in the dog pancreas during administration of purified anterior pituitary growth hormone has been studied. Fifteen mature normal mongrel dogs each received 3 mg/kilo of growth hormone daily subcutaneously. Two each were sacrificed on alternate days for 14 days. Eight animals were used as controls. It was found that sometimes glycogen is normally present in ductular epithelium but not in B cells. Increased vacuolization (glycogen infiltration) of intralobular and intercalated ducts developed prior to and generally in the absence of the appearance of glycogen in the B cells of the islets. These findings are taken to support the hypothesis that hydropic degeneration of B cells is not a degenerative lesion resulting from "functional exhaustion" but is rather a morphologic expression of hyperglycemia similar to that seen in other organs.

1. Allen, F., *J. Metab. Res.*, 1922, v1, 5.
2. Richardson, K. D., *Proc. Roy. Soc., London,* 1940, v128, 153.
3. Ham, A. W., and Haist, R. E., *Am. J. Path.,* 1941, v17, 787.
4. Duff, G. L., *Am. J. Med. Sci.,* 1945, v210, 381.
5. Toreson, W. E., *Am. J. Path.,* 1951, v27, 327.
6. Lazarus, S. S., and Bencosme, S. A., *Am. J. Clin. Path.,* 1956, v26, 1146.
7. Gomori, G., *ibid.,* 1950, v20, 665.
8. Bencosme, S. A., *A.M.A. Arch. Path.,* 1952, v53, 87.
9. Lillie, R. S., *Histopathologic Technique and Practical Histochemistry,* Blakiston Co., 1954.
10. Ohohashi, Y., Quoted by Toreson, W. E., *Am. J. Path.,* 1951, v27, 327.
11. Ehrlich, P., *Z. f. Klin. Med.,* 1883, v6, 33.
12. Robbins, S. L., *Am. J. Med. Sci.,* 1950, v219, 376.
13. Cruickshank, E. W. H., *Phys. Rev.,* 1936, v16, 597.
14. Babkin, B. P., *Secretory Mechanism of the Digestive Glands,* Paul B. Hoeber, 1944.

Received December 26, 1956. P.S.E.B.M., 1957, v94.

Effect of Dietary Lipid on Rat Serum and Liver Cholesterol and Tissue Mast Cells.*† (23027)

BENJAMIN W. GRUNBAUM, JOHN R. GEARY, JR., FRANCISCO GRANDE, JOSEPH T. ANDERSON AND DAVID GLICK

Histochemistry Laboratory, Department of Physiological Chemistry, and Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis.

It has been shown repeatedly in man(1-2) that increase in proportion of calories from dietary lipid causes elevation of concentration of serum cholesterol. Recent reports indicate that this effect does not follow when lipid consists of certain vegetable oils(3). Moreover certain oils rich in linoleic acid have been reported to produce a significant decrease of serum cholesterol concentration in man(4-8). In view of differences in cholesterol metabolism between man and rat, the present study on the rat is concerned first with the effect of different fat-diets on cholesterol concentration in serum lipoproteins and in liver.

Since heparin has a "clearing" action on lipemic serum, consideration was also given to the possibility that heparin might serve to some degree as a physiological regulator of blood lipid. Tissue mast cells contain heparin, and the question is whether their number, intact or undergoing disruption, is influenced by nature and concentration of blood lipid. This possibility was examined by counting mast cells in external ear tissue of rats maintained on various diets for study of serum and liver cholesterol.

Methods. Adult male albino rats of Sprague Dawley strain were used. The age-

* This study was supported in part by research grant from N.I.H., U. S. Public Health Service, and by grant from Louis W. and Maud Hill Family Foundation.

† The authors wish to express their appreciation for the technical assistance given by Georgiana Psik and Nedra Whittaker, and for pathological examinations of specimens by Dr. Lee Wattenberg.

