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Influence of Cod Liver Oil on Deposition of Ceroid in the Nutritional Rat Cirrhosis.

MAX WACHSTEIN. (Introduced by E. P. Pick.)

From the Laboratories of the Mount Sinai Hospital, New York City, and the Elizabeth A. Horton Memorial Hospital, Middletown, N.Y.

The production of cirrhosis of the liver in rats on a diet deficient in protein (Methionine) and free from choline, has been described by various investigators.[1,2,3,4] The cirrhosis is accompanied by the deposition of a peculiar insoluble pigment which has been given the name ceroid by Lillie and coworkers.[5] The staining properties of this pigment, which is found not only in the liver but also in lymph nodes, lungs, pancreas, spleen, bone marrow and adrenal cortex, have been extensively described by Endicott and Lillie.[6] Popper, György, and Goldblatt[7] found it to give a golden brown fluorescence which they thought to be very useful for its identification. An insoluble, acid-fast, ceroid-like substance was produced by Endicott[8] in rats by injection of cod liver and linseed oils. Endicott observed a similar substance in a human case of pneumonia due to aspiration of cod liver oil. Endicott, Daft, and Sebrell[9] have recently reported on the production of hepatic cirrhosis without ceroid in rats by feeding protein-deficient diets without cod liver oil. They concluded that a substance or substances present in cod liver oil cause the appearance of this pigment.

In similar experiments to be described, it was found that restriction of cod liver oil in the diet will lead to a marked reduction of ceroid in the experimental liver cirrhosis in rats. Complete omission of cod liver oil frequently leads to somewhat less severe cirrhosis, without any trace of ceroid in some cases, but with ceroid deposition in others. The use of peanut meal and especially of fibrin in combination with small amounts of cystine as protein source was found to produce marked cirrhosis after 90 days in nearly all surviving animals with very extensive ceroid deposition in those which received cod liver oil.

Experiments. (A) *Casein as Protein Source.* 30 white rats, weighing between 150 and 200 g, were placed on a diet composed of 67.8% sucrose, 20% hydrogenated cottonseed oil (Crisco), 8% casein (Smaco), 4% salt mixture No. 2 USP XII, and 0.2% cystine. They received in addition daily 20 μg of thiamine hydrochloride, riboflavin, and pyridoxine hydrochloride and 100 μg of calcium pantothenate, and weekly 1 drop of cod liver oil.

The rats were killed after 120 days with the exception of 2 which died after 112 and 116 days respectively. Twelve animals showed marked to very extensive cirrhosis, 9 fatty infiltration with beginning cirrhosis, and the remaining animals showed varying degrees of fatty changes. In this latter group, no ceroid deposition was seen. Among the animals with beginning cirrhosis, 3 showed traces and 1 a moderate amount. Of the 12 livers with marked cirrhosis, only in 2 moderate amounts of ceroid were seen, while in the remaining ones only traces to very small amounts of

1 György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185; 1942, **75**, 355; PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 492; György, P., *Am. J. Clin. Path.*, 1944, **14**, 67.

2 Webster, G., *J. Clin. Invest.*, 1942, **21**, 385.

3 Blumberg, H., and McCollum, E. V., *Science*, 1941, **93**, 598; Blumberg, H., and Grady, H. G., *Arch. Path.*, 1942, **34**, 1035.

4 Lillie, R. D., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1941, **56**, 1255.

5 Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V., *Pub. Health Rep.*, 1942, **57**, 502.

6 Endicott, K. M., and Lillie, R. D., *Am. J. Path.*, 1944, **20**, 149.

7 Popper, H., György, P., and Goldblatt, H., *Arch. Path.*, 1944, **37**, 101.

8 Endicott, K. M., *Arch. Path.*, 1944, **37**, 49.

9 Endicott, K. M., Daft, F. S., and Sebrell, W. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 330.

ceroid could be detected. No ceroid was seen in one of these livers. In previous experiments, using a similar diet with 2% cod liver oil, much larger amounts of ceroid were seen in livers showing early or progressive cirrhosis.

