3. Siegel, M., Cliffton, E. E., *J. Appl. Physiol.*, 1957, v10, 289.

4. Alkjaersig, N., Fletcher, A. P., Sherry, S., *J. Biol. Chem.*, 1958, v233, 86.

5. Kline, D. L., Fishman, J. B., *Ann. N. Y. Acad. Sci.*, 1957, v68, 25.

6. Ablondi, F. B., Hagan, J. J., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 195.

7. Sale, E. E., Priest, S. G., Jensen, H., *J. Biol. Chem.*, 1957, v227, 83.

8. Mullertz, S., *Biochem. J.*, 1955, v61, 424.

9. Troll, W., Sherry, S., *J. Biol. Chem.*, 1955, v213, 881.

10. Meyers, W. M., Burdon, K. L., *Am. J. Physiol.*, 1957, v190, 303.

Received August 24, 1959. P.S.E.B.M., 1959, v102.

Serum Lipoproteins and Glycoproteins in Aminopterin Treated Rats.* (25389)

KRISHNASUDHA ROHATGI AND SACHCHIDANANDA BANERJEE†
Dept. of Physiology, Presidency College, Calcutta, India

We observed(1) alterations in serum protein patterns of aminopterin treated folic acid deficient rats. Folic acid is involved in lipid metabolism(2-4). Lipids and the bulk of carbohydrates in blood are transported in association with proteins. The nature and composition of these lipoproteins and glycoproteins might reflect basic changes in metabolism and thereby give information on the nature of these changes. In the present investigation different electrophoretic components of serum glycoproteins and lipoproteins were determined in aminopterin-treated folic acid deficient and pair-fed normal rats. Total lipid, free cholesterol, ester cholesterol, phospholipid and neutral fat were also estimated in the serum of these animals.

Methods. Selection of rats, production of folic acid deficiency by intraperitoneal injection of aminopterin and collection of blood samples from carotid artery were the same as described previously(1). Only clear serum was used. Total glycoproteins in serum were determined by the method of Weimer and Moshin(5) as modified by Winzler(6). Cholesterol was estimated by the method of Sobel and Meyer(7). Phospholipid was estimated by determination of P of the serum extract(8) and multiplying the value by 25(9). Total serum lipid was determined by the method of Bragdon(10). Neutral fat was calculated by subtracting the figure for total cholesterol and phospholipid from the value for total lipid. Ionographic separation of lipoprotein and glycoprotein fractions of sera were carried out by the paper electrophoretic technic described earlier(1,11). For glycoproteins .01 cc and .04 cc serum were applied on different parallel paper strips and electrophoresis continued for 16 hours with a current of 4 ma and 200 volts in a barbiturate-barbituric acid buffer of pH 8.6 and ionic strength .05. The strip with .01 cc serum was stained with bromophenol blue for proteins, and used as reference strip in subsequent measurement of polysaccharides. Strips with .04 cc serum were stained for glycoproteins by the method of Koiw and Gronwall(12), modified by Roboz *et al.*(13), and based on histochemical technic described by Hotchkiss(14). Polysaccharides were indicated by violet bands. Densitometric evaluation of strips was performed immediately after staining, as the color was not permanent and the unstained portion of the paper turned pink after exposure for several hours in air. The densitometric curves of the different glycoprotein bands were measured by planimeter. For lipoproteins, the prestaining procedure of McDonald and Bermes(15) was followed. .05 cc of stained serum for lipoprotein and .005 cc of serum for protein determination were applied to parallel adjacent strips and electro-

* We are indebted to Lederle Laboratories (India) Ltd. for gift of aminopterin. Ministry of Scientific Research & Cultural Affairs, Govt. of India, granted a National Research Fellowship to KR.

† Present address: Prof. of Physiology, Medical College, Bikaner, India.

TABLE I. Serum Lipoproteins and Glycoproteins in Rats (mg/100 cc).