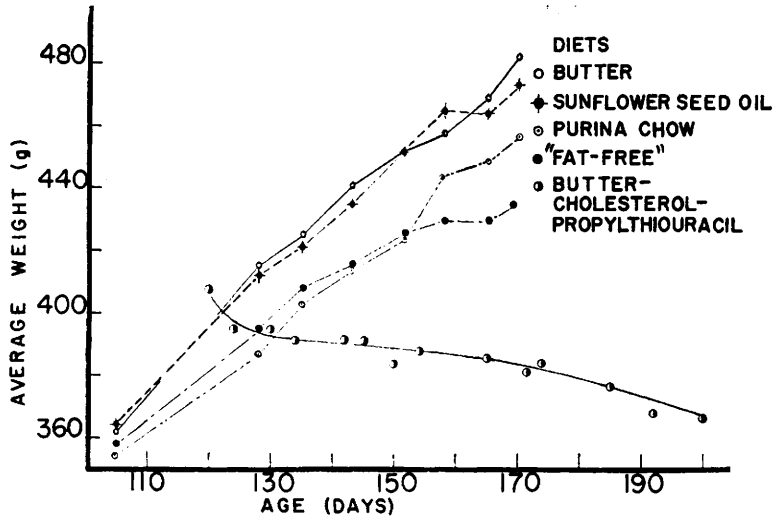

FIG. 1. Weight changes of rats maintained on different diets. Ralston Purina Chow diet: stated to have no less than 5% fat, 8 rats. "Fat-free" diet: Nutritional Biochemicals Co., 8 rats. Butter diet: 79% Purina Chow, 21% butterfat, 8 rats. Sunflower seed oil diet: 75% Purina Chow, 25% sunflower seed oil (iodine no. 131), 8 rats. Butter-cholesterol-propylthiouracil diet: 75% Purina Chow, 21% butterfat, 5% cholesterol (0.1% propylthiouracil added to mix), 16 rats.

weight relationships for animals on different diets are given in Fig. 1. Food and water *ad libitum* was furnished. Blood was obtained from tail or, for last sample, when animal was sacrificed, by heart puncture. Total serum cholesterol was estimated by the method of Abell, *et al.*(9) as modified by Anderson and Keys(10). Electrophoretic separation of lipoproteins was carried out by the method of Williams, *et al.*(11) with a Durrum Spinco, model R, apparatus (Beckman Instruments, Belmont, Calif.). From each sample of serum 20 μl were taken for protein electrophoresis and 100 μl for lipoprotein electrophoresis. Barbiturate buffer (Spinco buffer, B-2) of pH 8.6 was used. With rat serum, best separation was achieved when 11.5 ma at 150 volts were applied for 16.5 hours. After drying, the paper strip containing 20 μl was stained with bromphenol blue (Spinco dye, B-1) to indicate the various protein zones, and the strip with 100 μl was stained with Sudan black (2 g in 1 l 60% alcohol, refluxed 3-4 hours, decanted after cooling) to show lipoprotein zones. Two additional paper strips which carried 100 μl serum each, were subjected to electrophoresis and drying but not staining. The 20 μl of serum were deposited in one application on strip by means of striper supplied with the apparatus, while 100 μl in a graduated micropipette were delivered in 5 approximately equal portions onto paper strip by means of the striper. The unstained strips were cut into segments and analyzed for cholesterol using the stained strips as references. A piece 40 mm long, was cut from paper behind the starting line, where no serum components were present, to serve as a blank. Extraction of cholesterol from paper segments followed procedure of Anderson and Keys(10). The final volume of colored solution was 0.6 ml and was measured in Beckman DU spectrophotometer using Lowry-Bessey microcuvettes having 1 cm light path. Liver cholesterol was estimated in pieces (4 to 5 g) removed immediately after bleeding the animal. Liver samples were digested in 2 ml 30% KOH at about 90°C for 0.5 to 1.5 hours, or until the liver completely disappeared. After diluting to 50 ml with distilled water, 2 ml aliquots were added to 2 ml of absolute alcohol and 4 ml of petroleum ether. After shaking for a minute, the petroleum ether layer was removed. Extraction of the aqueous solution with a 4 ml portion of petroleum ether was repeated.

