurately than a comparison of percentage alone. The formula used for calculation of mg was as follows:

$$\frac{\% \text{ fatty acids}}{100 \text{ g}} = \frac{X}{\text{mean weight extracted fatty acid}}$$

X = gm fatty acids
X x 1000 = mg fatty acid

The amount of any one fatty acid synthesized or metabolized during the experimental period was determined by considering fat synthesis or metabolism of the control group. The amount of acid calculated for the control group was subtracted from all other groups. Results showing plus values indicated synthesis, minus values indicated metabolism.

EXPERIMENTAL PROCEDURES

Preparation of Supplements

Preparation of Linolenic Acid. Linolenic acid was prepared by heating 300 g of potassium hydroxide, 1200 ml of ethyl alcohol, and 100 ml of distilled water to nearly boiling on a steam bath. One thousand g of linseed oil was added to the hot solution and the entire mixture was refluxed for 30 minutes. The hydrolysate was cooled with tap water to about 40° C. and 1300 ml of cold distilled water was added. The solution was acidified with cold concentrated hydrochloric acid. The cold mixture was transferred to a large separatory funnel and shaken to decompose the soaps. After washing the fatty acids twice with water, one liter of ethyl ether was added and the fatty acids were washed again. When the water had been removed, the fatty acids were dried over sodium sulfate. One liter of ether was again added and the solution was allowed to stand overnight at -13° C. The sodium sulfate and saturated

acids were removed by filtering and another liter of ether
was added to the filtrate which was ready for bromination.

Preparation of Crystalline Hexabromostearic Acid. The
flask containing the fatty acids was clamped into an ice bath.
A mechanical stirrer agitated the solution. Bromine was added
slowly from a separatory funnel and the fatty acids were kept
at low temperatures. About 580 g of bromine was required for
complete saturation as determined by the persistence of the
bromine color. The flask was allowed to cool overnight at
-13° C. Crude crystalline hexabromostearic acid was collected
on a Buchner funnel, washed with ether and transferred to a
dry round bottom flask. Sufficient quantities of ether were
added to insure complete solution and the mixture was refluxed
on a steam bath. Twenty g of Norit was added, refluxing con-
tinued for a few minutes, then the hot solution was refil-
tered through a Buchner funnel. The filtrate was allowed to
stand overnight at -13° C. and the product was filtered on a
Buchner funnel and washed with ether. The white crystals
were dried at room temperature.

Preparation of Ethyl Linolenate. Two hundred g of hexa-
bromostearic acid and 200 g of zinc were mixed and put into a
found bottom flask. Following the addition of 200 ml of ethyl
alcohol and 2 ml of concentrated sulfuric acid, the solution
was refluxed for two hours. The mixture was cooled under tap
water and poured into a separatory funnel containing dis-
tilled water. After washing thoroughly, the mixture was allowed

to stand until the ester of linolenic acid separated from the
water. The aqueous solution was removed and extracted twice
with petroleum ether. The ester and the ether solutions were
washed with sodium carbonate solution and water. The ether
fraction was dried over sodium sulfate, filtered and freed
from solvent under reduced pressure.

Saponification

The rats were killed with ether and allowed to digest on
a steam bath with 100 cc of 30 percent potassium hydroxide per
100 gm of tissue. After six hours, one fifth volume of ethyl
alcohol was added and the digestion continued for another two
hours. The hydrolysate, freed from bones, was poured into
Erlenmeyer flasks and refluxed for an additional two hours.
The hydrolysate was then cooled and poured into large separa-
tory funnels. The non-saponifable material was removed by
two extractions with one third volume of Skellysolve F and
discarded. After removal of the non-saponifable material, the
solution was cooled under tap water and acidified with dilute
1:1 hydrochloric acid. The fatty acids were extracted three
times with one third volumes of Skellysolve F. Crude fatty
acids in the Skellysolve F layer were washed twice with dis-
tilled water to remove any excess hydrochloric acid and dried
over anhydrous sodium sulfate. When the fatty acids had been
partially freed from solvent under reduced pressure, the fatty

acids were poured into a weighed flask and the remaining solvent removed in a vacuum oven. Percentages of fatty acids were calculated when the tared flask and mixed fatty acids had been reweighed.

