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Generalized Edema in Chicks Prevented by d, l-Alpha Tocopherol.*

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The experiments here reported were designed to study the nutritive completeness of different samples of dried skimmilk for chicks. A basal ration was developed in which all the protein is supplied by dried skimmilk and the known deficiencies of dried skimmilk are corrected by the addition of small amounts of various supplements. This ration, referred to as Ration 3, has the following composition: dried skimmilk 54%, dextrinized starch 44%, ground limestone 1%, and NaCl 1%, plus 0.12% of ferric citrate, 0.012% of $MnSO_4 \cdot 4H_2O$ and 0.0012% of $CuSO_4 \cdot 5H_2O$. Cod liver oil is administered by pipette thrice weekly. Chicks consume this diet readily but grow at a somewhat subnormal rate.

When certain samples of dried skimmilk are fed as a part of this ration, a severe generalized edema develops in a large percentage of the chicks. The first manifestation of this condition is extreme subcutaneous edema in some chicks as early as 3 weeks of age, and sufficient in extent to be readily detected without handling the chicks. Many chicks develop a characteristic straddling stance, the legs being forced apart by the great accumulation of fluid under the skin of the ventral body surface. The subcutaneous edema may also extend to the neck and back of the head. No recoveries have been observed; all birds so affected have died within 2 or 3 weeks. Death is frequently preceded by stupor, seldom lasting longer than 10 to 12 hours. Labored breathing is frequently observed during this period.

A considerable proportion of the chicks die between the ages of 3 and 9 weeks without manifesting any subcutaneous edema. The most consistent post mortem finding in such chicks, as well as in those with subcutaneous edema, has been extreme distention of the heart and pericardium, the latter being filled with exudate. Other common post mortem findings have been ascites, edema of the brain and lungs, and coronary and intestinal hyperemia. In one case, in excess of 50 cc of ascitic fluid were removed from a 600 g bird.

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TABLE I.
Mortality and Incidence of Edema in Chicks Fed Ration 3 with and without Supplements.

Exp. No.	Skimmilk	Supplement	No. of chicks	% edema		% mortality	
				5 wk	8 wk	5 wk	8 wk
2	S*	0	13	62		62	
	W*	0	16	19		31	
3	S	0	30	37	53	50	73
	W	0	30	57	77	67	90
6	S	0	33	30	42	51	93
	W	0	32	31	53	59	94
	S	3% dehydrated grass	32	0	0	9	25
	W	3% " "	32	0	0	3	9
	S	6% yeast	32	25	50	41	72
	W	6% "	31	26	46	46	89

* S = Summer; W = Winter.

The first proof that the edema was the result of dietary deficiency was afforded by an experiment in which dehydrated cereal grass† was fed as a supplement to the basal ration. The results of this and other experiments are summarized in Table I. Samples of dried skimmilk prepared in summer and in winter were fed, but no difference in response was observed. The figures in the table establish the protective effect of grass and the lack of protective effect of yeast.

A trial of the effectiveness of d,l-alpha tocopherol was suggested by the report of Dam and Glavind[1] on its effectiveness in preventing the "alimentary exudative diathesis" described by them. Synthetic d,l-alpha tocopherol‡ was dissolved and suitably diluted with cod liver oil and administered thrice weekly to a group of 10 chicks in doses of such size as to approximate 7.5 μg per gram live weight per day. Dosing was begun when the chicks were 4 days of age and continued to the age of 32 days at which time the supply of alpha tocopherol was exhausted. The negative control group consisted of 15 chicks. The results of this experiment are shown graphically in Fig. 1 which shows the cumulative incidence of edema and the cumulative mortality associated with edema. The first case of edema did not appear in the treated group until the chicks were 9 weeks old, 4½ weeks after the administration of the last dose of alpha

† Supplied by the American Butter Company, Kansas City, Mo., through the courtesy of Dr. W. R. Graham, Jr.

‡ Supplied by Merck and Co., Inc., Rahway, N. J., through the courtesy of Dr. G. W. Lewis.

