hibition of cell penetration by virus, caprochlorone delayed release of newly formed virus from the cell, whereas l-adamantanamine · HCl and ammonium phosphate did not.

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1. Liu, O. C., Carter, J. E., Malsberger, R. G., DeSanctis, A. N., Hampil, B., J. Immunol., 1957, v78, 222.

2. Liu, O. C., Ferlauto, R. J., in H. P. Kuemmerle, P. Preziosi, P. Rentchnick, ed., Second Int. Symposium Chemotherapy, Napales, 1961. Part II, Antiviral Chemotherapy, S. Karger, New York, 1963, p308.

3. Liu, O. C., Malsberger, R. G., Carter, J. E., DeSanctis, A. N., Wiener, F. P., Hampil, B., J. Immunol., 1957, v78, 214.

4. Davies, W. L., Grunert, R. R., Haff, R. F., McGahen, J. W., Neumayer, E. M., Paulshock, M., Watts, J. C., Wood, T. R., Hermann, E. C., Hoffmann, C. E., Science, 1964, v144, 1.

5. Hoffmann, C. E., Neumayer, E. M., Haff, R. F., Goldsby, R. A., J. Bact., 1965, v90, 623.

6. Neumayer, E. M., Ha, R. F., Hoffmann, C. E., Proc. Soc. Exp. Biol. & Med., 1965, v119, 393.

7. Eaton, M. D., Scala, A. R., ibid., 1961, v108, 766.

8. Fletcher, R. D., Hirschfield, J. E., Forbes, M., Nature, 1965, v207, 664.

9. Jensen, E. M., Force, E. E., Unger, J. B., Proc. Soc. Exp. Biol. & Med., 1961, v107, 447.

10. World Health Org. Tech. Rep. Series No. 170, World Health Org., Geneva, 1959, p41.

11. Haff, R. F., Stewart, R. C., J. Immunol., 1965, v94, 842.

12. Haff, R. F., Wohlsen, B., Force, E. E., Stewart, R. C., J. Bact., 1966, v91, 2339.

13. Rapp, F., Benyesh-Melnick, M., Science, 1963, v141, 433.

14. Cooper, P. D., Virology, 1959, v7, 469.

15. Farnham, A. E., Newton, A. A., ibid., 1959, v7, 449.

16. Mandel, B., in Cold Spring Harbor Symposium on Quantitative Biology, Basic mechanisms in animal virus biology, Long Island Biological Assn., Cold Spring Harbor, 1962, v27, 123.

17. Payne, F. E., Kurtz, H., Ackermann, W. W., Arch. ges Virusforch., 1958, v8, 1.

18. Dales, S., Choppin, P. W., Virology, 1962, v18, 489.

19. Hoyle, L., Finter, N. B., J. Hyg., 1957, v55, 290.

20. Cartwright, S. F., Thorne, H. V., Nature, 1958, v182, 717.

21. Thorne, H. V., J. Bact., 1962, v84, 929.

22. Ackermann, W. W., Maassab, H. F., J. Exp. Med., 1954, v99, 105.

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Histopathology of Guinea Pigs with Cholesterol-Induced Anemia.* (32077)

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Hemolytic anemia, induced in guinea pigs by dietary cholesterol, was accompanied by enlargement of liver and spleen(1) and by hyperplasia of the bone marrow(2). Other organs did not show any gross changes, although the lipid composition of the liver, spleen(3), bone marrow(2), and adrenal(4), as well as that of lung, kidney and heart (unpublished), was greatly altered. We have now subjected these organs to a thorough microscopic examination in an attempt to determine the mechanism of the anemia.

Materials and methods. Male guinea pigs, weighing about 200 g, were fed a semisynthetic diet(2) with or without the addition of 1% cholesterol. Development of the anemia was monitored by red blood counts. The time required to produce the anemia varied from 10-14 weeks. When the red cell count had fallen to about 3.5 mil/mm^3, the animal was sacrificed and the liver, spleen, lung, kidney, adrenal, heart, pancreas, and testis were fixed in 10% buffered neutral formalin. The ends of

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the femurs were removed, and bone marrow smears were prepared from them. The smears were stained with Wright-Giemsa stain. Paraffin sections 7 μ thick were prepared from the formalin-fixed organs, and stained with hematoxylin-eosin, and Prussian blue—the latter for detection of hemosiderin. Sudan IV stain was used on frozen sections, for detection of lipid. Four anemic and 4 control guinea pigs, representing a random sample taken from groups of 40 cholesterol-fed and 40 control animals, respectively, were studied for pathological changes.