TABLE I. Effect of Diet on Total Cholesterol in Rat Serum and Liver.

Diet	Mean cholesterol (mg %) ± stand. error of mean Weeks on diet 1	9	9
	Serum		Liver
Purina chow	65 ± 3	58 ± 3	190 ± 12
"Fat-free"	85 ± 7*	73 ± 3†	192 ± 4
Butter	84 ± 4†	75 ± 4*	215 ± 8
Sunflower seed oil	88 ± 4†	84 ± 3†	366 ± 40†

* Significance of difference from corresponding Purina group, P = 0.05.
† Significance of difference from corresponding Purina group, P = 0.01.

The solvent was evaporated, the lipid was dissolved in alcohol-acetone(1:1), and cholesterol was precipitated as digitonide using aluminum chloride and ammonium hydroxide to speed the process as suggested by Brown, *et al.*(12). The method of Foldes and Wilson(13) was used to complete cholesterol determination. Because of high concentration of mast cells in the external ear and the ease of obtaining these specimens, samples of wide, mid-portion were routinely taken from each rat at time of sacrifice. Sections 12 μ, were prepared and stained with toluidene blue by the method of Smith and Atkinson(14) so that auricular cartilage appeared as a dark, central band with subcutaneous tissue and skin on each side. The mast cells in random fields, 0.17 x 0.17 mm, including subcutaneous tissue both dorsal and ventral to auricular cartilage were counted (Table IV).

Results. Mean weight curves for experimental groups are presented in Fig. 1. Animals on "fat-free" diet showed retarded growth after 7 weeks on this diet. Rats on butter-cholesterol-propylthiouracil diet showed progressive weight loss from beginning of experiment. In spite of weight effects all animals had a normal and healthy appearance.

Table I shows changes in serum and liver cholesterol for the first 4 groups of rats.

Rats on diets other than Purina chow showed increase in serum cholesterol concentration compared with the latter group. No change of liver cholesterol was observed in animals on "fat-free" and butter diets compared with those on Purina chow, while rats receiving sunflower seed oil showed a significant increase.

Serum and liver cholesterol of animals on butter-cholesterol-propylthiouracil diet is given in Table II. Both serum and liver cholesterol values were higher than those found on diets listed in Table I, and serum cholesterol increased with time. These increases are significant when comparisons are made with data from rats on Purina chow. All animals indicated in Table II had large fatty livers.

Cholesterol in serum lipoprotein fractions was measured on serum samples from 1 or 2 animals from each experimental group. Results are presented in Table III.

Distribution of cholesterol among serum protein fractions in the rat is strikingly different from that observed in man. While man carries about 80% of total serum cholesterol in the beta globulin(10), rats, on the Purina chow diet, had only about 20% in this fraction. These findings agree with those of Fidanza and Cresta(15). Changes in dis-

TABLE II. Total Cholesterol in Serum and Liver of Rats on Butter - Cholesterol - Propylthiouracil Diet.

No. of rats	Wk on diet	Mean cholesterol (mg %) ± stand. error of mean Serum	Liver
5	3	177 ± 20*†	568 ± 159*
5	6	243 ± 24*	820 ± 162*
6	11	306 ± 23*	675 ± 166*

* Significance of difference from Purina group (9 wk on diet), P = 0.01.
† One sample lost.

TABLE III. Distribution of Total Cholesterol among Serum Protein Fractions Separated by Paper Electrophoresis.

Diet	Wk on diet	Cholesterol (% of total) Albumin	α-globulin	β-globulin
Purina chow	9	30	51	19
"Fat-free"	9	50	35	15
Butter	9	37	47	16
Sunflower seed oil	9	44	40	16
Butter-cholesterol-propylthiouracil	3	20	53	27
Idem	6	23	57	20
"	11	29	45	26
"Normal" human serum		12	7	81

tribution observed in samples of serum from animals on "fat-free," butter, and sunflower seed oil diets appear to be unimportant. This indicates that increased cholesterol caused by special diets is distributed among all protein fractions.