	Normal (5)	Aminopterin treated (5)	"t"
α-lipoprotein	82 ± 22*	85 ± 18	.11
β- "	153 ± 23	319 ± 21	5.35
"O" fraction	227 ± 18	444 ± 31	6.03
Free cholesterol	12 ± 2	28 ± 2	6.40
Ester "	26 ± 3	40 ± 1	4.8
Phospholipid	183 ± 26	274 ± 76	1.1
Neutral fat	241 ± 45	506 ± 72	3.1
Total lipid	462 ± 30	848 ± 59	5.8
α_1-glycoprotein	98 ± 8	105 ± 8	.61
α_2- "	28 ± 2	27 ± 4	.25
β- "	47 ± 7	43 ± 3	.55

* Mean ± S.E. Figures in parentheses indicate No. of animals.

phoretic separation continued for 5 hours at 4 ma and 200 v. in a barbiturate-acetate buffer of pH 8.6. A better resolution with barbiturate-acetate buffer than barbiturate-barbituric acid buffer was observed. The lipoprotein zones appeared blue against a white background. The ionograms were scanned immediately after drying since the patterns were observed to fade with time, and areas of the different zones were measured. Swahn(16) had shown that uptake of Sudan Black B by whole serum on filter paper was correlated with total lipid content of serum. Total area of the plotted curve was, therefore, equivalent to total serum lipid content of sample used for electrophoresis as determined by the chemical method. Glycoprotein and lipoprotein concentrations of different fractions were calculated from total polysaccharides and lipid contents respectively. The significance of deviations from normal mean values was computed by the "*t*" test.

Results are given in Table I. Aminopterin treated rats showed significant increase in total lipid, free cholesterol, esterified cholesterol and neutral fat. Phospholipid values did not change. There was no change in the α-fraction but an increase in the β and O-fraction of serum lipoproteins. Glycoproteins showed 3 components migrating with the mobility of α_1, α_2 and β globulins as observed in man, dog and rabbit(17). There was no significant change in distribution of different glycoprotein fractions and in total glycoproteins in aminopterin treated rats.

Discussion. Hyperlipemia and hypercholesterolemia observed in the aminopterin treated folic acid deficient rats might be due to diminution of serum albumin(1,18) or to the lipotropic effect of folic acid(2,3). It appears that in folic acid deficiency there is a shift in distribution of fatty acids with a relative increase in neutral fat, free and esterified cholesterol. Folic acid accelerates methyl group neogenesis(19-22) and its deficiency is likely to affect the supply of choline and other methyl donors leading to changes in blood serum lipids. Lipids found in the "O" band are associated only to a small extent with γ-globulin. They consist mainly of substances which have been adsorbed on filter paper such as chylomicrons containing neutral fat and β-lipoproteins(23). The observed increase in O-fraction is possibly due to increased level of neutral fat which is also confirmed by chemical estimation. Serum cholesterol is mainly present in the β-lipoprotein(24). Increased serum cholesterol of aminopterin treated rats is possibly responsible for the increased β-lipoproteins as observed by paper electrophoresis. In contrast to lipids, distribution of protein bound carbohydrates did not change in aminopterin-treated rats. The magnitude of changes in distribution of serum lipids indicated more severe disturbances of lipid metabolism than their metabolism of carbohydrate in aminopterin-treated rats.

Summary. Different fractions of serum lipoproteins and glycoproteins were determined by paper electrophoresis in aminopterin-treated folic acid deficient and paired fed normal rats. Total lipid, free cholesterol, ester cholesterol and neutral fat were also estimated in serum of these animals. In aminopterin-treated animals there was significant increase in β-lipoprotein and O-fraction, no change in the α-lipoprotein and increase in total lipid, neutral fat and free and ester cholesterol. There was no change in glycoprotein fractions of serum. The lipid metabolism is much more disturbed than carbohydrate metabolism in folic acid deficiency.