Results and discussion. The cholesterol-fed animals were smaller than the controls and were more susceptible to infection(3). The peripheral blood showed severe anemia with reticulocytes, normoblasts, and a considerable proportion of abnormally-shaped red cells. The mean survival time of the red cells of the anemic animals was greatly reduced (data to be published separately). The *livers* were

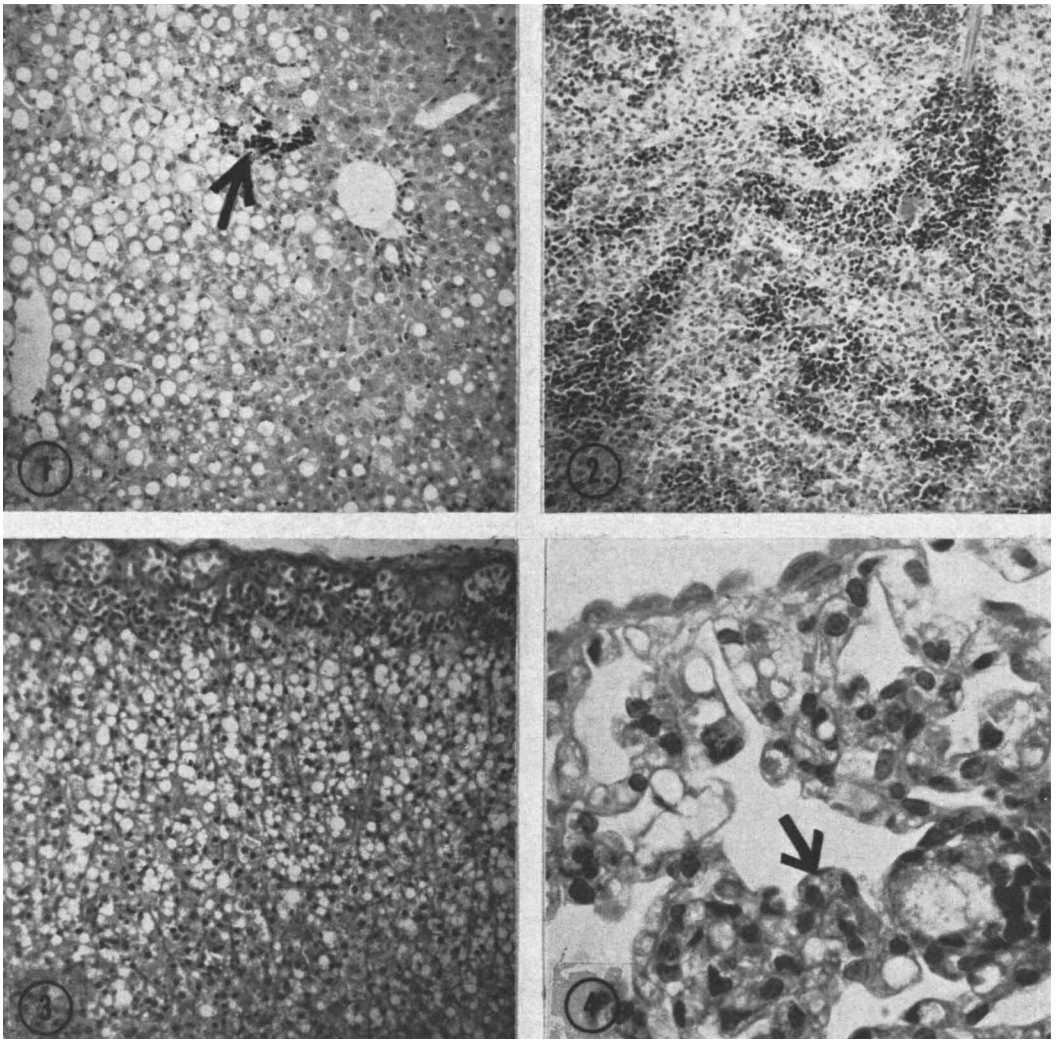

FIG. 1. Fatty liver from anemic guinea pig with erythropoiesis (arrow); hemotoxylin and eosin (× 110).

