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The Encephalomalacia Producing Effect of Arachidonic and Linoleic Acids

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With 1 figure and 1 table

(Received April 16, 1962)

In some of the early studies on vitamin E-deficiency in chicks it was observed that encephalomalacia only occurs when polyunsaturated fatty acids are present in the diet (1, 2).

In more recent experiments in which vitamin E-deficient diets containing 1.5% of the ethyl esters of linoleic and linolenic acids, respectively, were fed to chicks, encephalomalacia resulted when linoleate but not when linolenate was present (3).

The experiments to be described here show that encephalomalacia develops much faster with arachidonic than with linoleic acid.

Two recent developments facilitate this study: 1. The recent availability of synthetic ethyl arachidonate. 2. The circumstance that complications arising from occurrence of exudative diathesis can be avoided by addition of a trace of selenite to the diet (4, 5) without interference with the development of encephalomalacia (6).

Experimental

Day-old chicks (New Hampshire × White Leghorn) were fed a vitamin E-deficient "starter ration" (table 1) for five days[1]). Thereafter, 30 of the chicks were distributed into three groups (ten chicks per group) and given the experimental diets containing 1.5% ethyl arachidonate, 1.5% ethyl linoleate, or 30% lard, respectively, as indicated in table 1. The experiment was carried out in November and December 1961.

The ethyl esters of the fatty acids (containing 0.2% propylgallate) were incorporated into the basal diet every day, the portion of the diet left over from the foregoing day being discarded. The presence of the antioxidant propylgallate did not prevent peroxidation of the diets overnight on the food trays. Thus, in one trial, ethyl arachidonate and ethyl linoleate had peroxide values of 2200 and 575 μeq./g, respectively, after 24 hours. The lard (containing 0.01% propylgallate) was incorporated into the basal diet once a week; the unfed portion of the lard diet was stored in the ice box up to one week.

The chicks were inspected daily. Chicks showing clinical signs of encephalomalacia were killed, whereafter the disease was verified by examination of the brain.

[1]) The purpose of the starter ration was to obtain some gain in weight of the chicks before the beginning of the experimental feeding, and to permit a more uniform selection of chicks for the experiment. The starter ration did not supply vitamin E. Its content of lard is near the limit of what might introduce encephalomalacia over a period of several weeks, if the diet had not contained the yeast.

Table 1. Diets

	„Starter ration" g	Experimental diets g		
Casein (crude)[1])	20	—	—	—
Casein (Vitamin Test)[2])	—	30	30	30
FLEISCHMANN yeast 50 B[3])	20	—	—	—
Gelatine	3	3	3	3
Salt mixture[4])	5.17	5.17	5.17	5.17
Vitamin B mixture[5])	0.1	0.1	0.1	0.1
Choline chloride	0.2	0.2	0.2	0.2
Corn starch[6])	46.53	60.03	60.03	31.53
Lard	5	—	—	30
Ethyl arachidonate[7])	—	1.5	—	—
Ethyl linoleate[8])	—	—	1.5	—
	100.00	100.00	100.00	100.00
Vitamin K substitute [9])	1 mg	1 mg	1 mg	1 mg
Selenium dioxide		0.01 mg	0.01 mg	0.01 mg

[1]) "Dairinex", from A/S Dansk Mejeri Industri & Export Kompagni, Stege, Denmark.

[2]) From Genatosan Ltd., Loughborough, England.

[3]) From Standard Brands Inc., New York, U. S. A.

[4]) Secondary calcium phosphate, 2 aq., 2800 g; calcium carbonate, 875 g; desiccated magnesium sulfate, Ph. Dan., 404 g; potassium chloride, Ph. Dan., 460 g; sodium chloride, Ph. Dan., 500 g; ferric citrate (about 17.5% Fe), 100 g; manganese sulfate (water-free), 23 g; cupric sulfate, 5 aq., Ph. Dan., 2 g; zinc sulfate, 7 aq., Ph. Dan., 1 g; aluminium sulfate, 18 aq., Ph. Dan., 1 g; magnesium silicate, Ph. Dan., 1 g; diiodotyrosine, Ph. Dan., 1 g; cobalt carbonate, 0.05 g; total 5168.05 g.