1. Rohatgi, K., Banerjee, S., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 233.

2. Drill, V. A., *Ann. N. Y., Acad. Sci.*, 1954, v57, 654.

3. Kelly, B., Totter, S. R., Day, P. L., *J. Biol. Chem.*, 1950, v187, 529.

4. Dinning, J. S., Neatrour, R., Day, P. L., *ibid.*, 1954, v209, 717.
5. Weimer, H. E., Moshin, J. R., *Ann. Rev. Tuberc.*, 1953, v68, 594.
6. Winzler, R. J., *Method of Biochem. Analysis*, 1955, v2, 279.
7. Sobel, A. E., Mayer, A. M., *J. Biol. Chem.*, 1945, v157, 255.
8. Fiske, C. H., SubbaRow, *ibid.*, 1925, v66, 375.
9. Youngburg, G. E., Youngburg, M. V., *J. Lab. Clin. Med.*, 1930, v16, 158.
10. Bragdon, J. H., *J. Biol. Chem.*, 1951, v90, 513.
11. Banerjee, S., Chatterjee, K. P., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 474.
12. Koiw, E., Gronwall, A., *Scand. J. Clin. Lab. Invest.*, 1952, v4, 245.
13. Roboz, E., Hess, W. C., Forster, F. M., *Arch. Neurol. Psych.*, 1955, v73, 536.
14. Hotchkiss, R. D., *Arch. Biochem.*, 1948, v16, 131.
15. McDonald, H. J., Bermes, E. W., *Biochim. Biophys. Acta*, 1955, v17, 290.
16. Swahn, B., *Scand. J. Clin. Lab. Invest.*, 1953, v5, Suppl. 9.
17. Sohar, E., Bossak, E. T., Adlersberg, D., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 305.
18. Rosenman, R. H., Friedman, M., Byers, S. O., Smith, M. K., *J. Clin. Invest.*, 1956, v35, 522.
19. Totter, J. R., Kelley, B., Day, P. L., Edwards, R. R., *Fed. Proc.*, 1950, v9, 238.
20. Plant, G. W. E., Belheil, J. G., Lardy, H. A., *Abst. Am. Chem. Soc.*, Atlantic City, 1949, 65C.
21. Gordon, M., Ravel, J. M., Eakin, R. E., Shive, W., *J. Am. Chem. Soc.*, 1948, v70, 878.
22. Welch, A. D., Sakami, W., *Fed. Proc.*, 1950, v9, 245.
23. Adlersberg, D., Bossak, E. T., Sher, I. H., Sobotka, H., *Clin. Chem.*, 1955, v1, 18.
24. Oncley, J. L., Gurd, F. R. N., Melin, M., *J. Am. Chem. Soc.*, 1950, v72, 458.

Received September 14, 1959. P.S.E.B.M., 1959, v102.

Serum Amylase Changes Due to Antipancreas Serum. (25390)

P. F. POKORNY, J. F. RANSDELL AND J. T. BARRETT
(Introduced by Frank B. Engley, Jr.)
Depts. of Microbiology and Pathology, School of Medicine, University of Missouri, Columbia

Revival of interest in effects of antiorgan serum on normal animals has been directed largely toward the heart, kidney and thyroid. An additional endocrine organ, the pancreas, has been the subject of 2 conflicting reports. In one study murine pancreas demonstrated no serologic activity(1), in the other(2) it was shown that rat pancreas stimulates production of precipitating antisera in rabbits. The latter report suggested structural peculiarities of rat pancreas were in part responsible for its poor antigenicity, and the poor concentration of radiolabeled antibody when administered passively. The work presented here shows the discrete guinea pig pancreas is antigenic as determined by *in vitro* and *in vivo* serologic reactions and that its antiserum produces pancreas pathology and alters serum amylase levels.

Materials and methods. After removal of the pancreas from several ether-anesthetized guinea pigs, a 10% w/v homogenate was prepared in cold saline in Waring blendor. Immunization of male rabbits was performed according to Estes(3) with the complete homogenate. When the precipitating titer of undiluted immune serum reached 1:500 (supernatant of 10% suspension diluted 1:50), the rabbits were bled and serum collected. In view of ultimate use of antisera, a biologic assay seemed desirable and the passive cutaneous anaphylactic (PCA) method of Ovary (4) was chosen. Four hours after dermal sensitization with 0.1 ml of different antisera, 1 ml of a 50-50 mixture of 1% Evans blue and supernatant from the 10% pancreas extract was given intracardially. Reactive sites were measured after 45 minutes. To determine if injections of antiserum into normal guinea pigs would induce pathologic or enzyme changes, 2 intraperitoneal injections of 5 ml of pooled antiserum were given, one the same day and one the 4th day after base level serum amylase determinations were completed(5). On 5th day, serum amylase was again determined, the animals sacrificed, and the pan-