There were no appreciable differences between the degrees of fatty infiltration noted in the livers of the different groups. The amount of hepatic ceroid found in the rats fed the fat-free diet for 55–75 days was graded as a *trace*, for 105–126 days, as *2*. In the groups fed beef tallow or either one of the hydrogenated cottonseed oils, the average grading was only a *trace* at both times; in those receiving cocoa butter, *1*. In the ones given cottonseed oil or the corn oil-cod liver oil mixture, the values were *2* after 55–75 days and *3* after 105–126 days.

These observations indicate that, in the presence of 0.01% added α-tocopherol, the degree of unsaturation of the fat in the diet does influence ceroid formation in the livers of choline-deficient rats. That the fat-free diet had little effect upon the degree of unsaturation of hepatic or tissue fats is suggested by the appreciable amount of ceroid ultimately formed when this diet was fed. This is in agreement with the observations of NUNN AND MACLEAN[8] and KUMMEROW, PAN AND HICKMAN[9].

In this series of experiments, abnormal accumulation of lipids in the rats' livers was achieved by feeding hypolipotropic diets. Variation in the degree of unsaturation of these lipids was effected by feeding fats of different iodine numbers. A relative deficiency of antioxidants existed because of the small amount of α-tocopherol in the diet. The results indicate the importance of unsaturated lipids in the formation of hepatic ceroid and favour the suggestion[2] that this pigment is formed from unsaturated lipids by autoxidation. Studies concerning the effects of various antioxidants upon this process are described in the second report of this series[10].

REFERENCES

1 R. D. LILLIE, L. L. ASHBURN, W. H. SEBRELL, F. S. DAFT AND J. V. LOWRY, *U.S. Pub. Health Rep.*, 57 (1942) 502.
2 W. G. B. CASSELMAN, *J. Exptl. Med.*, 94 (1951) 549.
3 K. M. ENDICOTT, F. S. DAFT AND W. H. SEBRELL, *Proc. Soc. Exptl. Biol. Med.*, 57 (1944) 330.
4 M. WACHSTEIN, *Proc. Soc. Exptl. Biol. Med.*, 59 (1945) 73.
5 P. GYÖRGI AND H. GOLDBLATT, *J. Exptl. Med.*, 89 (1949) 245.
6 P. GYÖRGI AND H. GOLDBLATT, *J. Exptl. Med.*, 89 (1949) 73.
7 M. V. R. RAO, N. C. DATTA AND L. S. KRISHMAN, *Current Science*, 19 (1950) 14.
8 L. C. A. NUNN AND I. S. MACLEAN, *Biochem. J.*, 32 (1938) 2178.
9 F. A. KUMMEROW, H. P. PAN AND H. HICKMAN, *J. Nutrition*, 46 (1952) 489.
10 W. G. B. CASSELMAN, *Biochem. Biophys. Acta*, 11 (1953) 446.
11 C. H. BEST, C. C. LUCAS, J. M. PATTERSON AND J. H. RIDOUT, *Can. J. Med. Sci.*, 31 (1953) in press.

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FACTORS INFLUENCING THE FORMATION OF CEROID IN THE LIVERS OF CHOLINE-DEFICIENT RATS*

II. DIETARY ANTIOXIDANTS

by

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Since it was first named by LILLIE and his co-workers[1], *ceroid* has been associated with an insufficiency of dietary choline. Now, that it is generally considered identical with the lipid pigment appearing in vitamin-E deficiency, ceroid may also be associated with a deficiency of antioxidants in the diet. Indeed, increasing the amount of α-tocopherol fed to choline-deficient rats reduces the amount of this pigment formed in their livers[2,3]. This is in keeping with the suggestion that ceroid consists of the autoxidation products of pathologically accumulated, unsaturated fats[4,5]. Recent

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reports from DAM's laboratory[6,7,8,9,10,11] have described the vitamin-E-like activity of various compounds, especially methylene blue, in rats. This preliminary report concerns the similar inhibitory effects of α-tocopherol and methylene blue upon the formation of ceroid in the livers of choline-deficient rats.