It is evident (Table III) that the albumin zone contained a considerable fraction of total cholesterol. When the paper strips were stained with Sudan black, 2 distinct zones were visible corresponding to albumin and alpha globulins. The Sudan black stain is not sensitive enough to reveal quantities of lipids such as found in beta globulin zone of rat serum. In human serum beta globulin stains intensely, albumin less, and alpha globulin very little if at all.

In contrast with its action in man(6,8), sunflower seed oil at the 25% level in rat diet, produced an *elevation* of blood serum cholesterol similar to that observed in the group of animals receiving butterfat. Sunflower seed oil also caused an increase in liver cholesterol compared with Purina chow alone or plus 21% butterfat. The rats receiving "fat-free" diet showed an increase of blood serum cholesterol but no significant change of liver cholesterol. These results differ with those reported for fat-free diet by Alfin-Slater *et al.*(16), who found a decrease in blood serum cholesterol and an increase in liver cholesterol. The fat-free diet in our experiment differed from that of Alfin-Slater *et al.* in containing, respectively, 16% *vs.* 4% cellulose, 0.60% *vs.* 0.085% choline, and in continuing for 9 *vs.* 20 weeks. It appears from our experiment that elevation of serum and liver cholesterol in animals receiving sunflower seed oil can not be attributed to deficiency of essential fatty acids.

Addition of cholesterol and propylthiouracil to Purina-plus-butter diet enhanced hypercholesterolemia and caused an increase of cholesterol content of the liver. On fractionation of serum proteins by electrophoresis and subsequent determination of cholesterol in various protein fractions (Table III), it was found that with prolonged high blood cholesterol the proportion of cholesterol in the albumin zone decreased slightly while that in the beta globulin region increased slightly. Results on rats fed butter-cholesterol-propylthiouracil are similar to those of Page and Brown(17) on hypothyroid rats whose diets were supplemented with cholesterol. These authors reported increase of serum beta lipoprotein but no data were given for distribution of cholesterol among protein fractions.

TABLE IV. Mast Cell Counts on Tissue Sections from Ears of Rats on Different Diets. 100 microscopic fields counted for each diet (20 fields/rat, 5 rats/diet).

Diet	Mean No./field	Stand. dev.	Stand. error of mean
Purina chow	13.2	3.6	.4
"Fat-free"	12.8	3.8	.4
Butter	12.1	2.9	.3
Sunflower seed oil	11.6	2.9	.3
Butter-cholesterol-propylthiouracil	12.3	3.1	.3

Recently Fillios *et al.*‡ reported development of atherosclerosis in rats receiving purified diets supplemented with cholesterol, sodium cholate and thiouracil, and showing marked hypercholesterolemia and hyperbeta lipoproteinemia. No gross lesions were seen in arteries of any animals employed in our study. Both rat and dog, animals considered resistant to atherogenesis(17), show high levels of alpha and lower levels of beta lipoprotein(18).

It is apparent from Table IV that the different diets had no appreciable influence on mast cell counts in rat ears, and therefore it appears that a response in mast cell number does not occur under these conditions associated with varied levels of serum and liver cholesterol. Furthermore, no difference in proportion of abnormal or disrupted mast cells was found.

Summary. 1) An increase of blood serum cholesterol has been observed in rats subsisting on Purina chow diet when supplemented with butterfat, or sunflower seed oil. Animals on sunflower seed oil also showed increase of liver cholesterol. Rats on "fat-free" diet showed increase in serum cholesterol but no change in liver cholesterol. When Purina chow-butter diet was supplemented

‡ Reported in *Fed. Proc.*, 1956, 550.

with cholesterol and propylthiouracil, an even greater increase in both serum and liver cholesterol was found. 2) Separation of serum protein fractions by paper electrophoresis revealed that most of the cholesterol was associated with albumin and alpha globulin fraction in the rat, whereas, in man, the greatest proportion of cholesterol is found in the beta globulin fraction. This same general distribution was observed in all dietary groups regardless of their serum cholesterol levels, although rats receiving butter-cholesterol-propylthiouracil diet showed a slight shift of cholesterol toward the beta globulin fraction. 3) No diets induced a demonstrable change in mast cell count on tissue of rat ear.