[5]) Thiamine hydrochloride, 3 mg; riboflavine 4 mg; nicotinic acid, 50 mg; calcium pantothenate, 12 mg; pyridoxin, 3.5 mg; biotin, 0.1 mg; folic acid, 2 mg; sucrose, 924.4 mg; total 1000 mg.

[6]) The corn starch contained 0.31% of fat. 97% of the fat was fatty acids. Analysis of the fatty acid fraction by alkali isomerization showed that it contained 45.6% dienoic and 3.6% trienoic acids.

[7]) Generously furnished by F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland. The content of eicosatetraenoic acid determined by gas-liquid chromatography was 97% (November 1961). The content of trans fatty acids calculated as elaidic acid was 8% (December 1961).

[8]) From the Hormel Institute, Austin, Minnesota, U.S.A. The content of octadecadienoic acid determined by gas-liquid chromatography was 95.5% (February 1962). The content of trans fatty acids calculated as elaidic acid was 9.5% (February, March 1962).

[9]) Synkavit, Roche (di-calcium salt of 2-methyl-1,4-naphthohydroquinone-diphosphoric acid ester).

Vitamins A and D_3 were given in the form of 0.1 ml of an aqueous solution twice a week, corresponding to 250 i. u. vitamin A and 20 i. u. vitamin D_3 per day. The solution had the following composition: Crystalline vitamin A acetate (Roche), 1 g); crystalline vitamin D_3 (Roche), 0.0058 g; ethyl alcohol, 100 ml; "Tween 80", 64 g; and distilled water to make a total volume of 330 ml.

Results

The development of encephalomalacia within each group of ten chicks is recorded in fig. 1 in the following way:

Abscissae indicate number of days from the beginning of the experimental feeding, ordinates the number of chicks showing clinical signs of encephalomalacia within a given number of days. Each unit of the ordinate represents

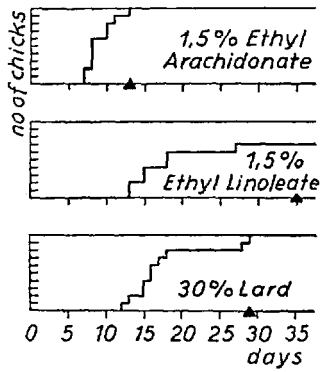

Fig. 1. The development of encephalomalacia within the three groups.
Abscissae: Days of experimental feeding. Ordinates: Numbers of chicks showing signs of encephalomalacia.

one chick. All the animals in this experiment showing evidence of encephalomalacia by autopsy showed clinical signs. Thus, a staircase-like frontline shows the development of the disease. A black triangle at the bottom marks the end of the experiment.

It is seen that encephalomalacia developed faster and more abruptly in the group receiving 1.5% ethyl arachidonate than in the groups receiving 1.5% ethyl linoleate or 30% lard.

Discussion

The observation that arachidonate and linoleate but, as shown previously, not linolenate produce encephalomalacia in the absence of vitamin E, indicates that encephalomalacia cannot *merely* be the result of autoxidation of unsaturated fatty acids. This might otherwise be expected since easily oxidizable fatty acids in the diet are necessary for the occurrence of the disease, and the feeding of certain antioxidants such as methylene blue (7), N,N'-diphenyl-p-phenylenediamine (DPPD) (8) or ethoxyquin (9,10) prevents or retards it.

When the effects of linoleic and linolenic acids in vitamin E deficiency were compared for the first time the diets contained no added selenium (3). It was then observed that both of these fatty acids greatly accelerated exudative diathesis and gave rise to formation of peroxides in the fat of the affected adipose tissue.