(B) *Peanut-Casein Diet.* Eight male weaning rats, weighing between 35 and 45 g, were given a diet consisting of 51.7% saccharose, 30% peanut meal (prepared according to Engel and Salmon),[10] 6% casein (Smaco), 6% hydrogenated cottonseed oil (Crisco), 4% salt mixture No. 2 USP XII, 2% cod liver oil, and 0.3% cystine. The food contained in addition 10 mg of riboflavin, thiamine hydrochloride, and pyridoxine hydrochloride respectively and 50 mg of calcium pantothenate in 1000 g.

Three animals died in the beginning of the experiment with signs of acute choline deficiency. The remaining 5 animals were killed after 90 days. They were in good condition and weighed between 60 and 120 g. Four animals presented cirrhosis, 2 quite progressed. The liver of all these animals showed a large amount of ceroid and large mesenteric lymph nodes, containing much ceroid, were seen in 2 cases. In 4 instances degenerative changes were found in the kidneys.

A second group of 11 male weaning rats were given a similar diet without cod liver oil. These rats received instead 1 drop of cod liver oil each week. Six animals survived the first month. Early cirrhosis with traces of ceroid deposition were seen after 55 and 63 days respectively. Distinct cirrhosis and a fair amount of ceroid was seen in 2 other rats after 78 and 83 days respectively. Of 2 animals killed after 90 days, one showed a moderate cirrhosis with a moderate amount of ceroid, while very little ceroid was found in the other rat. Degenerative kidney changes of varying degree were found in all cases.

A third group of 8 male weaning rats was placed on a similar diet without cod liver oil. These animals received 3 times weekly 40 μg carotene (Smaco) dissolved in liquefied cotton seed oil. No vitamin D was given since this vitamin is not essential for rats.[11] Six animals lived longer than 30 days. One animal died after 49 days, showing beginning cirrhosis without ceroid. The remaining 5 were killed after 90 days. They were in good condition and weighed between 65 and 90 g. Two of the livers showed moderate cirrhosis. Both contained a small amount of ceroid. Five out of 6 animals showed focal degenerative kidney changes.

(C) *Fibrin as Protein Source.* A group of 12 male weaning rats, weighing between 35 and 45 g, were placed on a diet of the following composition: 77.3% saccharose, 10% blood fibrin (Difco), 6% hydrogenated cottonseed oil, 4% salt mixture No. 2 USP XII, 2% cod liver oil, and 0.3% cystine. In addition, this food contained 10 mg of riboflavin, thiamine hydrochloride, and pyridoxine hydrochloride respectively, and 50 mg of calcium pantothenate in 1000 g.

Eight animals survived longer than 30 days. One died after 30 and another after 48 days. One was sacrificed after 63 days. This latter showed beginning cirrhosis without ceroid deposition. The remaining 5 animals were killed after 90 days. They had not gained any weight, were in poor general condition, and showed focal alopecia. Four of these 5 animals revealed marked and 1 moderate cirrhosis. One animal showed ascites. Enlarged brownish mesenteric lymph nodes were seen in all 5 cases. On microscopic examination all 5 livers contained large amounts of ceroid (Fig. 1). Mesenteric lymph nodes and spleen contained the same pigment. All 8 animals showed degenerative changes in the kidneys.

A second group of 12 male weaning albino rats were kept on a similar diet without cod liver oil. The animals received 3 times weekly 40 μg of carotene, dissolved in liquefied Crisco. Eight animals lived longer than 30 days. In 2, which lived for 57 and 65 days respectively, a moderate cirrhosis but no ceroid deposition was seen. One animal showed beginning cirrhosis and a small amount of ceroid after 86 days. Of the 4 remaining animals which were killed after 90 days, one showed marked and 3 moderate cirrhosis.

[10] Engel, R. W., and Salmon, W. D., *J. Nutrition*, 1941, **22**, 109.

[11] Griffith, J. Q., and Farris, E. J., *The Rat in the Laboratory Investigation*, 1942, J. B. Lippincott Co., p. 97.

