pregnenolone increased the synthesis of acetylcholine.

The authors wish to express their gratitude to Abbott Laboratories, Ayerst, McKenna & Harrison (U.S.), Ltd., Ciba Pharmaceutical Products, Inc., Roche-Organon, Inc., Schering Corporation, and Upjohn Company for the generous supply of hormones.

14800

Dietary Cirrhosis without Ceroid in Rats.

K. M. ENDICOTT, FLOYD S. DAFT, AND W. H. SEBRELL.

From the Pathology Laboratory and the Division of Physiology, National Institute of Health, Bethesda, Md.

Lillie, Daft, and Sebrell[1] among others have reported the production of hepatic cirrhosis in rats given a purified diet low in protein and low in choline. In their report and in the reports of other investigators[2,3] it was noted that the cirrhosis was always accompanied by the deposition of considerable amounts of a peculiar insoluble pigment in the fibrous trabeculations of the cirrhotic livers. The name "ceroid" has been given to this pigment.[4] Endicott and Lillie[5] have described in detail its staining properties and have noted that it is insoluble in all common solvents. Ceroid has not been reported in human liver cirrhosis nor in the experimental cirrhosis produced in the dog, the guinea pig, the pig, or the rabbit.

The question of whether or not ceroid is an essential part of the cirrhosis in rats has been the subject of much speculation. Ceroid was found to resemble the lipoid residues in cod liver oil pneumonia and similar substances were produced by chromate oxidation of cod liver oil and linseed oil but not by oxidation of various hydrogenated oils.[6] This suggested that dietary cirrhosis without ceroid might be produced by altering the dietary fat. Together with the work of Hass,[7] it suggested also the possibility that fatty acids found in both vegetable and animal oils and possessing more than one double bond might be involved in ceroid production. We have now achieved the production of cirrhosis without ceroid. Our results seem to indicate, however, that the most important dietary precursor of ceroid is not an ingredient of Wesson oil and that it is distinct from linoleic and linolenic as well as oleic, palmitic and stearic acids. It appears probable that the substance in question is present in cod liver oil but we have not as yet identified it further.

Weanling albino rats were given one of several purified diets containing leached and alcohol-extracted casein 4%, cystine 0.5%, and salt mixture No. 550[8] 4%. Diet No. 895 contained no fat or fatty acid, diet No. 954 contained 5% of Wesson oil, diet No. 953 contained 1% of a mixture of oleic, linoleic, linolenic, and saturated acids,* and

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

2 György, P., and Goldblatt, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 492.

3 Blumberg, H., and McCollum, E. V., *Science*, 1941, **93**, 598.

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

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

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

7 Hass, G. M., *Arch. Path.*, 1938, **26**, 956; 1939, **28**, 177.

8 Spicer, S. S., Daft, Floyd S., Sebrell, W. H., and Ashburn, L. L., *Pub. Health Rep.*, 1942, **57**, 1559.

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

* Armour and Company's "Neo-Fat No. 23." The composition given by the Company is oleic acid 30%, linoleic acid 56%, linolenic acid 10%, and saturated acids 4%.

diets No. 950, No. 951, and No. 952 contained 10% of palmitic, stearic and oleic acids respectively. Sucrose made up the remainder of the diets. Each rat received a daily supplement of 100 μg of thiamine hydrochloride, 50 of riboflavin, 20 of pyridoxine hydrochloride, 50 of calcium pantothenate and 1 mg of niacin. Each rat also received a weekly supplement of 3 mg of α-tocopherol in ethyl laurate and a bi-weekly supplement of 500 units of vitamin D in propylene glycol and 100 μg of carotene (S.M.A.) in ethyl laurate. A few of the rats on the fat-free diet received 20% alcohol *ad libitum* as the sole source of fluid; all of the other rats received water *ad libitum*. The animals were kept on these diets until their deaths which ranged from 10 days to 194 days from the beginning of the experiment (average of 56 animals on experiment, 66 days).

Histological examination of the liver, spleen, kidney, and adrenals of these 56 animals failed to reveal any trace of ceroid. Two of 5 of the animals receiving Wesson oil (deaths at 63 and 118 days), 1 of 5 receiving the mixture of oleic, linoleic, linolenic, and saturated acids (death at 110 days), 1 of 23 receiving the fat-free diet with water to drink (death at 111 days), and 2 of 8 receiving the fat-free diet and alcohol (deaths at 48 and 93 days) developed cirrhosis of the liver. None of the 15 rats receiving oleic, palmitic, or stearic acids (5 on each diet) developed cirrhosis but this may have been due to the short period of survival of these animals. Only 2 lived as long as 50 days on the experiment and the last survivor died at 70 days.

The cirrhotic livers showed fibrous trabeculation and distortion of lobular architecture. The distribution of the trabeculation was similar to that described by Lillie and coworkers.[4] The fibrous trabeculæ appeared to connect centrolobular areas, occasionally coursing through or near portal structures. Most liver cells contained large globules of fat.

It appears likely that the presence of cod liver oil in the ceroid-producing diets such as diet No. 545 employed in previous experiments[9,10,11] constitutes the important difference between these diets and the diets used in the present experiments, which did not cause the deposition of ceroid. Diet No. 954, containing 5% Wesson oil is very similar to diet No. 545 containing 2% cod liver oil and 3% Wesson oil. Diet No. 545 contains cornstarch instead of sucrose and Osborne and Mendel salt mixture instead of modified O. and M. salt mixture No. 550, but we have from time to time used many cirrhosis-producing diets containing sucrose and salt mixture No. 550, and have found that ceroid invariably accompanies the cirrhosis (unpublished experiments). In this connection the following statement by György[11] should be noted: "A survey of the nutritional conditions under which ceroid in experimental cirrhosis in rats was noted, reveals the presence of cod liver oil and the absence of large amounts of yeast or of other crude sources of the vitamin B-complex in the diet."

Although these results are clearly preliminary in nature, several points of interest are indicated. Perhaps first in importance is the fact that it is possible to produce dietary cirrhosis in rats without accompanying ceroid deposition. A second point of interest is the indicated probability that ceroid is related to some substance or substances present in cod liver oil. It is of some further interest that cirrhosis may be produced in rats on diets completely devoid of fat or fatty acids.

It is not clear as yet whether or not the formation and deposition of ceroid affects the incidence and severity of cirrhosis.

Conclusions. 1. Hepatic cirrhosis without ceroid has been produced in rats by feeding certain purified diets. 2. The type of fat given to rats in low-choline, low-protein diets appears to have an important influence on the deposition of ceroid. It seems probable that some substance or substances which are present in cod liver oil causes the appearance of this pigment. We have not observed ceroid in rats receiving palmitric, stearic, oleic, linoleic, or linolenic acids. 3. Cirrhosis may be produced on a completely fat-free diet.

10 Daft, Floyd S., Sebrell, W. H., and Lillie, R. D., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 1.

11 György, Paul, *Am. J. Clin. Path.*, 1944, **14**, 67.