IDIOPATHIC HYPERLIPEMIA: METABOLIC STUDIES IN AN AFFECTED FAMILY *

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The clinical syndrome of idiopathic hyperlipemia is characterized by lactescent blood plasma in the postabsorptive state. The milky appearance of the plasma is caused by its abnormally high content of triglyceride-rich lipoproteins of high molecular weight. Omission of fat from the diet of such patients decreases the concentration of triglycerides, suggesting that defective removal of exogenous fat from the circulation may be an important metabolic abnormality in this syndrome.

The heparin-activated enzyme, lipoprotein lipase, is thought to be concerned with removal of newly absorbed fat from the blood. The basis for this assumption rests on the accelerated removal of chylomicrons from the blood after injection of heparin, which liberates the enzyme into the circulation, resulting in lipolysis in the blood plasma itself (1); the retarded removal of chylomicrons from the blood after administration of antiheparin agents such as protamine sulfate (2); the preferential hydrolysis by the enzyme of triglycerides associated with lipoproteins (3); and the rapid conversion of the fatty acids of intravenously injected chylomicrons into circulating free fatty acids (4). A deficiency of lipoprotein lipase would be expected to result in excessive and prolonged chylomicronemia after ingestion of fat, and thus might be a basic cause of idiopathic hyperlipemia.

We undertook the present investigation to examine this hypothesis and to study lipid transport in the blood of three hyperlipemic siblings of a family of eight, who were previously described by Gaskins, Scott and Kessler (5). The results suggest that a deficiency of lipoprotein lipase is the cause of the hyperlipemic state in the affected siblings.

METHODS

Subjects. The subjects, who were members of one family, were W.P (father), D.P. (mother), F.P. (daughter), C.P. and A.P. (normal sons) and L.P., J.P. and P.P. (hyperlipemic sons). For purposes of comparison two unrelated men with idiopathic hyperlipemia were also studied. (For clinical data, see the Appendix.) Physicians and healthy volunteers hospitalized at the Clinical Center served as control subjects.

Procedures. The five hyperlipemic subjects, all males, were hospitalized in the Clinical Center and given measured diets designed to maintain their weight and containing 1 g of fat per kg of body weight per day. The diet was altered by varying the carbohydrate and fat content; calories were kept constant at the initial level unless otherwise noted. For studies of the effects of a single load of fat, the subjects were given a standard meal containing 1.5 g of fat per kg of body weight in the form of bacon, eggs and oleomargarine, but practically no carbohydrate (6).

Venous blood samples were taken routinely about 15 hours after the last meal. Serum was obtained from blood clotted at room temperature for lipid and lipoprotein analyses. Samples for estimation of plasma free fatty acids were mixed with solid sodium oxalate, 1.5 mg per ml of blood, and placed immediately in ice water. Samples taken after injection of heparin [1] (post-heparin samples) were centrifuged rapidly at 2° C and the plasma was extracted immediately thereafter. Blood samples for estimation of lipoprotein lipase activity were mixed with disodium ethylenediamine tetraacetate, 1 mg per ml of blood, and placed immediately in ice water.

Analytical methods. Blood serum was extracted in ethanol: acetone (1:1) or chloroform: methanol (2:1) and analyzed for total and free cholesterol (7), lipid phosphorus (8) and total lipids (9). Phospholipids were estimated as lipid phosphorus × 25, and triglycerides were calculated by difference (6). Lipoproteins were separated into three fractions at densities 1.019 (very low density, including chylomicrons), 1.063 (low density) and 1.21 (high density) in the preparative ultracentrifuge and analyzed as described previously (10). Free fatty acids were determined as described in previous publications (11, 12). Glycerol was measured by a modifica-

* Portions of this work were presented to the American Society for the Study of Arteriosclerosis (Circulation 1955, 12, 485) and at the Third International Conference on Biochemical Problems of Lipids, Brussels, July 1956 (The Blood Lipids and the Clearing Factor; Lederberg/Ghent, Erasmus, 1956, pp. 265–273).

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[1] Heparin sodium, 1,000 USP units per ml, Upjohn Co.

TABLE I

Serum lipoprotein fractions in P. family

Fraction	Total cholesterol	Phospholipids	Protein
	mg/100 ml	*mg/100 ml*	*mg/100 ml*
Very low density (< 1.019)			
L.P. } (hyperlipemic	216	220	79
J.P. } brothers)	341	298	75
P.P. }	401	416	93
D.P. (mother)	17	24	17
W.P. (father)	60	64	39
Healthy young men	26 (14–36)	31 (15–42)	~12
Low density (1.019–1.063)			
L.P.	9	19	30
J.P.	14	20	27
P.P.	5	13	30
D.P.	198	130	101
W.P.	162	108	105
Healthy young men	97 (61–133)	69 (45–79)	~62
High density (1.063–1.21)			
L.P.	4	36	81
J.P.	9	25	65
P.P.	5	25	69
D.P.	57	92	170
W.P.	30	68	134
Healthy young men	43 (31–48)	~84	~150
Residual (> 1.21)			
L.P.	0	18	
J.P.	0	25	
P.P.	0	17	
D.P.	0	22	
W.P.	0	27	
Healthy young men	0	~27	