It is possible, therefore, that the effect of linoleic and linolenic acids (and of polyunsaturated fatty acids in general) in promoting exudative diathesis is due to a destruction of vitamin E coupled with autoxidation of fatty acids in the tissue.

In encephalomalacia the effect of polyunsaturated fatty acids seems to be more complicated[1]). It seems that at least the structure in cis,cis-linoleic acid, comprising the 10 last carbon atoms counted from the terminal carboxyl group, is essential for the appearance of this particular manifestation of vitamin E deficiency. The same structure exists in all-cis arachidonic acid. The additional higher degree of unsaturation might be the cause of the superiority of arachidonic acid over linoleic acid as a promotor of encephalomalacia. Further, it is possible that linoleic and arachidonic acids which are capable of preventing tissue damage due to deficiency of essential fatty acids are being incorporated into other tissue elements (structural or chemical) rather than linolenic acid and its trans-isomer. This might also contribute to the different behavior of these polyenoic fatty acids with respect to encephalomalacia.

The content of dienoic acid in the brain of chicks is extremely low, even when linoleate is furnished in the diet (11). Dietary linoleate increases tetraenoic, and dietary linolenate increases hexaenoic acid in the brain of chicks (11). This might suggest that the effect of linoleate in promoting encephalomalacia is mediated via arachidonate. However, it must be taken into consideration that the amount of tetraenoic acid (about 8% of the total fatty acids) present in the brain of chicks reared on fat-free diets does not cause encephalomalacia in the absence of vitamin E.

The hypothesis that autoxidation occurring in the brain is the cause of encephalomalacia meets the following difficulties:

Attempts to detect peroxides in the brain of chicks with encephalomalacia by means of iodometric titration, leuco-1,4-dichlorophenylindophenol, thiocyanate or thiobarbituric acid have failed (unpublished results from the authors' laboratory).

Further, the brain of chicks is extremely low in vitamin E, even when this vitamin is supplied through the diet.

Unpublished results from our laboratory have shown that brains of chicks raised for 5 weeks on an encephalomalacia producing diet containing 30% lard and on the same diet supplemented with 10 mg% d,l-α- and 10 mg% d,α-tocopheryl acetate, respectively, contained 1.5, 1.9, and 2.2 micrograms of tocopherol per g tissue, whereas the corresponding figures for liver were 1.0, 14.7, and 21.2 micrograms per g.

Bieri (12) was able to demonstrate by the thiobarbituric acid test that peroxidation occurred in homogenates of chicken brain incubated in air at 37° C for 1 hour. The degree of peroxidation in brain homogenates was much higher than in homogenates of liver and muscle, and, moreover, the per-

[1]) As pointed out in our first communication on this subject (3), the sample of ethyl linolenate (from The Hormel Institute, Austin, Minnesota) with which these results were obtained (in May 1958) contained a certain amount of trans-isomers [see also (11)]. Even if the presence of these isomers might have contributed to the failure of the linolenate preparation in producing encephalomalacia, it is evident that the development of encephalomalacia is not merely dependent upon the degree of unsaturation of the dietary fatty acids.

The possible influence of stereo-isomers suggests the desirability of repeating, with pure cis,cis,cis-linolenic acid, not only the present but all other nutritional studies of the biological effects of linolenic acid hitherto carried out with preparations produced via the hexabromide.

oxidation in brain homogenate was independent of whether the chicks had received vitamin E or not. Although the diets on which the chicks had been raised were not of the markedly encephalomalacia producing type (their fat content was in one case only 1% and in another case only 4% of lard), these results suggest that tocopherol does not pass the blood brain barrier.

Therefore, if autoxidation of certain polyenoic fatty acids is a factor in the development of encephalomalacia (and this is still possible), it might be assumed that the autoxidative process occurs outside of the brain. The autoxidation which takes place on the food trays does not cause encephalomalacia when the diet contains tocopherol acetate, and peroxides from the food do not enter the intestinal lymph (13). However, some investigators have thought that encephalomalacia could be produced by intravenous injection of the hydroperoxide of methyl linoleate or of linoleic acid emulsified in serum (14). Attempts in our laboratory to produce encephalomalacia by this method have failed (15). Other investigators (16) have reported the production of encephalomalacia by feeding 12-oxo-9-octadecenoic acid in the presence of vitamin E. Our experiments with this substance which will be described in another communication have not confirmed this claim either.