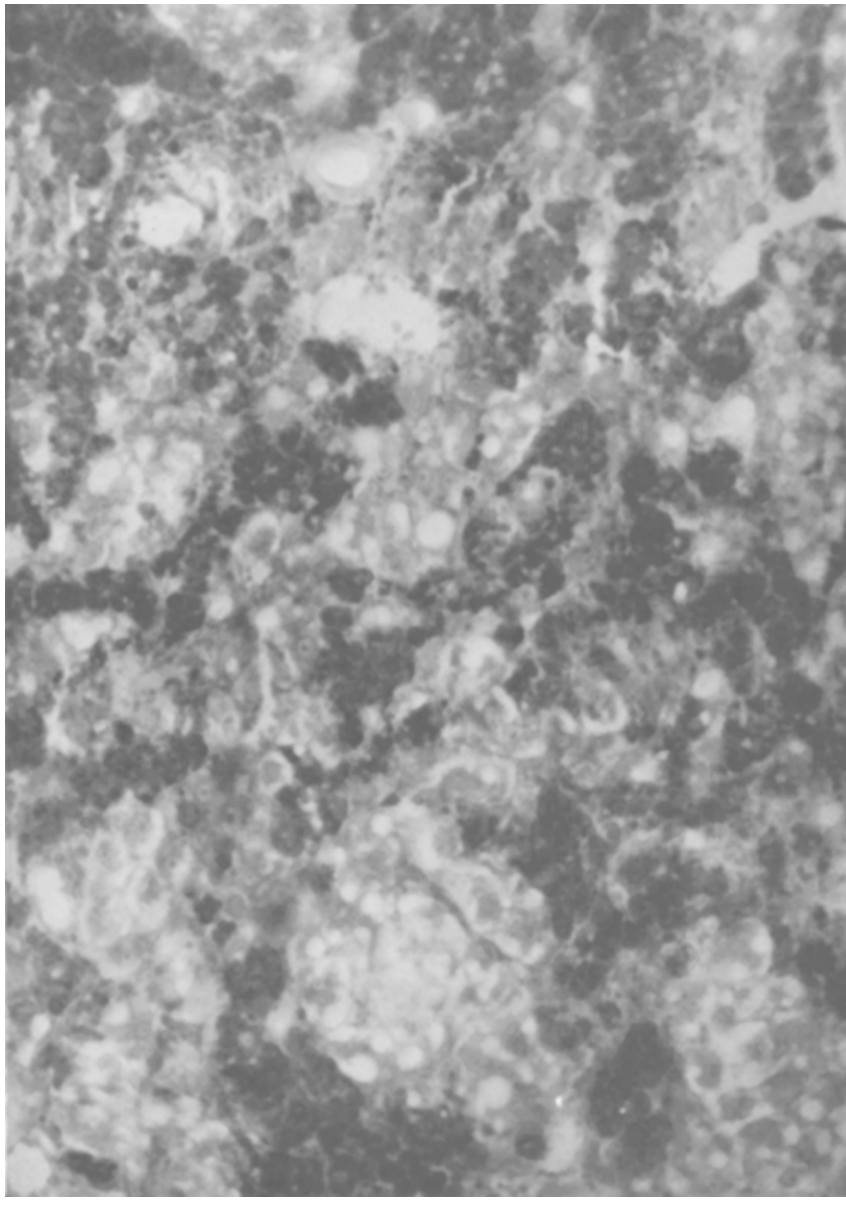

FIG. 1.
Liver showing extensive ceroid deposition in a rat after 90 days on a fibrin diet, containing 2% cod liver oil. Ziel-Neelson stain. × 150.

Two of these livers revealed a moderate amount of ceroid (Fig. 2). Seven animals showed varying degrees of degenerative kidney changes.