Iodine Value

Approximately 0.1 g of the mixed fatty acids was weighed into a glass stoppered iodine flask. Five ml of chloroform and exactly 15 ml of Wijs solution were added. The flasks were stoppered and placed in the dark. At the end of one hour, 10 ml of 15 percent potassium iodide solution was added and mixed thoroughly. The glass stopper and sides of the flask were rinsed with 10 ml of distilled water. The sample was titrated with N/10 sodium thiosulfate solution to a faint yellow. A few drops of starch solution were added and the mixture shaken well to free all the iodine. The solution was then titrated to a colorless end point. Two blank determinations were titrated with the unknowns.

Wijs Solution. Thirteen g of iodine was dissolved in a liter of glacial acetic acid. The solution was heated until all the iodine crystals were dissolved and then cooled. Chlorine gas was bubbled into the solution until the color was orange brown. The titration of the Wijs solution was double the titration of the original iodine solution when titrated with N/10 thiosulfate solution.

Potassium Iodide Solution. Fifteen g of potassium iodide was dissolved in 85 ml of distilled water.

N/10 sodium thiosulfate solution. Twenty four and eight tenths g of sodium thiosulfate were dissolved in one liter of distilled water and the exact normality determined by the following procedure: Exactly 10 ml of N/10 potassium dichromate was measured into an iodine flask and 5 ml of concentrated hydrochloric acid added. Ten ml of a 15 percent potassium iodide solution was added and the mixture was immediately titrated with thiosulfate solution to a greenish color. Starch solution was added and the titration continued slowly with occasional shaking until the solution turned bright, clear green in color.

The formulas used in calculating the iodine value were:

$$\text{Normality factor} = \frac{126.9 \times .1 \text{ (normality of dichromate)}}{\text{ml thiosulfate used}}$$

$$\text{Iodine value} = \frac{\text{normality factor} \times \text{(blank tit. - sample tit.)}}{\text{sample weight}}$$

Spectrophotometric Analysis

Approximately 0.1 g of mixed fatty acids were pipetted into small weighing vessels. The vessels were placed in marked ignition tubes. Three ml of freshly prepared ethylene glycol containing 7.5 percent potassium hydroxide were added to each sample. Nitrogen was blown into each tube to replace air and the tubes were stoppered with glass tops and placed in a wire

basket. The basket was placed in preheated oil bath at 180° C. and the tubes held at this temperature for 30 minutes. Two blank determinations were run with each set of unknown samples.

After heating for 30 minutes, the basket was removed from the bath and the oil was wiped from the tubes with a piece of cloth. Contents of the tubes were transferred quantitatively to 100 ml volumetric flasks using 95 percent ethyl alcohol. The solution was diluted to 100 ml. Each sample was mixed. The volumetric flasks were allowed to stand overnight in the cold room so that the silica from the glass precipitated. The samples were removed from the cold room and allowed to stand until they reached room temperature. Each sample was filtered into an Erlenmeyer flask. A 10 ml aliquot was carefully transferred to a 250 ml volumetric flask, diluted to volume and mixed thoroughly. The samples were read on the Beckman spectrophotometer and readings were recorded at 2320, 2340, 2620, 2680, 2740, 3100, 3160 and 3220 $\mathring{A}$. If samples could not be read at the above wavelengths, suitable dilutions were made as previously described. Blanks were diluted by the same method as the unknowns.

Ethylene Glycol. Fifteen g of ground potassium hydroxide was dissolved in 180 cc of ethylene glycol.

The formulas used for calculating the fatty acid compositions were:

$$K_2 = \frac{K_{2320}}{\text{wt sample per liter}} - .04$$

$$K_3 = \frac{2.8}{\text{wt sample per liter}} (K_{2680} - K_{2620} \quad K_{2740}/2)$$

$$K_4 = \frac{2.5}{\text{wt sample per liter}} (K_{3160} - K_{3100} \quad K_{3220}/2)$$

The preceding formulas were those used for unisomerized samples which were used as correction factors.