tion of the method of Lambert and Neish (13).[2] Unless otherwise noted, optical clearing of plasma was measured as follows: reagents and plasma were warmed to 37° C; 0.1 ml of 0.5 per cent coconut oil emulsion (made by diluting Ediol, Schenley Laboratories), 0.1 ml of 0.5 per cent disodium ethylenediamine tetraacetate, 0.3 ml of saline-phosphate buffer (pH 7.4, ionic strength 0.1), and 0.5 ml of plasma were mixed in a colorimeter tube and placed in a water bath at 37° C. Serial readings of optical density were made in a Coleman, Jr. spectrophotometer at 500 mμ. Respiratory quotient was measured after subjects had fasted overnight.

RESULTS

Nature of the lipemia

The total lipid concentration of serum from the hyperlipemic siblings when on their usual diet was 3,000 to 5,000 mg per 100 ml. When the serum was kept at 3° C for a few days, the turbid material separated as a creamy layer, leaving an optically clear subnatant fluid which contained only 100 to 200 mg of lipid per 100 ml. Table I shows the distribution of certain constituents of lipoprotein fractions separated from these sera and from sera obtained from the subjects' parents and from healthy 20 to 30 year old men (10). In Table II the composition of the very low density lipoprotein fraction (14) in the sera of the three brothers and of the two unrelated men with idiopathic hyperlipemia is compared with that of chylomicrons isolated by flotation from pooled serum obtained from the American Red Cross Blood Donor Service. The concentration of very low density lipoproteins in the blood of the hyper-

[2] We are indebted to Dr. Edward Korn for performing these analyses.

TABLE II

Composition of very low density lipoproteins in sera from individuals with idiopathic hyperlipemia

Subject	Component: % by weight				
	Cholesterol esters	Free cholesterol	Phospholipids	Triglycerides	Protein
L.P.	5.3	2.9	6.2	83.4	2.2
J.P.	6.4	3.4	7.0	81.6	1.6
P.P.	6.9	3.3	6.6	81.5	1.7
F.M.	7.9	4.3	12.1	73.5	2.2
L.W.	13.0	4.3	13.6	64.0	5.1
Pooled serum chylomicrons	6.1	3.1	7.1	81.3	2.5

lipemic brothers was markedly increased. The concentration of the low density and high density fractions was considerably reduced, compared with that in normal men, and their content of cholesterol was disproportionately low (Table I). In one of the two unrelated subjects the percentage of cholesterol esters in the very low density lipoproteins was considerably greater than that of chylomicrons; in both, the concentration of phospholipids was greater, while the proportion of triglycerides was less (Table II).

Effects of diet

Since the results of dietary changes were similar in the three brothers, detailed results of studies on L.P. only are presented (Figure 1). With the initial diet, which contained 1 g of fat per kg body weight [3] per day, serum triglyceride concentration fell rapidly to about 1,300 mg per 100 ml. After 21 days the dietary fat was reduced by 90 per cent. Serum triglyceride concentration fell rapidly to about 250 mg per 100 ml, and the serum became optically clear on Day 24. No significant change was produced by cutting caloric intake in half for five days (Days 28 to 33). Respiratory quotient during this period was 0.73. When the diet containing 1 g of fat per kg per day was resumed (Day 39), triglyceride concentration rose slightly above the previous value. Later (Day 61) fat intake was increased to 3 g per kg per day, and triglyceride concentration rose strikingly to 5,000 mg per 100 ml. The concentration fell dramatically again when the dietary fat content was reduced to 0.5 g per kg (Day 67). Changes in the total and free cholesterol and phospholipid concentrations of the serum paralleled the changes in triglycerides but were less pronounced.

Data on the serum lipoprotein fractions during this experiment are shown in Figure 2. In the very low density lipoprotein-chylomicron fraction the concentrations of total cholesterol, phospholipids and protein followed closely the rise and fall of serum triglycerides. However, their concentrations remained considerably above the normal values shown in Table I. In the low density lipoproteins the changes were in the opposite direction. Alterations in the high density lipoproteins were variable. Total cholesterol concentration changed relatively little; variations in phospholipid concentration could not be correlated definitely with alterations in diet. The concentration of phospholipids in the residual serum proteins was unaltered throughout the study. A comparative study was made on one of the unrelated subjects with

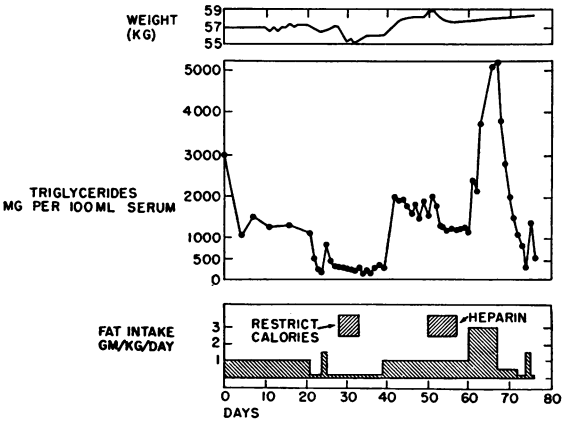

FIG. 1. EFFECTS OF ALTERATIONS IN DIET AND ADMINISTRATION OF HEPARIN, 200 MG SUBCUTANEOUSLY EVERY 12 TO 24 HOURS, ON SERUM TRIGLYCERIDE CONCENTRATION IN HYPERLIPEMIC SUBJECT L.P. A single fat-rich meal was fed on Days 23 and 74.