1 Dam, H., and Glavind, J., *Nature*, 1939, **143**, 810.

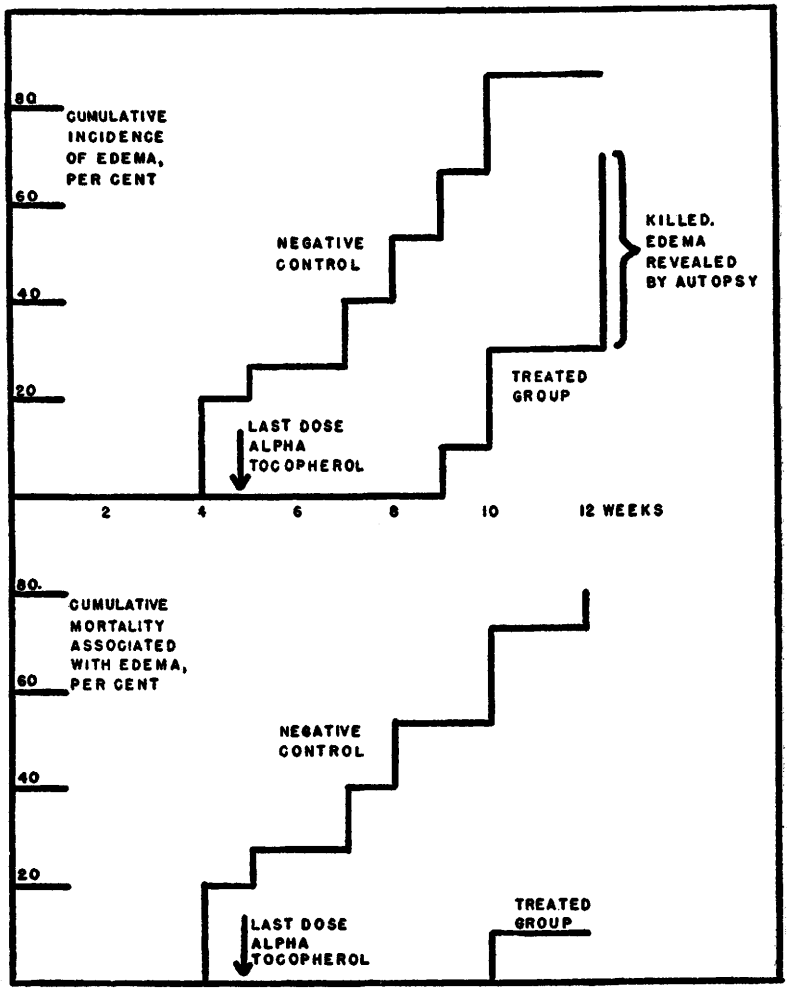

FIG. 1.

Effect of administration of d,l-Alpha Tocopherol on incidence of edema and mortality associated with edema in chicks fed Ration 3.

tocopherol. At this time 10 of the 15 untreated birds had become edematous. The first death associated with edema in the treated group occurred at 10 weeks of age, at which time 11 of the 15 untreated chicks had died with edema. These results would seem to show conclusively that this disease is a manifestation of a deficiency of d,l-alpha tocopherol.

Comparison of the symptoms described here with those described in detail by Dam and Glavind[2] would seem to indicate that the differ-

[2] Dam, H., and Glavind, J., *Skand. Arch. für Physiol.*, 1939, **82**, 299.

ences are differences of degree. The Danish workers report exudation in subcutaneous tissues and, rarely, in the cavum peritoneum; and they report, further, that the disease as observed by them terminates frequently in recovery and occasionally in death. From this standpoint as well as from the standpoint of ease of preparation, the ration used in these studies would appear to offer greater possibilities for vitamin E assay than the ration used by Dam and Glavind, who have suggested that an assay method might be developed on the basis of their experiments. Further experiments, designed to explore these possibilities, are in progress.

It is of interest to point out that localized edema is consistent and conspicuous among the histopathological changes in the brains of encephalomalacic chicks, as reported by Pappenheimer *et al.*,[3] since Dam *et al.*[4] have found this disease also to be preventable by d,l-alpha tocopherol. It should be pointed out also that Pappenheimer *et al.* reported a very low incidence of subcutaneous edema in encephalomalacic chicks observed by them. In these experiments edema of the brain was frequently noted, but in no case were the symptoms of encephalomalacia observed in the living edematous birds. Two cases of encephalomalacia were observed among the groups in which generalized edema was prevented by feeding dehydrated grass. This is in agreement with Dam's statement that encephalomalacia occurs on a higher intake of vitamin E than does exudative diathesis.

Dam and Glavind[2] have pointed out that the edema-producing diets used by them were very low in fat, and have discussed the relationship of this fact to the finding of Pappenheimer *et al.*[3] that the incidence of encephalomalacia was increased by increasing the fat content of the diet. They postulate further that vitamin E may act in two different ways against encephalomalacia and exudative diathesis respectively. It may be noted that the edema-producing diet described in this paper is also low in fat although none of the constituents were subjected to extraction to lower their fat content.

The results of the experiments with alpha tocopherol are of some interest from the standpoint of method of administration since it has been reported by Madsen *et al.*[5] and by Morris[6] that cod liver oil favors the development of muscular dystrophy in rabbits and

[3] Pappenheimer, A. M., Goettsch, M., and Jungherr, E., *Conn. Agr. Exp. Sta. Bul.*, 229, 1939.

[4] Dam, H., Glavind, J., Bernth, O., and Hagens, E., *Nature*, 1938, **142**, 1157.

[5] Madsen, L. L., McCay, C. M., and Maynard, L. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1434.

[6] Morris, S. G., *Science*, 1939, **90**, 424.

guinea pigs and hence may be assumed to exert a destructive effect on vitamin E. Such destruction was not evidenced under the conditions of this experiment, and administration of alpha tocopherol in cod liver oil appears to be an entirely practical procedure in experiments of this kind. Nondestearinated U.S.P. cod liver oil was used, and 100 mg of alpha tocopherol were dissolved and diluted in this oil at one time. Such an amount was sufficient to last for 7 to 10 days. It was kept in the refrigerator when not in use.

Summary. A large percentage of chicks fed a laboratory diet of dried skimmilk, dextrinized corn starch, cod liver oil, and mineral salts develop a generalized edema and die. The most consistent post mortem finding is edema of the heart and pericardium. This disease can be prevented by administration in cod liver oil of synthetic d,l-alpha tocopherol. The disease has not been observed in any chicks fed practical rations.

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Effect of Amino Acids, of Vitamin B Complex and Other Compounds on Respiration of Bakers' Yeast.

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In our laboratories a considerable amount of work has been done on the effects of various fractions from yeast and other sources on the respiration and proliferation of yeast and tissues. In this connection experiments have been performed with pure substances some of which may be present in these preparations. The results of these experiments are the subject of the present paper.

Although we have used pure cultures of yeast in some of our respiration work (pure cultures are always used in proliferation studies), it has been desirable to have a readily available and reasonably constant source of yeast in quantity. We have found that Fleischmann's bakers' yeast answers these requirements fairly well. (Anheuser-Busch bakers' yeast has also been used with equal satisfaction in later work, but the experiments herein reported deal with

* Assistance in some experiments was given by Vincent Sacksteder.