Effect of Fatty Acids on Chick Encephalomalacia.* (25254)

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The intake of easily oxidized unsaturated lipids appears to be important in producing encephalomalacia in Vit. E deficient chicks (1). Our studies(2,3) and those by Dam *et al.*(4) and Machlin *et al.*(5) suggested that the amount of linoleic acid-containing oils ingested was of primary importance in the etiology of this disorder. Addition of coconut oil to a diet containing a low level of corn oil increased the incidence of encephalomalacia in tocopherol-deficient chicks, although high levels of dietary coconut oil alone had no effect (3). Effects of various dietary lipids are reported here, using appearance of symptoms of encephalomalacia and cerebellar hemorrhages as end-point. Evidence was obtained that fatty acids other than linoleic acid modify the appearance of symptoms.

Materials and methods. One-day-old Indian River crossbred cockerels in electrically heated brooders were given the diet described by Bosshardt *et al.*(6) with slight modifications(3). Substitutions of lipids other than at 4% level were made isocalorically with dextrose. The low-tocopherol corn oil was the same as that used in our studies of human tocopherol requirements(7,8), having been stripped of most of the tocopherols by vacuum distillation† and stirred with air at 95°C until a peroxide number (PN) of 50 was reached. Lard was similarly stripped and oxidized to PN of 25. Commercially available linseed, coconut, and cod liver oils, and butter were also aerated just sufficiently to oxidize the tocopherols. The lipids were analyzed for peroxide number, polyunsaturated fatty acids, iodine number, and total, alpha, and gamma tocopherol levels as described elsewhere(3), and in addition, some fatty acid compositions were determined by gas chromatographic methods using an ionization detector(9,10). The saturated fatty acids used had the following purities: lauric acid, 98.6%; myristic acid, 97.6%; palmitic acid, 96%; and stearic acid, 99%. Oleic acid assayed at 76.2%, the chief impurities being palmitoleic acid, 9.8%; palmitic acid, 4.2%; and myristic acid, 3.1%; with only 1.8% linoleic acid. Lipids and water-soluble vitamins were added to the diets daily to minimize oxidation. Vit. A (2500 IU) and D (600 IU) were fed weekly, and d-α-tocopherol acetate supplements were given in drops twice/week. Birds having ataxia, clonic, and tonic convulsions, head twisting, or paralysis after 14 and up to 28 or 30 days were sacrificed and the cerebella examined.

Results. The "diene" contents of diets used (Table II) compare favorably with linoleic acid levels reported by Hilditch(11). Approximately 70% of chicks developed characteristic symptoms with cerebellar lesions when fed a low-tocopherol synthetic diet containing 4% stripped corn oil (Table I). The relationship between level of corn oil in the diet and tocopherol necessary to prevent encephalomalacia has been previously described(3). Soy bean oil, cottonseed oil, safflower oil, and lard were also capable of inducing encephalomalacia in tocopherol-deficient chicks when the diets contained at least 14 g of linoleic acid/kg(3). However, neither olive oil nor linseed oil induced encephalomalacia, even when amounts equivalent to 23 or 18 g of linoleic acid/kg diet, respectively, were fed. Ten % cod liver oil by itself, although furnishing high levels of pentaene and hexaene fatty acids, did not produce cerebellar lesions. No direct relationship between iodine number and incidence of encephalomalacia was noted.

When combined in the diet with safflower oil, linseed oil inhibited appearance of encephalomalacia, while olive oil had an insignificant effect. The effects of combinations of

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TABLE I. Effects of Various Dietary Lipids on the Incidence of Encephalomalacia.

Dietary lipid (vit. E removed)	PN*	Iodine No.	"Diene," g† per kg diet	Linoleic acid, g‡ per kg diet	Incidence
4% stripped corn oil	58	50	22	12–22	48/70
4% " lard	28	28	5	4– 5	0/10
15% " "	28	92	17	12–20	10/20
20% " "	28	128	22	16–26	15/21
15% butter	0	63	2.3	1.5–6.0	0/16
20% coconut oil	1	20	3.6	2.6–4.8	0/10
10% olive oil	60	89	11	4–20	2/37
15% " "	60	133	17	6–30	0/19
20% " "	60	178	23	8–40	"
10% linseed oil	120	183	12§	9–22	"
15% " "	120	274	18§	14–33	0/10
10% cod liver oil	45	160	(~1.6)		0/18
10% oleic acid		89	2.2		0/ 9

* PN reached after heating with aeration sufficiently to destroy virtually all tocopherol.
† Diene content, as determined by alkaline isomerization and UV absorption.
‡ Calculated values from Hilditch(11).
§ Linseed oil also furnished 54 and 81 g of linolenic acid, respectively, to 10% and 15% diets.

natural lipids and various fatty acids with corn oil are given in Table III.