[3] Hereafter written as g per kg (of body weight understood).

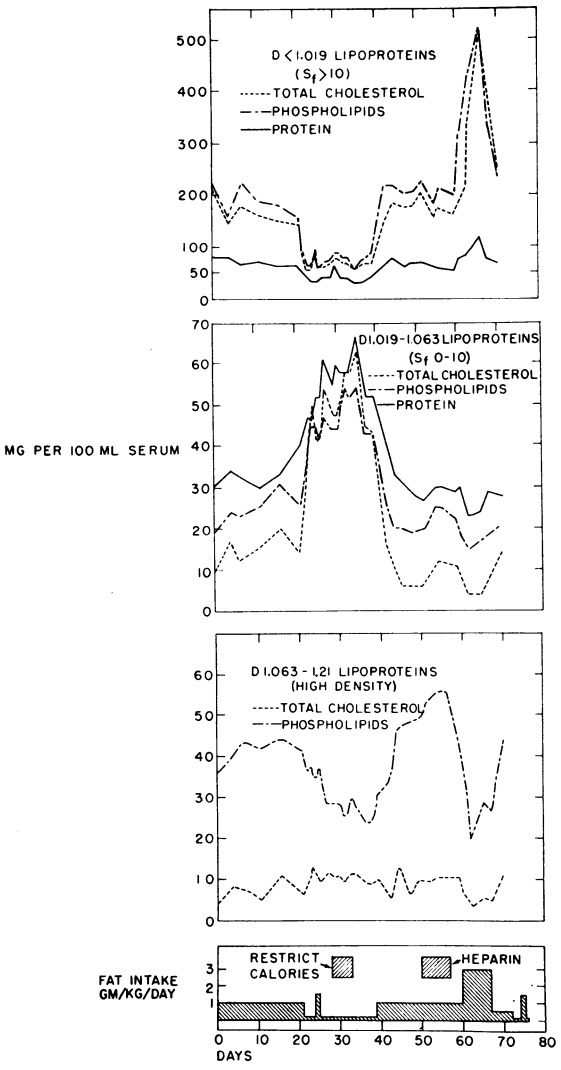

FIG. 2. EFFECTS OF ALTERATIONS IN DIET AND ADMINISTRATION OF HEPARIN ON CHEMICAL CONSTITUENTS OF THREE SERUM LIPOPROTEIN CLASSES IN HYPERLIPEMIC SUBJECT L.P.

idiopathic hyperlipemia. As shown in Figure 3, reduction of fat intake was associated with a fall in triglyceride concentration and reciprocal changes in cholesterol concentration in the very low and low density lipoprotein fractions. Serum cholesterol concentration remained practically unchanged.

Effects of single loads of fat

After a period of stabilization on the diet containing 0.1 g of fat per kg per day, the subjects were fed single meals containing 1.5 g of fat per kg. The results of a representative study are

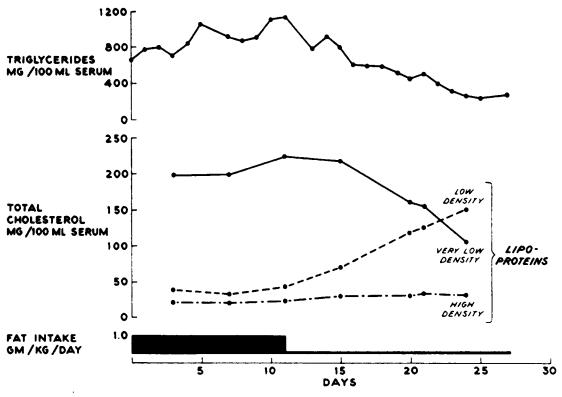

FIG. 3. EFFECT OF ALTERATIONS IN DIET ON TRIGLYCERIDE CONTENT OF SERUM AND ON CONCENTRATION OF TOTAL CHOLESTEROL IN THREE SERUM LIPOPROTEIN CLASSES IN HYPERLIPEMIC SUBJECT L.W.

shown in Figure 4. In the affected siblings such loads of fat resulted in inordinate increases in serum triglyceride concentration, which persisted for two or three days. Smaller, but significant increases occurred in cholesterol and phospholipids (Figure 4). In Subject L.P. even greater changes took place after ingestion of fat in the form of corn oil. Triglyceride concentration rose from 270 to 1,190 mg per 100 ml in 8 hours and to 1,340 mg in 24 hours; after 48 hours it had dropped to 500 mg per 100 ml. These effects resulted from alterations in the amount of very low density lipo-

