

## 14123

# Effect of Cod Liver Oil and Rancidity on Certain Vitamin E Deficiency Symptoms.\*

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It is known that cod liver oil added to a vitamin E deficient diet will accelerate the development of such symptoms as the muscular dystrophy in rabbit and guinea pigs,<sup>1</sup> whereas hydrogenated cod liver oil has no such effect.<sup>2</sup> Earlier suggestions that the effect of cod liver oil might be due to a destruction of traces of vitamin E in the diet through rancidity of the unsaturated fatty acids, either before or after their ingestion, have received added support by the recent studies of Mattill and Golumbic<sup>3</sup> who found that rancidity of the fat aggravated the symptoms. It would obviously be of interest to investigate how far the same considerations might be applied to another manifestation of vitamin E deficiency, namely, the increased capillary permeability in chicks.

Earlier studies<sup>4</sup> have already shown that lard and cod liver oil given in sufficient amount will accelerate the appearance of both exudates and encephalomalacia. These studies have since been extended to comprise an investigation of the influence of rancidity as well as that of certain fatty acids on the symptoms. It has thereby been possible to draw certain conclusions as to the role played by fats.

**Procedure.** The vitamin E deficient diet to

which the various fats were added consisted of: alcohol-extracted casein 15 g, McCollum's salt mixture No. 185 supplemented with Cu and Mn 7.2 g, ether-extracted dried yeast 10 g, gelatin 8 g, gum arabic 5 g, choline chloride 0.1 g, cystine 0.1 g, sucrose 54.6 g, diacetate of 2-methyl-1,4-naphthohydroquinone 1 mg. The animals received, twice a week, one drop of a concentrate of vitamins A and D dissolved in cod liver oil (28.6 of each 29 mg drop consisted of cod liver oil) during the first 5 weeks, providing 48 I.U. of A and 5 chick units of D per day. This diet is not complete for chicks but adequate for the purposes of these experiments. With the exception of fats which were to be made rancid in the diet, all fats were added to the base every day. The chicks were day-old White Leghorns.

**Results.** The data presented in Table I demonstrate that fresh cod liver oil is extremely effective in accelerating the capillary symptoms; whereas cod liver oil which has been made thoroughly rancid, either before or after it is mixed with the diet, has no such effect.

It may therefore be concluded that cod liver oil has an effect on the capillary symptoms which is not limited to a destruction through rancidity of possible traces of vitamin E in the diet, or to the presence of rancidity products in the diet. A rancidity product, heptaldehyde, tested directly showed no effect. Furthermore, since addition of fresh cod liver oil to a diet containing rancid cod liver oil will also accelerate the symptoms, it may be assumed that rancidity does not result in the formation of a factor which protects against the symptoms.

Cod liver oil and lard are more effective in accelerating the capillary symptoms than fatty acids from linseed oil, while oleic acid is ineffective (Table II). This indicates that the effect is due to the presence of highly

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<sup>1</sup> Madsen, L. L., McCay, C. M., and Maynard, L. A., *Cornell Univ. Agric. Exp. Sta. Memoir*, No. 178, 1935.

<sup>2</sup> McCay, C. M., Paul, H., and Maynard, L. A., *J. Nutr.*, 1938, **15**, 367.

<sup>3</sup> Mattill, H. A., and Golumbic, C., *J. Nutr.*, 1942, **28**, 625.

<sup>4</sup> Dam, H., Glavind, J., Prange, I., and Ottesen, J., *Kgl. Danske Videnskabernes Selskab, Biologiske Meddelelser* (Royal Danish Academy of Science, Biological Communications), 1941, **16**, 7.

TABLE I.  
Influence of Fresh and Rancid Cod Liver Oil on Exudate and Encephalomalacia in Chicks.

| Additions to the basal diet                                                        | No. of birds with symptoms within 28 days |                  | No. of birds without symptoms within 28 days |
|------------------------------------------------------------------------------------|-------------------------------------------|------------------|----------------------------------------------|
|                                                                                    | Exudate                                   | Encephalomalacia |                                              |
| 1. None                                                                            | 0                                         | 0                | 10                                           |
| 2. 5% fresh cod liver oil (iodine value 173)                                       | 8                                         | 1                | 1                                            |
| 3. 5% cod liver oil, made rancid* and added to diet (iodine value 86)†             | 0                                         | 0                | 9                                            |
| 4. 5% cod liver oil, made rancid in diet‡ (iodine value 62)                        | 0                                         | 0                | 9                                            |
| 5. As No. 4 + an additional 5% of fresh cod liver oil                              | 8                                         | 0                | 2                                            |
| 6. 5% cod liver oil made slightly rancid§ before adding to diet (iodine value 149) | 9                                         | 0                | 1                                            |
| 7. 0.5% heptaldehyde                                                               | 0                                         | 0                | 7                                            |

\* By rapidly aerating at 100°C for one week.

† The decrease in iodine value (Rosenmund-Kuhnhehn method) gives some indication of the degree of rancidity.

‡ By standing on shallow trays at 50°C for 1 week.

§ By slowly aerating at 100°C for 24 hours.

|| The heptaldehyde was mixed with the food several times during the day, because of the volatility of the compound.

