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Effect of Dietary Fats on Infection by *Escherichia coli* in Chicks.*† (31094)

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Ross and Adamson(1) reported that chicks deficient in essential fatty acids (EFA) developed aspergillosis more readily than those fed a diet containing corn oil. Hopkins *et al* (2) reported that chicks fed an EFA-deficient diet developed a respiratory disease while those which received the diet supplemented with soybean oil did not. The causative agent of the disease was not identified. Nagai *et al* (3) showed that EFA-deficient mice were susceptible to bacterial infections but added linoleate conferred protection. Dubos(4) reported peanut oil increased survival of tuberculosis-infected mice, while Hedgecock(5) found olive oil to be ineffective against that agent compared with coconut oil. The experiments reported here were conducted to determine if the EFA-deficient chick is more susceptible to *E. coli* infection than chicks given diets supplemented with various oils of widely varying fatty acid content and to determine the comparative activity of purified fatty acids.

Experimental. White Plymouth Rock chicks from flocks of the Poultry Disease Research Center were selected at random and placed in wire-floored electrically heated batteries. All chicks were fed the prescribed ration from the time of hatching. The basal ration was a casein-gelatin-cerelose diet described by Edwards(6). Supplementary oils: coconut, corn, menhaden and linseed, were added at the rate of 2%. The fatty acid composition of these oils as determined by gas-liquid chromatography is shown in Table I(7). Methyl oleate (18:1) or methyl linoleate (18:2) were added at the 1% level, approximately the amount present in the corn oil diet. The methyl oleate was prepared from olive oil by low temperature crystallization(8) and the methyl linoleate by the urea adduct method (9). The methyl oleate was 98% pure, containing traces of myristic acid, palmitic acid and palmitoleic acid. The methyl linoleate was 96.5% pure, the contaminant being almost entirely methyl oleate. A commercial starter ration free of medicaments was also used in one trial.

TABLE I. Fatty Acid Composition of Oils.

Fatty acid	Corn oil	Coconut oil	Linseed oil	Menhaden oil
	% Dry wt of total fatty acids			
8:0		.4		
10:0		6.9		
12:0		28.8		
14:0		24.4	.7	11.8
14:1				.4
16:0	13.5	16.3	7.7	23.4
16:1				15.7
18:0	2.9	5.1	4.7	4.8
18:1	30.8	13.8	22.0	15.8
18:2	51.1	4.2	18.2	1.5
18:3	1.7		46.7	2.4
18:4				1.6
20:3				.7
20:4				.8
20:5				9.9
22:5				1.3
22:6				9.7

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TABLE II. Mortality from *E. coli* Among Chicks Fed Rations of Different Fat Composition.

Dietary oil	Age inoculated 1 wk	2 wk	3 wk	4 wk
None	14/18*	10/17	10/16	11/17
Coconut oil	13/18	7/15	10/17	9/14
Linseed "	14/18	8/18	5/17	—
Menhaden "	16/18	6/16	3/17	6/16
Corn "	17/18	5/16	2/18	0/11

* No. dead/No. inoculated.

Means joined by vertical lines are not significantly different. $p > .05$.

TABLE III. Mortality from *E. coli* Among Chicks Fed Rations of Different Fat Composition.

Dietary oil	Age at inoculation 3 wk	4 wk
Coconut oil	9/18*	5/21
None	7/15	3/18
Linseed oil	2/18	2/20
Corn "	1/18	1/21
Menhaden "	1/18	0/20

* No. dead/No. inoculated.

Means joined by vertical lines are not significantly different. $p > .05$.

Escherichia coli serotype Olab was grown in Trypticase Soy broth for 24 hours at 37°C and diluted to contain from 5.5×10^8 to 1.1×10^9 viable cells per ml as measured photometrically. Chicks were inoculated intraperitoneally with 0.1 ml and mortality was recorded for 6 days. Mortality data were analyzed by the method of least significant difference.

Results. Results of the first experiment are shown in Table II. At 1 week of age there were no differences due to diet, but by 2 weeks, the chicks fed the basal ration showed some increased mortality. High mortality at 1 week was attributed to too low dilution of inoculum for the age of chicks used. At 3 weeks of age, the chicks fed basal or basal plus coconut oil experienced 63 and 59% mortality, respectively, while those fed the corn or menhaden oil diets had significantly lower mortality rates. Similar results appeared at 4 weeks of age.