Apparently, more elaborate experimentation is required in order to elucidate the mode of action of arachidonic and linoleic acids (and perhaps certain other polyenoic acids) in the development of encephalomalacia.

Summary

Vitamin E-free diets containing 1.5% of the ethyl esters of linoleic and arachidonic acids, respectively, and a vitamin E-free diet containing 30% lard were fed to groups of 10 chicks from the age of 5 days through a period of maximally 35 days.

All chicks in the arachidonate group developed encephalomalacia within 13 days.

In the linoleate group, encephalomalacia began after 13 days. A total of 7 of the chicks in this group had encephalomalacia at the end of the feeding period.

In the group receiving 30% lard, encephalomalacia developed somewhat faster than in the linoleate group but slower than in the arachidonate group.

Earlier experiments in which 1.5% of ethyl linoleate and 1.5% of ethyl linolenate were compared showed that ethyl linolenate did not give rise to encephalomalacia within the feeding period.

The integrity of that part of the linoleic acid molecule comprising the 10 last carbon atoms (counted from the carboxyl group) seems to be of importance for the development of encephalomalacia. A corresponding structure is present in arachidonic but not in linolenic acid. The additional unsaturation of arachidonic acid may contribute to the superiority of this fatty acid as encephalomalacia producing agent. Further, it is possible that the encephalomalacia producing effect of dietary linoleic acid is mediated by arachidonic acid formed in the tissues from the ingested linoleic acid.

The possibility of explaining the development of encephalomalacia on the basis of *in vivo* autoxidation of fatty acids is discussed.

Zusammenfassung

Gruppen von 10 Küken wurden mit künstlichen, Vitamin-E-freien Nahrungen, enthaltend 1,5% Äthyl-linoleat, 1,5% Äthyl-arachidonat oder 30% Schweineschmalz, gefüttert.

Die Küken waren 5 Tage alt beim Versuchsbeginn. Die Fütterung dauerte bis zu 35 Tagen.

Sämtliche Küken in der Arachidonat-Gruppe entwickelten Enzephalomalazie innerhalb von 13 Tagen.

In der Linoleat-Gruppe begann die Entwicklung von Enzephalomalazie nach 13 Tagen. Insgesamt 7 der Küken in dieser Gruppe hatten Enzephalomalazie beim Versuchsabschluß.

In der Schweineschmalz-Gruppe entwickelte sich die Enzephalomalazie etwas schneller als in der Linoleat-Gruppe, aber langsamer als in der Arachidonat-Gruppe.

In früheren Versuchen, in welchen 1,5% Äthyl-linoleat und 1,5% Äthyl-linolenat verglichen wurden, zeigte es sich, daß Linolenat Enzephalomalazie nicht herbeiführte. Es scheint somit, daß der Teil des Linolsäure-Moleküls, welcher die 10 letzten Kohlenstoffatome (gerechnet von der Carboxylgruppe) umfaßt, für die Entstehung der Enzephalomalazie von Bedeutung ist. Eine entsprechende Struktur ist in Arachidonsäure vorhanden, nicht aber in Linolensäure. Die höhere Ungesättigtheit der Arachidonsäure könnte zur Überlegenheit dieser Fettsäure als Enzephalomalazie-Erreger beitragen. Es ist auch möglich, daß die Enzephalomalazie-erregende Wirkung der mit der Nahrung zugeführten Linolsäure durch die aus dieser Fettsäure entstandenen Arachidonsäure vermittelt wird.

Die Möglichkeit, die Entstehung von Enzephalomalazie auf Grund einer *in vivo* Autoxydation von Fettsäuren zu erklären, wird diskutiert.

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