$$K'_2 = \frac{K_{2320}}{\text{wt sample per liter}} + .07$$

$$K'_3 = \frac{4.1}{\text{wt sample per liter}} (K_{2680} - \frac{K_{2620} \quad K_{2740}}{2})$$

$$K'_4 = \frac{2.5}{\text{wt sample per liter}} (K_{3160} - \frac{K_{3100} \quad K_{3220}}{2})$$

where K_{2320}, K_{2620} etc. designated the spectrophotometric readings of the fat sample at that particular wave length.

$$K''_2 = K'_2 - K_2$$
$$K''_3 = K'_3 - K_3$$
$$K''_4 = K'_4 - K_4$$

Percent linoleic acid $= 1.125 \times K''_2 - (1.27 \times K''_3) + .04 \, K''_4$

Percent linolenic acid $= 1.87 \times K''_3 - 4.43 \times K''_4$

Percent arachidonic acid $= 4.43 \times K''_4$

Percent oleic acid = Iodine number x 100 (181.5 x percent linoleic acid - 273.5 x percent linolenic acid - 333.5 x percent arachidonic acid)/90.

Percent saturated acids = 100 - percent linoleic acid - percent linolenic acid - percent arachidonic acid - percent oleic acid.

RESULTS

In all groups which did not receive pyridoxine and/or calcium pantothenate, a loss of body weight and aggravation of dermal condition was noted. When ethyl linolenate and ethanolamine were fed a loss of 13.5 gm mean body weight occurred. Ethanolamine alone caused a severe aggravation of the skin condition. The addition of pyridoxine and calcium pantothenate brought about large weight gains in all cases. Ethyl linolenate supplemented with the two vitamins produced a gain of 47 gm and caused improvement of the symptoms; ethyl linolenate alone caused a weight loss of 6.0 gm. Yet, when pyridoxine and calcium pantothenate were given without a source of essential fatty acids, no change in dermal index was noticed although the animals gained a considerable amount of weight.

Although the mean weight of mixed fatty acids extracted varied (Table 4), two trends were established. Without pyridoxine and calcium pantothenate, ethyl linolenate was a necessary factor for the deposition of fat. However, when choline or ethanolamine was given in combination with ethyl linolenate only 0.2 and 0.6 g fat respectively were extracted. Therefore, the possible role in metabolism of the two components of phospholipids might be suggested. The second trend established by the data showed that the amount of fat extracted increased greatly when pyridoxine and calcium pantothenate were

added. Ethyl linolenate with the two vitamins caused a deposition of 3.9 percent of fat.

Variation in the iodine value was great. Animals fed ethyl linolenate and tocopherol showed the largest and the group fed pyridoxine and calcium pantothenate with no fatty acid showed the smallest iodine value or 118.0 and 70.6, respectively.

Spectrophotometric analysis of the mixed fatty acids indicated large differences in composition of the deposited fat. Linolenic acid is of principal importance in this study. Table 5 indicates that groups receiving no supplement, ethanolamine, and tocopherol deposited no linolenic acid since there was no dietary source available. With linolenic acid, tocopherol showed the largest synthesis with 49.0 mg while linolenate alone showed 39.6 mg. Choline and ethanolamine seemed to bring about a reduction in the deposition of linolenic acid even though a dietary source was present.

When pyridoxine and calcium pantothenate were added to the diet the results were altered by the presence of the two vitamins. Except for one group no linolenic acid was found in spite of the dietary source. The animals receiving ethyl linolenate and only calcium pantothenate showed linolenic acid had been deposited in the tissue. However, when linolenate was fed with both vitamins all traces of this acid were removed.

As the linolenic acid fluctuated, arachidonic and lino-

leic acids showed changes. The greatest amount metabolized
was indicated by the group which received linolenate and
choline. In this group, however, the total percent of fat
was low. The corresponding group with ethanolamine was also
low, but a very slight synthesis was shown for both linoleic
and arachidonic acids. Again, pyridoxine and calcium panto-
thenate seemed to be functional in the synthesis of these
acids as shown by the group supplemented with the two vitamins
and ethyl linolenate. This group synthesized 120.7 mg arachi-
donic acid and 64.3 mg linoleic acid over the control.