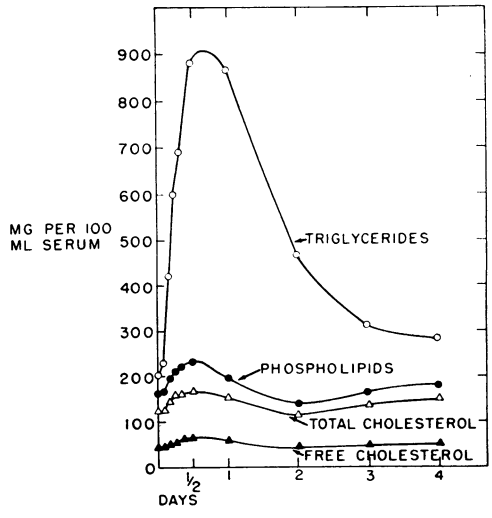

FIG. 4. EFFECT OF A SINGLE MEAL CONTAINING 1.5 G OF CREAM FAT PER KG OF BODY WEIGHT ON SERUM LIPID CONSTITUENTS IN HYPERLIPEMIC SUBJECT L.P. Subject's diet throughout test period contained less than 0.1 g of fat per kg of body weight per day.

proteins; the only other change was a small increase (10 mg per 100 ml or less) in phospholipids of high density lipoproteins.

Effects of administration of heparin

Subject L.P. was given 230 mg of heparin intravenously over a 12 hour period, followed by subcutaneous injections of 200 mg of heparin in aqueous solution (200 mg per ml) every 12 to 24 hours for seven days (Days 50 to 57, Figure 1). His diet during this time contained 1 g of fat per kg. Serum triglyceride concentration decreased slightly after the first three days of the experiment. The decrease appeared to be caused by heparin; it was, however, associated with a slight loss of weight, and no further change occurred in the three days after cessation of heparin during which intake of fat was constant.

Plasma free fatty acid (FFA) concentration

Plasma FFA concentrations were determined in samples taken after the subjects had fasted overnight. The concentrations in sera from the three brothers and the two other hyperlipemic subjects were similar and showed no relationship to serum triglyceride concentration (Table III). Similar determinations were made on two separate occasions on samples taken 10 minutes after intravenous administration of 1 mg of heparin per kg to the hyperlipemic subjects. The increases in plasma FFA concentration were much less in the three brothers than in the other hyperlipemic subjects. In two healthy subjects and the mother of the brothers, increases in FFA concentration after administration of heparin were similar to those of the other hyperlipemic subjects.

Removal of very low density lipoprotein from the circulation

To determine whether the mechanism for removing newly ingested fat from the circulation was impaired in the three brothers, the following experiment was performed. L.P. was given a high fat diet for three days; 2 U of blood was then drawn in acid-citrate-dextrose. The plasma contained 5,200 mg of lipid per 100 ml (81 per cent triglycerides), of which 98 per cent was contained in very low density lipoproteins. Two days before this plasma was obtained from L.P., a 23 year old healthy volunteer and J. P. were started on diets

TABLE III

Effect of administration of heparin on plasma free fatty acid (FFA) concentration

Subject	Heparin	Triglyceride fatty acids	FFA	Increase in FFA after heparin
	mg/kg body wt	*mEq/L*	*mEq/L*	*mEq/L*
Normal				
R.H.	0.2	4.2	0.31	0.50
	1.0	3.7	0.54	0.31
R.G.	1.0	4.3	0.37	0.39
Idiopathic hyperlipemia				
L.W.	1.0	24.7	0.73	0.81
	1.0	37.6	0.55	0.69
F.M.	1.0	91.6	0.75	0.52
	1.0	35.3	0.48	0.49
P. family				
D.P.	0.2	5.0	0.90	0.39
L.P.	1.0	10.8	0.26	0.02
	1.0	9.3	0.29	0.09
J.P.	1.0	13.7	0.31	0.08
	1.0	96.6	0.37	0.18
P.P.	1.0	31.1	1.03	0.17
	1.0	16.0	0.34	0.03

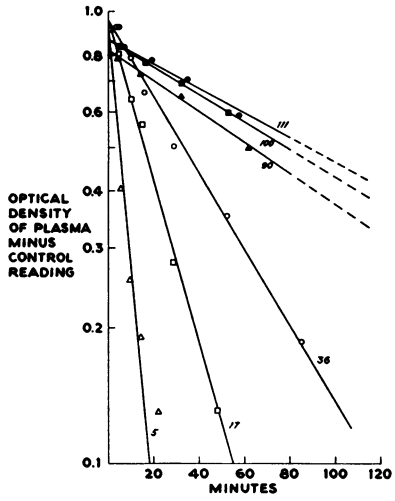

FIG. 5. CHANGES IN OPTICAL DENSITY OF PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION OF LIPEMIC PLASMA FROM L.P. TO HIS HYPERLIPEMIC BROTHER, J.P. (CLOSED SYMBOLS) AND TO A HEALTHY VOLUNTEER (OPEN SYMBOLS). Squares, no pretreatment; triangles, after 0.5 mg of heparin per kg body weight; circles, after 2 mg of protamine sulfate per kg of body weight. Numbers indicate half-times of removal.

