in his conditions the "liberation" was complete before incubation of the tissue was begun; only with muscle from hypophysectomised animals, lacking any endogenous growth hormone, would it then be possible to demonstrate an *in vitro* effect.

REFERENCES

1 J. H. OTTAWAY, *Nature*, 167 (1951) 1064.
2 M. E. KRAHL, *Ann. N.Y. Acad. Sci.*, 54 (1951) 649.
3 C. R. PARK, *Phosphorus Metabolism*, Vol. II, 1953. Johns Hopkins Press, Publishers.
4 W. C. STADIE AND J. A. ZAPP, *J. Biol. Chem.*, 170 (1947) 55.
5 C. R. PARK, D. A. BROWN, M. CORNBLATH, W. H. DAUGHADAY AND M. E. KRAHL, *J. Biol. Chem.*, 197 (1952) 151.
6 E. H. KASS AND B. A. WAISBREN, *Proc. Soc. Exptl. Biol. Med.*, 60 (1945) 303.
7 M. SOMOGYI, *J. Biol. Chem.*, 174 (1948) 597.

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

I. DIETARY FATS

by

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The golden yellow, lipid pigment which may occur in the cirrhotic livers of choline-deficient rats was named *ceroid* by LILLIE and his associates[1]. Studies on the *in vitro* preparation and histochemical properties of substances resembling it support the suggestion that this material might be formed by the autoxidation of pathologically accumulated, unsaturated fats[2]. Through their influence upon tissue lipids, therefore, dietary fats might be expected to affect the formation of ceroid in the livers of choline-deficient rats. Cod liver oil favours this[3,4,5], while replacing the lard in the diet by butter decreases it and by hydrogenated cottonseed oil prevents it[6]. Even reduction in the amount of fat has an effect[7]. This preliminary note concerns a systematic study of the influence of the degree of unsaturation of the dietary fat upon the amount of ceroid formed in the livers of choline deficient rats.

Seven similar groups of male, Wistar rats, each consisting of 10 animals initially weighing 58–65 g, were fed choline-deficient diets***. The group receiving the basal, fat-free diet was given methyl linoleate, 50 mg per 100 g body weight, orally twice weekly. The other 6 groups were fed diets in which 10% of the sucrose in the basal mixture was replaced by 10% of a fat: hydrogenated cottonseed oils (Iodine Number = 12 or 36), cocoa butter (I. No. = 36), beef tallow (I. No. = 40), cottonseed oil (I. No. = 111), or a mixture of 4 parts cod liver oil (I. No. = 151) and 6 parts corn oil (I. No. = 125). To reduce the incidence of fatal renal lesions, the diets were supplemented with choline chloride, 0.08% for the first 10 days, and 0.03% for the next 14 days, but not any thereafter. Individual food consumptions were determined daily, body weights twice weekly. Rats from each group were sacrificed at random between the 55th and 126th days. Sections from 4 regions of each of their livers were graded for the amount of ceroid present (paraffin sections, Oil Red O method).

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*** Basal choline-deficient diet contained: extracted peanut meal, 30%; vitamin and fat-free casein, 6%; powdered sucrose, 49.90–49.98%; salt mixture[11], 3%; vitamin mixture[11], 1%; choline chloride (see text), 0.00–0.08%; cod liver oil concentrate (200,000 I.U. vitamin A and 50,000 I.U. vitamin D per g), 0.01%, and α-tocopherol acetate, 0.01%. When fat was added, this replaced 10% of the sucrose.

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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II. DIETARY ANTIOXIDANTS

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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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