FIG. 2. Enlarged spleen from anemic guinea pig with erythropoiesis and lipid-laden histiocytes; hematoxylin and eosin (× 110).

FIG. 3. Adrenal from anemic guinea pig; note fat vacuoles; hematoxylin and eosin (× 110).

FIG. 4. Lung from anemic guinea pig; note lipid-laden histiocytes (arrow); hematoxylin and eosin (× 450).

grossly enlarged, and contained numerous large, single vacuoles in the hepatic cells (Fig. 1). Frozen sections stained with Sudan IV showed that these vacuoles were due to the presence of lipid globules. The centrolobular areas were more affected than were the periportal areas. Under polarized light, many cholesterol-like "crystals" were seen in the lipid globules as well as in the macrophages. The exact physical phase of the cholesterol was not determined. The cholesterol crystals varied in shape from long and spindly to rectangular. Similar structures were seen in the sections from spleen and lung, both of which had accumulated considerable amounts of cholesterol(3). Many foci of erythropoiesis were scattered throughout the liver (Fig. 1), presumably a response to the acute hemolytic anemia. Normoblasts of various ages were present in these foci, and an occasional megakaryocyte was seen. Hemosiderin was present in moderate amounts in the cells of the periportal areas, indicating storage of excess iron released by the hemolytic process. Some spotty necrosis and an increase of mitotic activity occurred. Scattered throughout the liver were neutrophils, indicating a moderate degree of inflammation. These findings suggest a highly dynamic state of the liver, in which fatty infiltration, spotty necrosis, and regeneration occurred simultaneously. No bile stasis nor red cell congestion was observed, nor any fibrosis. *Spleens* were greatly enlarged to approximately 14 times normal size, and contained 20 times the normal level of cholesterol ester(3). They were soft and pliable, and showed no gross necrosis. The white pulp had decreased in size and there was widespread erythropoiesis throughout the red pulp (Fig. 2), with a predominance of polychromatic and orthochromatic normoblasts and a few megakaryocytes. Much fatty deposition appeared in the red pulp, especially in the reticuloendothelial cells. The white pulp was less affected. Under polarized light, cholesterol was seen throughout the organ, although not in the concentration seen in the liver. In sections stained with Prussian blue, much hemosiderin, as well as many engulfed red cells, was seen in the macrophages. The venous sinuses showed moderate red cell congestion, but not sufficient to account for the splenomegaly. A few areas of microscopic necrosis were seen, with numerous inflammatory cells. The capsule and trabeculae were normal, without evidence of fibrosis.

The *adrenals* showed a selective lipid infiltration of the outer two-thirds of the zona fasciculata (Fig. 3). The cortical cells in this area showed numerous large, single, fat globules throughout, with the nuclei pushed to one side of the cell. The zona glomerulosa and zona reticularis were normal. Frozen sections stained with Sudan IV showed a considerable increase of lipid globules and cholesterol crystals as compared with controls. The medulla was essentially normal.

Lungs showed many fatty deposits and some cholesterol crystals. Chemical analysis showed a 21-fold increase of esterified cholesterol and only a moderate increase in free cholesterol (unpublished). The lipid was found in small droplets in the histiocytes and as fat emboli in the capillaries. In spite of the emboli, there was no indication of respiratory impairment in the animals. The pleura was reduced to about half its normal thickness, with some areas only one cell thick. Some thickening of the alveolar walls occurred, due mostly to lipid-laden histiocytes (Fig. 4). Also affected was the epithelial lining of the bronchioles and bronchi, which showed papillary hyperplasia and some desquamation of the epithelium.

The *heart* was remarkably normal, with no sign of hypertrophy in spite of the severe anemia. The coronary artery and the aorta showed no degenerative lesions, and the endocardium and myocardium were essentially normal.

The *pancreas* was normal. The Islets of Langerhans were normal in size and appearance. The acinar cells, with many zymogen granules, appeared to be functioning normally. The duct of Wirsung and its branches showed no pathological changes.