containing 0.1 g of fat per kg. On the fourth day of the diet both were given plasma from L.P. (1 ml per kg) intravenously over a 2 minute period; serial blood samples were taken during this time and chilled immediately in ice water. On successive days the procedure was repeated after intravenous administration of 0.5 mg of heparin and 2 mg of protamine sulfate per kg, respectively. Measurements of optical density of the plasma are shown in Figure 5. The turbid lipoproteins were removed rapidly from the circulation of the healthy volunteer; the rate of removal was significantly reduced by administration of protamine. The rate of removal after injection of heparin was much more rapid, although part of the decrease in optical density probably resulted from continuing lipolysis in the drawn blood. In J.P. the rate of lipoprotein removal was much slower and did not appear to be affected by either heparin or protamine. Similar results were obtained in the first transfusion experiment by determination of serum triglyceride concentration in both subjects.[4]

[4] Experiments in dogs have also shown that changes in optical density and triglyceride concentration of plasma after intravenous administration of chylomicrons parallel each other.

Since these measurements are indirect and require subtraction of an appreciable baseline value, the results were considered less precise as an index of the rate of removal of turbid lipoproteins from the blood.

Plasma lipoprotein lipase activity

The preceding studies on the affected brothers suggested either that the lipoprotein lipase liberated by heparin was unable to effect lipolysis of plasma triglycerides or that heparin failed to release the enzyme from body tissues. To determine the cause of the subjects' failure to respond to heparin, the following experiments were carried out.

Substrate studies. While Subject L.P. was receiving the low fat diet, he and a healthy volunteer were given 0.2 mg of heparin per kg intravenously; blood was drawn 10 minutes later. Two aliquots of plasma from each subject were incubated, one with a saline suspension of chylomicrons isolated from pooled serum obtained from the American Red Cross and one with a saline suspension of very low density lipoproteins obtained from L.P. when he was on his usual diet. As shown in Figure 6, both substrates cleared readily

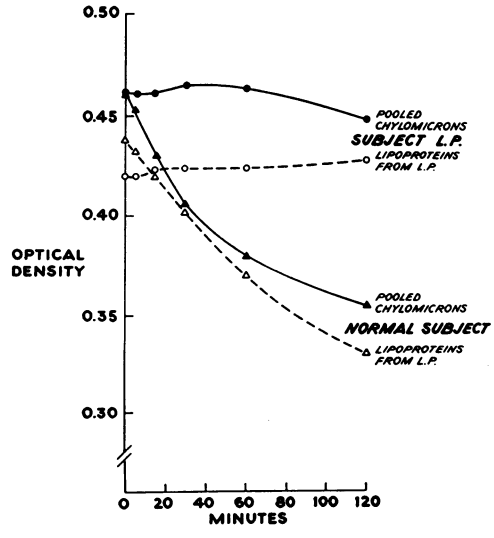

FIG. 6. CHANGES IN OPTICAL DENSITY OF TURBID LIPOPROTEINS DURING INCUBATION WITH POST-HEPARIN PLASMA FROM HYPERLIPEMIC BROTHER L.P. AND A NORMAL SUBJECT. Each incubation tube, containing 0.1 ml plasma and 0.9 ml of a saline suspension of substrate, as noted, was incubated at 37° C; optical density was measured at 500 mμ.

in plasma from the healthy volunteer, whereas neither cleared in plasma from L.P. Similar results were obtained with coconut oil emulsion as substrate on a separate occasion. L.P. was given 1 and the volunteer 0.2 mg of heparin per kg. The standard method of incubation was used. In one hour, 0.50 μmole of glycerol was produced by post-heparin plasma from the healthy volunteer and optical density decreased 0.283 unit. Under the same experimental conditions no glycerol production was detected in post-heparin plasma from L.P. and optical density increased 0.008 unit.

Inhibition studies. To determine whether inhibitory substances in the plasma of the hyperlipemic subjects were preventing optical clearing of triglyceride-rich substrate, the following experiments were performed. Blood samples were drawn before and 10 minutes after administration of 1 mg of heparin per kg to Subjects J.P. and P.P. (lipemics) and of 0.2 mg per kg to D.P. (not affected). Aliquots of both plasma samples were incubated with post-heparin plasma from D.P. as shown in Figure 7. Both pre- and post-heparin plasma from J.P. and P.P. inhibited optical clearing in post-heparin plasma from D.P. The degree of inhibition decreased with time.