One of the largest variations was shown in a comparison
of the amounts of oleic and saturated acids which were de-
posited. Previously mentioned trends were repeated. The
combination of linolenate and choline effected a metabolism
of 312 mg oleic acid, always common to the animal body, and
137 mg of saturated acid. Linolenate and ethanolamine, as
has been shown with other acids, produced similar results but
to a lesser degree. The addition of pyridoxine and calcium
pantothenate caused large amounts of oleic and saturated acids
to be synthesized. Animals receiving linolenate and the two
vitamins produced 2188 mg oleic acid and 114 mg saturated
acids. The group receiving only pyridoxine and calcium panto-
thenate also synthesized significant amounts of the two acids.
The lower degree of unsaturation was in agreement with the
lower iodine values of these groups. It is also interesting
to note that the group receiving only linolenate and choline

had one of the highest iodine numbers and metabolized the
largest amounts of oleic and saturated acids.

Table 1. Diet V.

Ingredients	Pounds/100# ration
Casein	18
Wesson salts	4
Cerelose	78
Thiamin hydrochloride	200 mg
Riboflavin	400 mg

<div align="center">Wesson salts</div>

Sodium chloride	105.00 gm/kg
Potassium chloride	120.00
Potassium hydrogen phosphate	310.00
Calcium diphosphate	149.00
Calcium carbonate	210.00
Magnesium sulfate	90.00
Ferric pyrophosphate	59.50
Manganese sulfate	.20
Potassium aluminum sulfate . $24H_2O$	.09
Cupric sulfate . H_2O	.39
Potassium iodide	.05

Table 2. Stages of dermatitis.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Lips	Normal	Hyperemia, usually with small ulcers at corners	Slight swelling, redness along vertical grove	Medium swelling desquamation to nostrils	Severe swelling, raw sores around mouth and nostrils
Eyes	Very slight scaliness	Lids wet, slight crust at corners	Slight swelling of lids, crust in corners	Medium swelling, lids adhere with gummy exudate	Severe swelling, lids swollen shut, crusts on lids
Fore paws	Very slight scaliness	Slightly scaly redness, desquamation between digit	Slight swelling, digits desquamated and sore	Medium swelling, digits raw and sore, pus sores	Severe swelling, desquamated and sore, gangrenous
Hind paws	Same as fore paws	Same as fore paws	Same as fore paws, desquamation of large areas on paws	Same as fore paws, skin cracked and ulcerated	Severe swelling, large red areas on paws and inner thighs
Ears	Very slight swelling and slight scaliness	Hyperemia, slight swelling	Pinnae swollen and thickened due to scale formation	Medium thickening at lips, large scales sloughing	Severe thickening, necrosis
Tail	Very slight scaliness	Scaly with small ringlets	Very scaly	Very scaly, necrosis near tip	Sections of tail may drop off

Table 3. The effect of supplements on dermal lesions and weight.

Group	Supplements	Mean body weight change in 3 weeks	Dermal index Initial	3 weeks	Description
	I. Without pyridoxine and calcium pentothenate*				
1	None	- 7.0	5.0	7.5	Negative
2	18 mg ethanolamine	- 9.3	4.3	8.0	Negative
3	14 mg tocopherol**	- 8.3	4.3	8.0	Negative
4	87 mg et. linolenate	- 6.0	4.6	6.0	Negative
5	87 mg et. linolenate 20 mg choline	-11.5	3.0	6.0	Negative
6	87 mg et. linolenate 18 mg ethanolamine	-13.5	4.0	5.5	Negative
7	87 mg et. linolenate 14 mg tocopherol**	- 6.0	3.6	5.0	Negative
	II. With pyridoxine and calcium pentothenate*				
8	87 mg et. linolenate pyridoxine only	24.5	0.0	0.0	-
9	87 mg et. linolenate only Ca pentothenate	12.0	5.3	5.5	Negative
10	None	41.0	4.3	4.3	No change
11	87 mg et. linolenate	47.0	4.0	2.6	Improvement
12	87 mg et. linolenate ethanolamine	32.6	6.4	2.2	Improvement

* 20 gamma pyridoxine and 50 gamma Ca pentothenate daily.
** Amount given weekly. Other supplements listed were given daily.

Table 4. The effect of supplements on total fat.