The 3- and 4-week treatments were repeated and results are shown in Table III. Again, the chicks fed basal or basal plus coconut oil diet had significantly higher mortality rates at 3 weeks of age than those fed the other diets. In all experiments, uninoculated chicks in each pen survived the term of the experiment.

Table IV shows that mortality is significantly lower when the basal diet is supplemented with corn oil or linoleic acid. Oleic acid supplementation of the basal ration in preventing mortality was relatively ineffective.

Discussion. These studies indicate rather conclusively that the chicks which have received the EFA-deficient diet for 2 to 4 weeks are more susceptible to mortality from challenging with *E. coli* than chicks fed a corn oil-supplemented diet. The results also indicate that the extent of protection afforded by various supplemental oils cannot be quantitatively correlated with their linoleic acid content. The menhaden oil gave considerable protection although it contains very small amounts of linoleic acid. The coconut oil that contains more linoleic acid offered much less protection. Linseed oil contains approximately half as much linoleic acid as corn oil and it appeared to have a protective effect. However, it should be kept in mind that menhaden oil contains considerable quantities of extremely long-chain polyunsaturated fatty acids and that linseed oil contains a large amount of linolenic acid that is rapidly converted by the chicks to 20- and 22-carbon fatty acids; therefore there is no fatty acid It is possible that these fatty acids along with arachidonic acid play a part in the protection afforded by these oils. Coconut oil contains primarily short- and medium-chain saturated fatty acids, therefore there is no fatty acid present in large amounts that the chick can rapidly convert to long chain polyunsaturated fatty acids as it does with linoleic acid in

TABLE IV. Mortality from *E. coli* Among Chicks Fed Diets Containing Corn Oil or Purified Fatty Acids.

Diet	Mortality/No. inoculated
Basal	12/24
Oleic acid	7/24
Commercial	3/17
Linoleic acid	2/24
Corn oil	1/24

Age at inoculation—2 wk.

Means joined by vertical lines are not significantly different. $p > .05$.

corn oil or linolenic acid in linseed oil. The time factor in these studies is interesting in view of the report of Edwards and Marion (12) indicating that large amounts of eicosatrienoic acid (20:3) first begin to appear in the chick liver lipids of EFA-deficient chicks after they have been fed the fat-free diet for 3 weeks from time of hatch. The present studies indicate that susceptibility to *E. coli* challenge follows somewhat the same pattern. There have gradually evolved from research two general theories of function of essential fatty acids, *viz.*, (A) as precursors of the hormones prostaglandins (review 13), and (B) as integral parts of phospholipids in various coenzymes and lipoproteins of subcellular structure(14).

Unfortunately, the studies reported here do not prove which way the fats tested are functioning in offering protection against the *E. coli* infection. It might be reasoned that even the small amount of linoleic acid in coconut oil should be able to supply sufficient amounts of linoleic acid for the formation of 11a, 15-dihydroxy-9 keto prosta-5, 13 dienoic acid (PGE_2) the most active prostaglandins so far tested(15). However, this same line of reasoning acknowledges that the chicks fed the basal diet or the basal diet plus coconut oil would have very low total levels of extremely long chain polyunsaturated fatty acids that might be available as precursors of prostaglandins, while those fed corn oil, linseed oil or menhaden oil would have large amounts of long chain polyunsaturated fatty acids available. Some information(15) indicates that prostaglandins that originate from fatty acids possessing low essential fatty acid activity have some biological activity although it is much lower than the activity of prostaglandin (PGE_2) that originates from arachidonic acid. Further studies using purified fatty acid preparations are in progress to obtain information as to the mode of action of these fats in protecting the chickens from the *E. coli* infection.

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Antigen Production in Hyperoxic Organ Cultures.* (31095)

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Many current approaches aimed at establishing allograft tolerance in the adult depend upon administration of large amounts of donor antigen(1,2,3,4). Using inbred strains of mice, practically unlimited amounts of strain-specific tissue are available, and have been

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