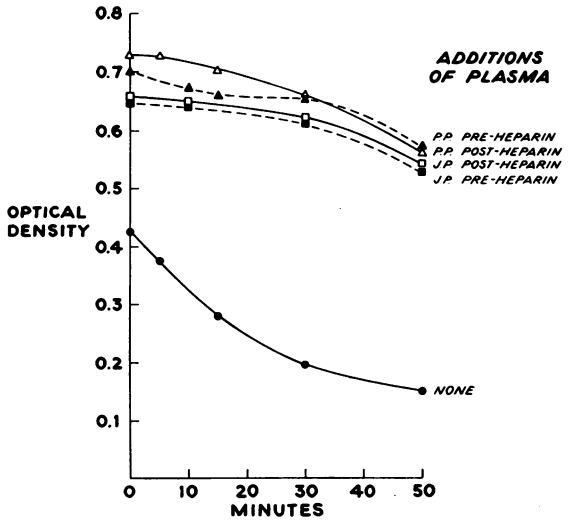

FIG. 7. INHIBITORY EFFECT OF PLASMA FROM HYPERLIPEMIC SUBJECTS ON OPTICAL CLEARING OF COCONUT OIL EMULSION BY POST-HEPARIN PLASMA. Each colorimeter tube contained 0.1 ml of a 0.5 per cent coconut oil emulsion, 0.1 ml of 1 per cent disodium ethylenediamine tetraacetate and 0.25 ml of post-heparin plasma from D.P. (mother); 0.25 ml of pre- or post-heparin plasma from J.P. or P.P. (hyperlipemic sons) was added as indicated, plus saline-phosphate buffer to give a final volume of 1 ml.

A study was then undertaken to determine whether the inhibition resulted from competition between coconut oil and relatively nonturbid, triglyceride-rich lipoproteins in the plasma of the hyperlipemic subjects for the lipoprotein lipase of the post-heparin plasma of D.P. Post-heparin plasma was obtained from hyperlipemic subject L.W. at a time when his plasma (postabsorptive) was only slightly opalescent. A portion of the plasma was centrifuged for 30 minutes at 137,000 × G to remove most of the very low density lipoproteins. Aliquots of the clear infranatant plasma and the uncentrifuged plasma were incubated with coconut oil emulsion. Measurements showed that optical clearing in 60 minutes was 0.215 unit in the tubes containing the infranatant plasma and 0.136 unit in the tubes containing the uncentrifuged plasma; glycerol production was 0.50 and 0.62 μmole, respectively. Thus, lipolysis was not impaired in the presence of very low density lipoproteins as measured by liberation of a product of triglyceride hydrolysis, whereas optical clearing was inhibited considerably. To demonstrate inhibition of optical clearing in post-heparin plasma of a healthy subject by very low density lipoprotein from plasma of a hyperlipemic subject, the following experiment was performed. An aliquot of plasma taken from L.W. before administration of heparin was similarly cleared of very low density lipoproteins. Aliquots of the clear infranatant plasma and uncentrifuged plasma were incubated with plasma obtained from a healthy volunteer 10 minutes after injection of 0.2 mg of heparin per kg. In addition, the post-heparin plasma was incubated alone and with plasma from another healthy subject. As shown in Figure 8, uncentrifuged plasma from L.W. caused the same initial inhibition of optical clearing as that produced by plasma from L.P. and J.P. Clear infranatant plasma from L.W., however, caused only slight initial inhibition of clearing, similar to that produced by plasma from a healthy subject.

Production of free fatty acids in vitro. Since optical clearing proved to be an inadequate measure of lipoprotein lipase activity in hyperlipemic plasma containing turbid lipoproteins, further studies were carried out using production of FFA as a measure of enzyme activity. Pre- and post-

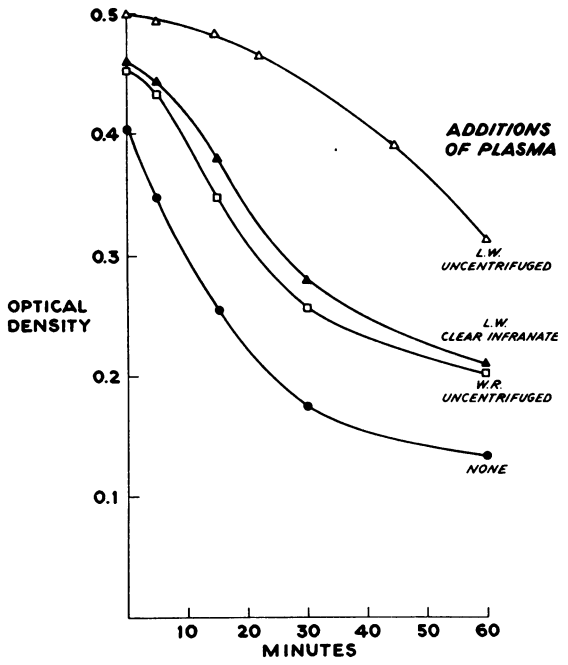

FIG. 8. EFFECT OF ADDITION OF PLASMA FROM HYPERLIPEMIC SUBJECT L.W. AND NORMAL SUBJECT W.R. ON OPTICAL CLEARING OF COCONUT OIL EMULSION BY POST-HEPARIN PLASMA. Experimental conditions were as described for Figure 7.

heparin blood samples were taken from the subjects as described previously. Duplicate 2 ml samples of plasma were mixed with 0.2 ml of 0.5 per cent coconut oil emulsion. Both samples were extracted for FFA analysis, one immediately and the other after incubation at 37° C for 15 minutes. As shown in Table IV, production of free fatty acids was much less in the sera of the three brothers than in the sera of the other hyperlipemic subjects or normal individuals. A study was then carried out to determine the effects on fatty acid production of mixing pre- and post-heparin (0.5 mg per kg) plasma of L.P. and a healthy individual. As shown in Table V, after 15 minutes' incubation, pre-heparin plasma from L.P. caused no inhibition of fatty acid production in post-heparin plasma from the healthy individual, although optical clearing was considerably inhibited. Addition of pre-heparin plasma from the healthy individual to post-heparin plasma from L.P. failed to increase the production of fatty acids.