1. Keys, A., Anderson, J. T., Fidanza, F., Keys, M. H., and Swahn, B., *Clin. Chem.*, 1955, v1, 34.

2. Keys, A., Anderson, J. T., Mickelsen, O., Adelson, S. F., and Fidanza, F., *J. Nutrition*, 1956, v59, 39.

3. Beveridge, J. M. R., Connell, W. F., Mayer, G., Firstbrook, J. B., and DeWolfe, M., *Circulation*, 1954, v10, 1954.

4. Ahrens, E. H., Jr., Blankenhorn, D. M., and Tsaltas, T. T., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 872.

5. Ahrens, E. H., J., Tsaltas, T. T., Hirsch, J., and Insull, W., *J. Clin. Invest.*, 1955, v34, 918.

6. Bronte-Stewart, B., Antonis, A., Eales, L., and Brock, J. F., *Lancet*, 1956, v270, 521.

7. Kinsel, L. W., Partridge, J., Bolling, L., Margen, S., and Michaels, G., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 909.

8. Keys, A., Anderson, J. T., and Grande, F., *Lancet*,

9. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., *J. Biol. Chem.*, 1952, v195, 357.

10. Anderson, J. T., and Keys, A., *Clin. Chem.*, 1956, v2, 145.

11. Williams, F. G., Jr., Pickles, E. G., and Durrum, E. L., *Science*, 1955, v121, 829.

12. Brown, H. H., Zlatkis, A., Zak, B., and Boyle, A. J., *Anal. Chem.*, 1954, v26, 397.

13. Foldes, F. F., and Wilson, C., *ibid.*, 1950, v22, 1210.

14. Smith, E. W., and Atkinson, W. B., *Science*, 1956, v123, 941.

15. Fidanza, A., and Cresta, M., *Boll. Soc. Ital. Biol. Sper.*, 1955, v31, 1108.

16. Alfin-Slater, R. B., Aftergood, L., Wells, A. F., and Deuel, H. J., *Arch. Biochem.*, 1954, v52, 180.

17. Page, I. H., and Brown, H. B., *Circulation*, 1952, v6, 681.

18. Bossak, E. T., Wang, C. I., and Adlersberg, D., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 637.

Received January 16, 1957. P.S.E.B.M., 1957, v94.

Experimental Production of Tuberculous Pericarditis.* (23028)

R. W. POSTLETHWAIT, J. R. WILSON, M. H. SALEM, D. C. GROSSKREUTZ, W. C. SEALY, AND D. T. SMITH

Departments of Thoracic Surgery and Microbiology, School of Medicine, Duke University, and Veterans Administration Hospital, Durham, N. C.

We were interested in producing tuberculous pericarditis in animals to study frequency of development of the constrictive phase, to determine the effect of antituberculous drugs and to investigate the proper time for pericardiectomy. Prior to this study, no reference could be found concerning experimental tuberculous pericarditis, but after completing our work the report of Kinoshita(1) was found. Kinoshita produced tuberculous pericarditis in rabbits with or without preceding sensitization, and his results were similar to those noted below.

Procedure. New Zealand white rabbits weighing approximately 5 kg were sensitized by subscapular injection of 1 cc dried heat killed bovine tubercle bacilli. Three mg of the ground material were suspended in each cubic cm of Bayol F. From 6 to 12 weeks later, skin tests with commercial tuberculin were carried out and approximately one-third of the original group discarded because of negative skin tests. In the remaining sensitive animals, 0.5 cc of milky suspension of

* Supported in part by grant from N. Car. Medical Fn.