Group:	Supplements	Mixed fatty acids		Iodine value
		Mean weight	Percent	
	I. Without pyridoxine and calcium pentothenate			
1	None	.72	1.43	86.8
2	Ethanolamine	.69	1.29	94.5
3	Tocopherol	.68	1.13	85.2
4	Et. linolenate	1.32	1.95	97.3
5	Et. linolenate choline	.24	.44	115.4
6	Et. linolenate ethanolamine	.55	.88	105.9
7	Et. linolenate tocopherol	1.30	1.58	118.0
	II. With pyridoxine and calcium pentothenate			
8	Et. linolenate pyridoxine only	3.71	6.31	88.2
9	Et. linolenate Ca pentothenate only	2.63	1.19	84.5
10	None	3.51	3.13	70.6
11	Et. linolenate	4.24	3.86	74.2
12	Et. linolenate ethanolamine	3.73	3.75	77.5

Table 5. Spectrophotometric studies of mixed fatty acids from carcasses of fats.

Group:	Supplements	Arachidonic : %	: Mg*	Linolenic : %	: Mg*	Linoleic : %	: Mg*	Oleic : %	: Mg*	Saturated : %	: Mg*
					I. Without pyridoxine and calcium pantothenate						
1	None	5.6	0	0	0	4.0	0	67.5	0	22.9	0
2	Ethanolamine	7.0	12.3	0	0	5.5	9.1	67.9	-20	19.6	- 27
3	Tocopherol	5.2	- 2.0	0	0	3.8	- 3.0	67.6	-27	23.4	- 2
4	Et. linolenate	6.5	45.3	3.3	39.6	4.0	24.1	65.7	381	20.4	106
5	Et. linolenate — choline	7.8	-19.2	5.0	12.0	6.1	-14.4	71.8	-31.2	9.3	-137
6	Et. linolenate — ethanolamine	8.0	2.6	2.7	14.7	5.6	2.0	68.5	-108	15.2	- 76
7	Et. linolenate — tocopherol	9.7	81.0	3.8	49.0	7.1	62.2	69.2	415	10.2	- 28
					II. With pyridoxine and calcium pantothenate						
8	Et. linolenate — B6 only	3.4	55.0	0	0	2.9	105.1	72.5	1201	21.1	399
9	Et. linolenate — Ca panto only	3.8	58.5	.89	23.1	3.2	54.4	71.2	1364	28.7	581
10	None	1.0	- 5.2	0	0	2.0	41.4	68.5	1860	27.8	824
11	Et. linolenate	3.8	120.7	0	0	2.2	64.2	63.9	2188	30.0	1114
12	Et. linolenate — ethanolamine	3.4	86.5	0	0	1.8	38.3	69.5	2112	24.4	750

* Milligrams of a fatty acid synthesized (plus values) or metabolised (minus values) as calculated from percent and mean weight of extracted fatty acids.

DISCUSSION

The biological effectiveness and metabolism of linolenic acid seem to be dependent on the presence of pyridoxine and pantothenic acid. The nutritive value of the various fatty acids has been discussed by Hume et al. (6). The relative potency of ethyl linolenate compared with other esters of fatty acids is not important here, but of significance is the improvement in dermal symptoms and weight. The general state and well being of the animals were markedly improved when linolenate, pyridoxine and calcium pantothenate were given together. These results are in general agreement with those found by Burr and Burr (5) who had added yeast, a rich source of both vitamins, to their basal diet.

Hume et al. (7) have concluded that linolenic acid is changed upon entering the body. Nunn and Smedley-MacLean (12) have further concluded that linolenic acid is not stored as such in the animal body unless large amounts are fed. These conclusions are again illustrated in the present experiment and here, as in the work of Nunn and Smedley-MacLean (12), pyridoxine and pantothenic acid were present. However, without the vitamins, linolenic acid evidently was not changed upon entering the body since the three double bond acid is found as such in the animal body fat.

Removal of linolenic acid seems not only of value in prolonging the onset of rancidity as suggested by Kummerow

et al. (8) but seems also essential for the life of the rat.
When ethyl linolenate was given to the animals, two out of
three died before the three weeks' experimental period had
elapsed. Analysis of these animals showed that linolenic
acid was present in extracted fat. Yet, when pyridoxine and
calcium pantothenate were added with linolenate the deficiency
disease was cured and the animals returned to normal. Analysis
of the extracted fat showed no linolenic acid. Similar re-
sults were indicated when only linolenate and pyridoxine
were fed, however, growth was slightly impaired without cal-
cium pantothenate. Pyridoxine was evidently functional in
removing the linolenic acid. Pyridoxine was also a stimulus
to growth, although the weight gain was slightly sup-optimum.