Hypolipotropic diets were given to 3 similar groups of 8 male rats of the Wistar strain, weighing 180–200 g. One group was fed *ad libitum* the basal mixture* also lacking vitamin E. The other 2 were group-pair-fed against the first and received the basal mixture supplemented with 0.05% α-tocopherol acetate or 0.1% methylene blue. To reduce the incidence of fatal renal lesions, the diets were supplemented with 0.02% choline chloride for the first 18 days and with 0.01% for the next 14 days but not any thereafter. The individual daily food consumptions were determined. Pair-feedings were calculated and the animals weighed twice weekly. The rats were sacrificed at random between the 52nd and 62nd days. Portions from 4 regions of each of their livers were fixed in Bouin's fluid and graded for the ceroid demonstrable in paraffin sections by coloration with Oil Red O.

None of the rats grew normally. Those receiving the basal diet gained an average of 30 g (16%) by the 28th day but lost it by the 60th. Those fed the α-tocopherol-supplemented diet showed no appreciable gain and lost some 5 g (3%) by the 58th day. The rats fed the diet with methylene blue lost weight, an average of 14 g (8%) by the 58th day. There were no differences between the groups insofar as the degree of fatty infiltration noted in their livers was concerned. The average degree of ceroid formation for those on the basal diet was 3, for those on the α-tocopherol-supplemented one, 2, and on the methylene blue-supplemented diet, 1.

These results indicate that the addition of α-tocopherol or methylene blue to a hypolipotropic vitamin-E-deficient diet fed to rats decreases the amount of hepatic ceroid formed within 52–62 days. This observation, complementing those given in the preceding paper of this series[5], further supports the thesis that ceroid is formed from tissue lipids which have accumulated in abnormal amounts under conditions favouring autoxidation[4].

These experiments extend the similarity between α-tocopherol and methylene blue reported by the Copenhagen investigators to include inhibiting the deposition of ceroid in the livers of choline-deficient rats. The inequality in their effects in these experiments may be attributable to equimolar amounts not being used. Not only depression of growth but actual weight loss was seen in the choline-deficient rats. DAM AND GRANADOS[11] explained growth depression in rats fed diets not deficient in choline on the basis of the dye not always substituting directly for vitamin E. Perhaps concurrent choline deficiency accentuates this difference.

REFERENCES

1 R. D. LILLIE, L. L. ASHBURN, W. H. SEBRELL, F. S. DAFT AND J. V. LOWRY, *U.S. Pub. Health Rep.*, 57 (1942) 502.
2 VICTOR AND PAPPENHEIMER.
3 P. GYÖRGI AND H. GOLDBLATT, *J. Exp. Med.*, 89 (1949) 245.
4 W. G. B. CASSELMAN, *J. Exp. Med.*, 94 (1951) 549.
5 W. G. B. CASSELMAN, *Biochim. Biophys. Acta*, 11 (1953) 445.
6 F. CHRISTENSEN AND H. DAM, *Acta Pharm. et Toxicol.*, 7 (1951) 167.
7 E. AAES-JORGENSEN, H. DAM AND H. GRANADOS, *Acta Pharm. et Toxicol.*, 7 (1951) 171.
8 H. DAM AND H. GRANADOS, *Acta Pharm. et Toxicol.*, 7 (1951) 181.
9 H. DAM, I. PRANGE AND E. SONDERGAARD, *Experientia*, 7 (1951) 184.
10 H. DAM, I. PRANGE AND E. SONDERGAARD, *Acta Pharm. et Toxicol.*, 8 (1952) 23.
11 H. DAM AND H. GRANADOS, *Acta Pharm. et Toxicol.*, 8 (1952) 47.
12 C. H. BEST, C. C. LUCAS, J. M. PATTERSON AND J. H. RIDOUT, *Can. J. Med. Sci.*, 31 (1953) in press

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* The basal diet contained: extracted peanut meal, 6%; alpha soya protein, 6%; cystine, 0.15%; celluflour, 2%; beef tallow, 15%; corn oil, 5%; salt mixture[12], 3%; vitamin mixture[12], 1%; sucrose, 61.73–61.75%; choline chloride (see text) 0.02, 0.01, or 0.00%; cod liver oil concentrate (200,000 I.U. vitamin A and 50,000 I.U. vitamin D per g) 0.015%. In the supplemented diets 0.1% methylene blue or 0.05% α-tocopherol acetate replaced the appropriate amount of sucrose.