TABLE II.  
Influence of Different Fats and Fatty Acids on Exudate and Encephalomalacia in Chicks.

| Additions to basal diet              | No. of birds with symptoms within 35 days |                  | No. of birds without symptoms within 35 days |
|--------------------------------------|-------------------------------------------|------------------|----------------------------------------------|
|                                      | Exudate                                   | Encephalomalacia |                                              |
| 1. None                              | 0                                         | 0                | 10                                           |
| 2. 4.8% oleic acid                   | 0                                         | 0                | 10                                           |
| 3. 4.8% fatty acids from linseed oil | 4                                         | 0                | 6                                            |
| 4. 5% lard                           | 6                                         | 2                | 2                                            |
| 5. 5% cod liver oil                  | 8                                         | 2                | 0                                            |

unsaturated fatty acids. The nature of this accelerating effect is under investigation at present.

The question arises as to whether exudates or encephalomalacia can be produced by diets absolutely free from highly unsaturated fat. In the experiments of Dam, Glavind, Prange and Ottesen<sup>4</sup> a few such symptoms were observed when no fat was added to the diet, but the latter was not so thoroughly extracted as that used in the present experiments. The fact that no capillary symptoms appeared in chicks reared on the basal diet used in the present studies, even after period longer than the 5 weeks indicated in Table II, suggests the importance of dietary fat in the genesis of the symptoms characteristic of vitamin E deficiency in the chick. The fact that the rancid

state of fats does not accentuate the capillary symptoms in chicks, but appears to have a marked effect on muscular lesions in rabbits,<sup>3</sup> suggests that the way in which fats influence these types of lesions may be different. This, in turn, suggests the need for information concerning the effect of fatty acids and their rancidity products on muscular disturbances in the chick and related species.

*Summary.* The exudative diathesis, characteristic of vitamin E deficiency in chicks, readily occurs with diets containing 5% of fresh cod liver oil, slightly rancid cod liver oil, or a mixture of fresh and completely rancid cod liver oil; and to a lesser extent by similar proportions of lard or fatty acids from linseed oil. On the other hand, the symptoms do not occur if the diet contains a similar proportion

of oleic acid or thoroughly rancid cod liver oil, or if the diet is rigidly freed of fats. These observations indicate that the appearance of symptoms of increased capillary permeability is dependent upon the presence of highly

unsaturated fatty acids in the vitamin E deficient diet, and that rancidity of the dietary fat does not contribute to the development of the symptoms.

## 14124

## Use of Thioglycollate Media for Testing Disinfectants.\*

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The results which we obtained with an *in vivo* "infection-prevention" method for evaluating skin disinfectants<sup>1</sup> indicated that the mercurial compounds tested were actually quite ineffective, despite their accepted high disinfecting power as determined by the standard *in vitro* methods. The conclusion was drawn that failure of growth in the test tube probably reflected only bacteriostatic action, and that dilution of the mercurial or its combination with substances present in circulating blood or lymph might free the bacteria of the mercurial and result in infection and death of a test animal.

Brewer has suggested that the addition of sodium thioglycollate to media interferes with the bacteriostatic action of mercurials.<sup>2</sup> Acting on this suggestion, the National Institute of Health has recommended that this substance be added to media used for testing the sterility of biologicals containing mercurial preservatives.

On this basis, it was decided to determine whether the addition of sodium thioglycollate to media used in testing disinfectants by an *in vitro* method would give results similar to those obtained by the "infection-prevention" method.

The mercurial compounds used in the experiments to be discussed included mercuric

chloride, mercuric oxycyanide, and aqueous solutions of metaphen, merthiolate, mercresin, and mercurochrome; the non-mercurial disinfectants were phenol, Lugol's iodine, and aqueous phemerol. A loopful of a broth culture of *Staphylococcus aureus* was placed in 5 ml of various concentrations of each of the disinfectants. After 10 minutes, these solutions were subcultured both into extract broth, and into extract broth containing 0.1% sodium thioglycollate.

The results summarized in Table I indicate that, with the non-mercurial disinfectants, essentially the same results were obtained whether or not thioglycollate was present in the medium. However, without exception, in the case of the mercurial disinfectants, the percent of positive growths was much greater when subcultures were made to the thioglycollate-containing media.

In the first experiments, the 0.1% concentration of thioglycollate was arbitrarily selected. However, the optimum concentration for inhibiting the bacteriostatic effect of the mercurial compounds was later determined to be between 0.05 and 0.10% (Table II). A greater concentration, 0.20%, retarded the growth of the test organism.

In further experiments, sodium thioglycollate was added to tubes of plain broth to give 0.1% concentration at intervals after they had been inoculated with staphylococci previously exposed to the various disinfectants for 10 minutes. It will be noted in Table III, which gives representative results of such

\* Aided by a grant from Parke, Davis and Company.

<sup>1</sup> Nungester, W. J., and Kempf, Alice H., *J. Infect. Dis.*, 1942, **71**, 174.

<sup>2</sup> Brewer, John H., *J. A. M. A.*, 1939, **112**, 209.