TABLE IV

Production of free fatty acids during incubation of plasma in vitro with coconut oil emulsion

Subject	Increase in free fatty acids	
	Pre-heparin	Post-heparin
	mEq/L	
Normal		
R.H.	−0.03	1.67
R.G.	+0.02	1.81
Idiopathic hyperlipemia		
L.W.	+0.04	2.30
F.M.	−0.04	1.80
P. family		
L.P.	+0.02	0.24
J.P.	+0.06	0.54
P.P.		0.37

TABLE V

Lipoprotein lipase activity of post-heparin plasma from L.P. and a normal subject

Additions	Experiment number			
	I	II	III	IV
			ml	
0.5% Coconut oil emulsion	0.4	0.4	0.4	0.4
Plasma				
Normal pre-heparin	1.0			1.0
Normal post-heparin	1.0	1.0		
L.P. pre-heparin		1.0	1.0	
L.P. post-heparin			1.0	1.0
			mμ	
Decrease in optical density	0.149	0.070	0.003	0.000
			mEq/L	
Increase in free fatty acids	1.68	1.57	0.12	0.11

Lipoprotein lipase activity in other members of P. family. Blood samples were taken from the father, the mother, the daughter and one son (A.P.) and from a healthy volunteer 10 minutes after intravenous injection of 0.2 mg of heparin per kg. In all cases the post-heparin plasma caused rapid optical clearing of coconut oil emulsion and equivalent production of glycerol (about 0.5 μmole per hour).

Fatty acid mobilization

Since mobilization of fat stores during caloric restriction had resulted in no increase in serum lipid concentrations, studies were undertaken to determine whether mobilization of FFA was carried out normally in the hyperlipemic subjects. FFA concentration was no different in L.P., J.P., and P.P. than in normal subjects (Table III). Results of oral glucose tolerance tests were normal in all the hyperlipemic brothers except P.P., who

showed no rise in blood sugar after orally administered glucose and a normal curve in the intravenous test. In all subjects FFA concentration fell normally (11) during the glucose tolerance test.

DISCUSSION

The major defect in lipid metabolism in the three siblings described in this report appears to be a greatly diminished ability to remove newly absorbed fat (chylomicrons) from the circulation. The lipoproteins present in increased concentration in their blood had the physical and chemical characteristics of chylomicrons, and their concentration in plasma varied directly with the fat content of the diet. Furthermore, at a time when fasting serum triglyceride concentrations were only slightly elevated, a fat-rich meal produced an abnormally intense and prolonged lipemia; also, the rate of removal of intravenously administered chylomicron-like lipoproteins from the blood was greatly impaired. It was necessary to lower plasma triglyceride concentrations prior to performing these tests to reduce the size of the "pool" of triglycerides with which the absorbed or injected triglycerides were mixing, since an expanded pool might alter the rate of removal of added triglycerides in spite of a normal or even increased total removal rate.

Almost total elimination of fat from the diet of these subjects failed to reduce the concentration of triglycerides to normal. This finding, however, does not establish the presence of an additional defect in lipid transport, since diets high in carbohydrate and low in fat can cause an increase in triglyceride concentrations in healthy persons (15). The possibility remains, however, that a defect in removal of endogenously-produced triglycerides from the blood is also present. The most likely source of such endogenous triglycerides is the liver, since in hepatectomized dogs the appearance of labeled triglycerides in plasma after parenteral administration of labeled acetate (16) and long chain fatty acids (17) is practically eliminated.

The cause of the reduced concentrations of low density and high density lipoproteins is not entirely clear. Low density lipoproteins contain, in part, the same protein as very low density lipoproteins (18). In the hyperlipemic subjects in this study, as well as in subjects with other hyperlipemic states (19, 20), the concentration of low density and very low density lipoproteins was inversely related. Thus, the low density lipoproteins may serve, at least in part, as "building blocks" for very low density lipoproteins rich in triglycerides or, alternatively, may be liberated by their breakdown. Also, the protein moiety of high density lipoproteins is known to be contained in chylomicrons (18), so that an inverse relationship between the concentrations of chylomicrons and high density lipoproteins might be expected. In this study, however, high density lipoprotein concentration did not increase when the subjects' diets were low in fat. The higher concentration of high density lipoprotein phospholipids when the subjects' intake of fat was normal or high was similar to the increase noted in healthy individuals after ingestion of fat (6), although less marked.