Comment. Extensive and progressive cirrhosis of the liver can be produced by feeding male weaning rats a choline-free diet in which the protein source is either 10% fibrin or 30% peanut meal plus 6% casein, and to which 0.3% cystine is added. In nearly all animals surviving 90 days, fatty infiltration and progressive cirrhosis was found. Beginning cirrhosis was occasionally seen in less than 60 days. The changes were most distinct on the fibrin diet. Very large amounts of ceroid were found under these experimental conditions. The microscopic appearance of the cirrhotic livers corresponded closely to that described by various investigators. The normal lobular architecture was destroyed and small and larger areas of liver tissue were separated by fibrous bands. Single as well as

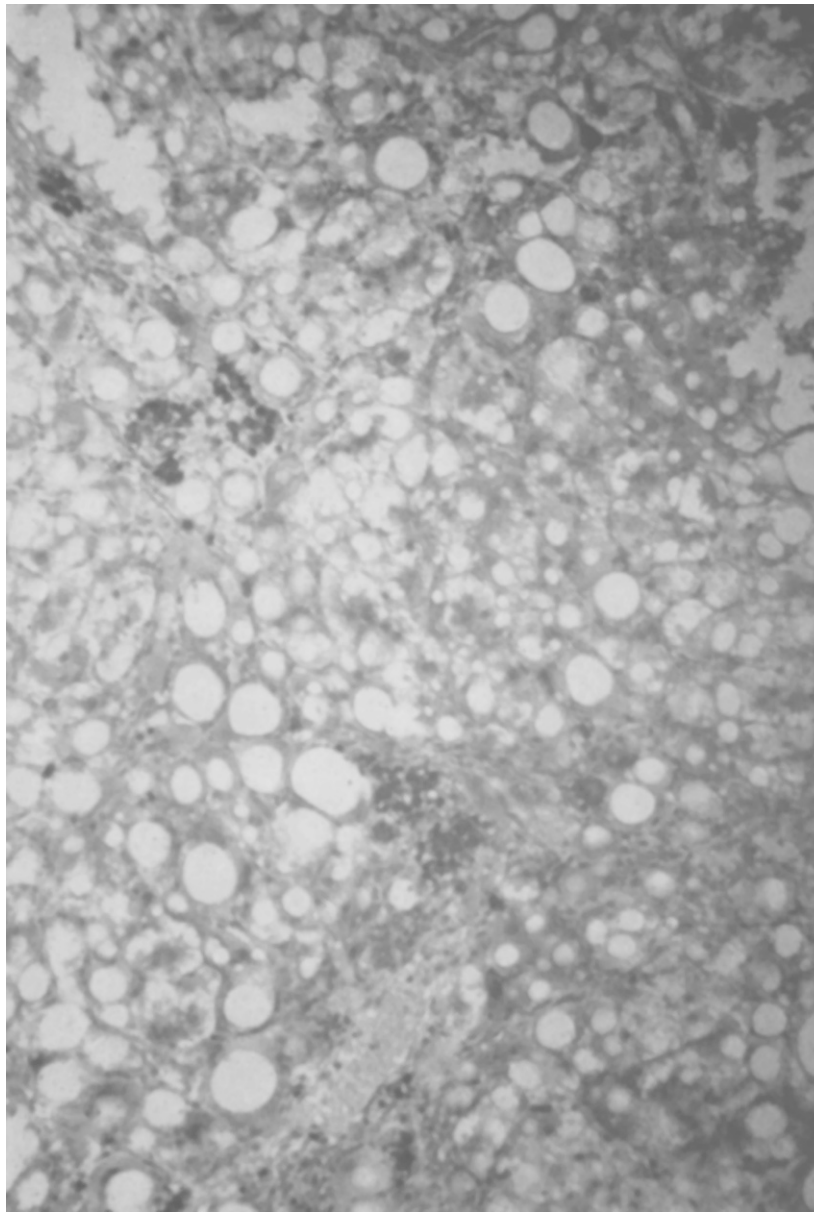

FIG. 2.
Liver showing moderate ceroid deposition in a rat after 90 days on a fibrin diet without cod liver oil. Ziel-Neelson stain. × 150.

islands of liver cells, some of them necrotic, were seen enclosed in the proliferating connective tissue. Many liver cells contained large amounts of fat.