The *testes* of the anemic animals were smaller than those of the controls. Although this could have been due to the smaller size of the anemic animals, it was reflected by poorly developed seminiferous tubules. In a few

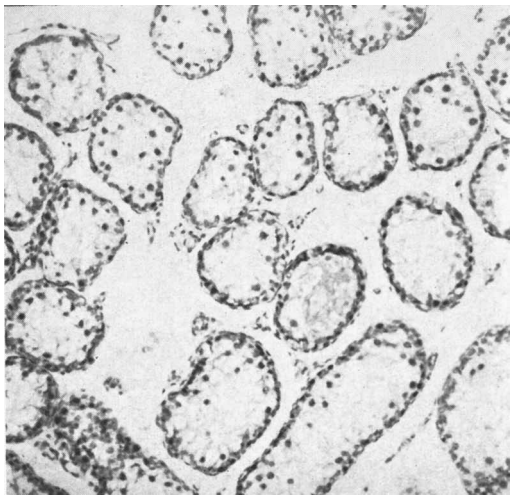

FIG. 5. Atrophic testis from anemic guinea pig; hematoxylin and eosin (× 110).

tubules, much acidophilic amorphous material was present in the lumen. Spermatogenesis was greatly reduced as shown by the reduced number of cells in the tubules (Fig. 5).

The *kidney* showed hemosiderin in the proximal convoluted tubules, fat globules in the glomeruli and juxtaglomerula apparatus, and a striking proliferation of the mesangium of the glomeruli(5).

The *bone marrow* showed an altered lipid composition, both quantitatively and qualitatively(2). Most of the yellow marrow was replaced by red marrow. The number of nucleated cells had increased greatly, while fat cells normally present in large numbers had virtually disappeared. The ratio of erythrocytic to myelocytic elements was changed from 1:3 to 1:1, with most of the increase in the erythrocytic cells resulting from polychromatic normoblasts. This bone marrow hyperplasia is a typical response to a hemolytic anemia and is apparently independent of the hypercholesteremia.

In the light of these findings, dietary cholesterol in the guinea pig apparently leads to an accumulation of cholesterol and to subsequent alterations of the cellular integrity of various organs. The inability of the liver to metabolize, eliminate, or esterify the cholesterol is probably the primary physiological burden on the animal. The limitations of a severely fatty liver may have caused some of the pathological changes seen in some of the organs. The extra-medullary erythropoiesis and the deposition of hemosiderin are manifestations of the hemolytic process and are in themselves secondary symptoms.

Summary. The response of guinea pigs to dietary cholesterol is unique among small animals. Instead of developing cardio-vascular lesions, the guinea pig becomes acutely anemic. The anemia is accompanied by enlarged liver and spleen, and fatty infiltration of liver, spleen, lung, and kidney. Most of the organs showed striking pathological changes when examined microscopically. The liver showed lipid globules, erythropoiesis, spotty necrosis, accumulation of hemosiderin and cholesterol, and diffuse regeneration. Splenomegaly was due to erythropoietic proliferation, red cell congestion and, to a lesser degree, to fatty deposits. The primary change in the adrenal was a selective fatty infiltration of the zona fasciculata. The lungs showed fat globules in the histiocytes as well as fat emboli in the capillaries. The testes were small and poorly developed. Spermatogenesis was greatly impaired. The bone marrow was hyperplastic, and the kidney showed glomerulosclerosis. The pancreas and heart were unaffected. The changes observed resulted primarily from alterations in the cellular structure caused by the accumulation of cholesterol, and secondarily from impaired liver function and the effects of the hemolytic process.

1. Okey, R., Greaves, V., J. Biol. Chem., 1939, v129, 111.

2. Ostwald, R., Darwish, O., Irwin, D., Okey, R., Proc. Soc. Exp. Biol. & Med., 1966, v123, 220.

3. Ostwald, R., Shannon, A., Biochem. J., 1964, v91, 146.

4. Ostwald, R., Shannon, A., Miljanich, P., Lyman, R. L., J. Nutrition, 1964, v82, 443.

5. French, S., Yamanaka, W., Ostwald, R., Arch. Path., 1967, v83, 204.

Received February 14, 1967. P.S.E.B.M., 1967, v125.