As Burr and Burr (5) have suggested, a dietary source of
unsaturated fatty acids must be provided. However, in the
presence of pyridoxine and calcium pantothenate, the rat
organism seems capable of converting the furnished fatty acids
to other unsaturated acids such as linoleic and arachidonic
acids. Nunn and Smedley-MacLean (12) have pointed out that
unless linoleic or linolenic acids are present, the rat is not
capable of synthesizing the more highly unsaturated and longer
chain fatty acids which seem necessary for life. They further
suggest that linolenic and/or linoleic acids function as
building blocks for the higher acids. Furthermore, Nunn and
Smedley-MacLean (12) suggested that linolenic acid is converted
to a C_{22} acid with five double bonds. They believed that the

body accomplished the conversion not by desaturation but by building longer chains from linolenic acid, double bonds being formed in the process. The present data cannot confirm this suggestion since at present the spectrophotometric method of analysis does not include unsaturation higher than four double bonds. However, the idea seems possible in the presence of pyridoxine and pantothenic acid. This work does show that the vitamins and ethyl linolenate combine to synthesize considerable amounts of linoleic and arachidonic acids. The possibility that arachidonic acid is built as has been suggested for a C_{22} acid is feasible.

Quachenbush and Steenbock (14) were unable to explain lower iodine values obtained when rats were fed supplements of pyridoxine and calcium pantothenate. A partial hydrogenation catalyzed by the two vitamins appears to be one possible explanation. Yet, when the vitamins were given without a fatty acid, the iodine value was lowered. The possibility that pyridoxine and calcium pantothenate stimulated the production of fat from carbohydrate and/or protein also would seem logical. Determination of the mechanism by which the synthesis of the more saturated acids occurred was not possible here. However, after further investigation, the alteration of metabolism toward deposition of more saturated fatty acids might prove valuable as an approach to the delay of rancidity.

The function of choline and ethanolamine seemed to be

quite similar. In both cases, the supplements prevented the deposition of fat. When only ethanolamine was given the percentage of extracted fat was higher than when linolenate was supplied in the diet with ethanolamine. In some undetermined way the ethanolamine and choline evidently caused the fat to be metabolized. However, when the two were present, the smaller amount of fat which was deposited seemed to be of a more unsaturated nature. When ethanolamine, ethyl linolenate, pyridoxine and calcium pantothenate were given together a competition probably existed between the vitamins and the ethanolamine. The percentage of fat deposited was slightly lower, the iodine value slightly higher, and the fatty acid composition slightly more unsaturated than when ethanolamine was omitted from a similar group.

Hume et al. (7) have suggested that no significant results were obtained by including tocopherol in the diet. They state that skin lesions were healed and weight increases appeared to be promoted as well in the absence as in the presence of tocopherols. The present results show that vitamin E made no significant contribution to the state of the animal.

SUMMARY

Analysis of extracted carcass fat showed that linolenic
acid was not deposited in the tissue when pyridoxine and cal-
cium pantothenate were given with ethyl linolenate. However,
without the two vitamins, linolenic acid was deposited and
the animals died. On supplements of linolenate, pyridoxine
and calcium pantothenate, the amounts of linoleic and arachi-
donic acids were higher than in groups not receiving the vita-
mins. A possible conversion of linolenic acid to linoleic
and arachidonic acids was suggested.

Iodine values of groups receiving pyridoxine and cal-
cium pantothenate were lower than those of groups not supple-
mented with the vitamins. Analysis of fatty acid composition
showed more oleic and saturated acids had been deposited by
rats receiving the two vitamins.

Chlorine and ethanolamine supplementation caused a low-
ering of the percentage of fat extracted. Although the
amount of fat deposited was considerably less, a higher de-
gree of unsaturation was noticed in agreement with higher
iodine values. The results indicated a possible role of
ethanolamine and choline in linolenic acid metabolism.

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