Discussion. Encephalomalacia is induced in tocopherol-deficient chicks when the linoleic acid content of the diet is above a certain minimum(3), but this incidence of encephalomalacia is enhanced, unaffected, or inhibited by various fatty acids. The ability of lauric and myristic acids to increase this incidence of encephalomalacia may be due to their being metabolized first, leaving more linoleic acid in tissues, while palmitic and stearic acids, which are not as well absorbed (12), may be less capable of "sparing" linoleic acid. Ability of some lipids and fatty acids to inhibit occurrence of encephalomalacia in corn oil-fed chicks appeared to be related to unsaturated fatty acids which they provide, other than linoleic. Thus, lard and olive oil both furnish equal amounts of

TABLE II. Effects of Various Lipids in Combinations with Safflower Oil on Incidence of Encephalomalacia.

Dietary lipids (treated to remove tocopherols)	Incidence
3% safflower oil	7/11
Idem + 12% olive oil	3/10
" + 12% linseed oil	0/11
5% safflower oil	6/10
Idem + 12% olive oil	3/11
" + 12% linseed oil	0/11

TABLE III. Effects of Various Lipids and Fatty Acids in Combinations with Stripped Corn Oil upon the Incidence of Encephalomalacia.

Dietary lipids (treated to remove tocopherol)			Incidence
2%	corn oil		7/40
4	"		48/70
2	"	+ 8% coconut oil	40/51‡
2	"	+ 8 butter	9/34
2	"	+ 8 olive oil	9/20
4	"	+ 6 "	14/20
2	"	+ 8 linseed oil	0/20
4	"	+ 6 "	6/19
2	"	+ 8 cod liver oil	0/20
4	"	+ 6 "	1/20‡
4	"	+ 6 oleic acid	0/19‡
2	"	+ 8 lauric acid	21/35‡
		+ 8 myristic "	10/20†
		+ 8 palmitic "	3/20
		+ 8 stearic "	7/19
		+ 8 fatty acid like coconut oil*	13/14‡
		+ 8 fatty acid like butter*	2/19
10%	lauric acid		0/5
10	myristic "		0/10
10	palmitic "		0/7
10	stearic "		0/9
10	fatty acid like coconut oil*		0/11

* Fatty acids like coconut oil were mixed to contain 64% lauric acid, 22% myristic acid, 12% palmitic acid, and 2% stearic acid. Fatty acids like butter were mixed to contain 2.5% lauric acid, 12.5% myristic acid, 37.5% palmitic acid, 12.5% stearic acid, and 35% oleic acid.
† $p < .01$ and
‡ $p < .001$ in comparison with corresponding results from 2 and 4% corn oil alone.

linoleic acid, but olive oil, which has about 75% oleic acid, fails to produce encephalomalacia, whereas lard, which contains about 40% oleic acid, balanced by an approximately equal amount of saturated fatty acids, does provoke cerebellar pathology. Similarly, ability of coconut oil to enhance appearance of encephalomalacia seems to be related to its high content of lauric and myristic acids, whereas butter, having mostly a mixture of oleic, palmitic, myristic, and stearic acids, has no such effect.

Summary. Corn oil or lard from which tocopherol had been removed, promoted chick encephalomalacia, whereas coconut oil, butter, linseed oil, and cod liver oil did not produce symptoms, and olive oil had a questionable effect. Dietary combinations of 2% corn oil with either 8% coconut oil, lauric acid, myristic acid, or a mixture of saturated fatty acids like coconut oil significantly increased the incidence of encephalomalacia over 2% corn oil alone, while 6% linseed oil, cod liver oil, or oleic acid inhibited the effect of 4% corn oil. Olive oil, butter, fatty acids like butter, palmitic acid, and stearic acid had no net effects upon the incidence of encephalomalacia induced by corn oil. The intake of linoleic acid appears to be a primary factor in the etiology of encephalomalacia, but some of the other fatty acids may secondarily increase or decrease this effect.

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A Method for Detection and Estimation of Penicillin in Poliomyelitis Vaccine.* (25255)

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During the manufacture of poliomyelitis vaccine, penicillin is introduced into the tissue culture medium at a concentration of 200 units/ml. Most of this penicillin is either removed or degraded during the processing of the vaccine. In recent medical literature there has been occasional reference to development of symptoms suggestive of penicillin sensitivity as a result of injecting poliomyelitis vaccine. However, data obtained during field trials in which this vaccine was tested, as well as experiences with subsequent mass immunizations, have shown that the incidence of sensitivity reactions is extremely low. Only 0.4% of subjects vaccinated in field trial studies, showed reactions to the vaccine. If only major reactions are tabulated, the incidence decreases to 0.004% (1). Although most of the penicillin introduced into poliomyelitis vaccine is removed or degraded to form microbiologically inactive products, it is of interest to determine residual pencillin in the finished vaccine. Microbiological assay indicated that residual penicillin would be present at low concentration. Because of this low concentration and because of presence in the vaccine of preservatives that interfere with microbiological assay, special precautions

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