A peanut-casein diet had been used by Engel[12] for the production of liver cirrhosis in rats. However, Engel kept his animals for 16 months on the experimental diets. Engel fed small amounts of choline in the beginning of the experiments, in order to avoid the detrimental influence of choline deficiency on the kidneys. While some animals died in the beginning of the experiments here reported, due to acute choline deficiency, a good number survived. Most of the surviving animals showed the typical degenerative kidney lesions

[12] Engel, R. W., *Fed. Proc.*, 1943, **2**, 62.

found in choline deficiency.[13,14,15]

Most investigators reported the occurrence of cirrhosis in rats only after a longer period of feeding the experimental diets. Daft, Sebrell, and Lillie[16] found, however, moderate to marked cirrhosis in weaning rats on a diet containing 4% casein and 0.5% cystine, after an average period of 83 days. In the experiments here reported cirrhosis can be produced in a short time even by giving large amounts of protein, deficient in methionine and enriched by cystine.

If the diet used in these experiments contained 2% cod liver oil, very large amounts of ceroid are deposited. If, however, the cod liver oil intake is restricted to 1 drop weekly, markedly less ceroid is formed. Hepatic cirrhosis is also observed in rats which are fed the experimental diets without any cod liver oil. However, the cirrhosis seems less severe in the majority of cases. In some instances, no ceroid was found in these cirrhotic livers, thus confirming the results of Endicott and co-workers. In other cases, however, ceroid deposition occurred, although in much smaller amounts than in the controls receiving cod liver oil. Cod liver oil can, therefore, not be the only reason for the occurrence of ceroid.

So far ceroid has only been observed in the livers of rats fed a choline-free, protein-deficient diet. It has, however also been found in the livers of mice in which cirrhotic changes had been produced by means of carbon tetrachloride[17] and O-aminoazotoluene.[18] These animals were on a full normal diet without added cod liver oil.

Summary. Regular and severe liver cirrhosis can be induced in male weaning rats on a choline-free, protein-deficient diet after 90 days, if the protein source is either fibrin or peanut meal plus casein. Early cirrhosis is occasionally observed in less than 60 days. Very large amounts of ceroid are deposited by the use of these diets, containing 2% cod liver oil. The amount of the deposited ceroid is markedly reduced if the intake of cod liver oil is restricted to one drop weekly. On the same diet without any cod liver oil, the produced cirrhosis is less severe. Some of these livers contain no ceroid, others small amounts of this pigment. Cod liver oil is therefore not the only cause for ceroid pigmentation in nutritional cirrhosis. It enhances, however, its formation very markedly.

13 György, P., and Goldblatt, H., *J. Exp. Med.*, 1940, **72**, 1.

14 Christensen, K., *J. Biol. Chem.*, 1940, **133**, xx; *Arch. Path.*, 1942, **34**, 633.

15 Wachstein, M., *Arch. Path.*, 1944, **38**, 297.

16 Daft, F. S., Sebrell, W. H., and Lillie, R. D., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 228.

17 Edwards, J. E., and Dalton, A. A. J., *J. Nat. Cancer Inst.*, 1942, **3**, 19.

18 Andervont, H. B., Grady, H. G., and Edwards, J. E., *J. Nat. Cancer Inst.*, 1942, **3**, 131.

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Serial Peritoneoscopic Examinations as a Means of Studying Experimental Liver Damage in Dogs.*

F. W. HOFFBAUER, HECTOR DUCCI,† AND R. E. EMMONS. (Introduced by C. J. Watson.)

From the Department of Medicine, University of Minnesota, Minneapolis, Minn.

Most experimental observations of the histological changes occurring in the liver as the result of the administration of toxic agents have been based upon postmortem findings. Such a method necessitates either sacrifice of the animal or waiting until the effects of the agent are lethal.

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† Santiago, Chile, S.A. Traveling Fellow, Rockefeller Foundation.