The effects of heparin in the affected siblings differed greatly from its effects in healthy persons and in other subjects with idiopathic hyperlipemia, as noted in this and other studies (21–25). The very low density lipoproteins in their plasma were indistinguishable from normal chylomicrons in susceptibility to lipolysis, as well as in chemical composition. Also, the rate of removal of these lipoproteins from the blood of a healthy subject was similar to that of chylomicrons injected intravenously in dogs (4). In the other hyperlipemic subjects the very low density lipoproteins had a higher proportion of constituents other than triglycerides, and mean particle size presumably was smaller. Such lipoproteins probably would be cleared from the circulation more slowly (26) than those of the affected brothers and might be less susceptible to lipolysis, as in the case described by Carlson and Olhagen (25).

The present studies demonstrated that clearing activity of plasma is an unsatisfactory measure of the lipolytic activity of post-heparin plasma containing appreciable quantities of very low density lipoproteins which are less turbid than the substrate used for the test. This phenomenon was first pointed out by Brown, Boyle and Anfinsen (27). Defective lipemia clearing activity in post-heparin plasma has been noted in a variety of hyperlipemic states; for example: in hyperlipemia induced in rats by administration of alloxan (28);

in rabbits by administration of uranium acetate (29) and cortisone (30) and after excessive bleeding (31); and in man, in the nephrotic state, glycogen storage disease and idiopathic hyperlipemia (32). Klein and Lever, in a series of studies (32–34), found that the inhibitory material was associated with very low density lipoproteins. They also reported that after administration of heparin, serum glycerol levels rose less in hyperlipemic subjects than in normal subjects. Robinson and Harris (31), however, found no inhibition of glycerol production in post-heparin plasma of rabbits after excessive bleeding, and Day and Peters (30) noted that free fatty acid levels were higher in post-heparin plasma from rabbits made hyperlipemic by cortisone than in similar control samples, despite marked inhibition of *in vitro* clearing in the hyperlipemic plasma. Our studies have demonstrated clearly that the inhibition of optical clearing observed in post-heparin plasma of some subjects with idiopathic hyperlipemia results from the presence of relatively large quantities of very low density lipoproteins and is not associated with deficient lipoprotein lipase activity as measured by production of free fatty acids. Since it has been established that lipoprotein lipase forms an enzyme-substrate complex with very low density lipoproteins, it is quite probable that relatively nonturbid very low density lipoproteins in the plasma of hyperlipemic subjects on fat-restricted diets would effectively compete with added substrate for the enzyme. Such lipoproteins are always present in small quantities in blood plasma of healthy humans and in somewhat greater amounts in plasma of patients with manifest coronary heart disease. Similar inhibition of optical clearing without inhibition of fatty acid production has been observed in post-heparin plasma of patients with coronary heart disease, although to a lesser degree than in idiopathic hyperlipemia (35). It appears unwise, therefore, to base estimates of lipoprotein lipase activity of post-heparin plasma on measurements of optical clearing.

In the affected siblings studied here, defective lipolysis in post-heparin plasma, as measured by production of glycerol and free fatty acids *in vitro*, was striking. In addition, these studies showed that this defect did not result from the presence of inhibitors of lipoprotein lipase activity or from lack of a cofactor necessary for lipolysis. We therefore concluded that heparin failed to release lipoprotein lipase from tissue sites and that the enzyme in tissues was probably abnormal or greatly reduced in quantity. When adequate methods for assaying lipoprotein lipase in human tissues, particularly adipose tissue, become available, this problem can be attacked directly.

The present studies cast further light on the normal function of lipoprotein lipase. They strongly support the concept that this enzyme plays an important role in the removal of chylomicron triglycerides from the circulation. They cast considerable doubt on the concept that it promotes hydrolysis of triglycerides in adipose tissue, with formation of FFA for release into the circulation. Instead, our data suggest that another enzyme may carry out this function. This hypothesis is understandable if lipoprotein lipase is assumed to be located at the capillary wall (1, 36) or cell surface and another lipolytic system in the cytoplasm of the adipose tissue cell.

The removal of chylomicrons from the circulation of the liver probably does not involve lipoprotein lipase activity (1). It is likely, therefore, that in the affected siblings the liver is the major site of removal. This could account for fatty infiltration of the liver found in some subjects with idiopathic hyperlipemia. The findings of splenomegaly and fatty infiltration of the bone marrow could be explained by phagocytosis of some of the chylomicrons by reticuloendothelial cells.

In contrast to the defective removal of triglycerides from their blood, the metabolism of free fatty acids in the affected subjects appeared to be normal. In *L.P.*, the lack of increase in serum triglyceride concentration when dietary fat and calories were restricted suggests that fatty acids were being mobilized from adipose tissue as free fatty acids, in accordance with modern concepts of fatty acid transport, rather than as triglycerides.

It is clear that in most hyperlipemic subjects, like the others studied here, release of lipoprotein lipase into the circulation after administration of heparin is not impaired. The nature of the defect in lipid transport in such subjects is not clear, but the finding that the lipolytic activity of their plasma was normal after administration of heparin does not necessarily imply that the enzyme was normally active in body tissues. The present findings, however, strongly suggest the existence of

more than one causative factor for the clinical syndrome of idiopathic hyperlipemia. The affected siblings in this study differed from other patients with idiopathic hyperlipemia not only in their inability to release lipoprotein lipase into the circulation after administration of heparin, but in a number of other ways (see Appendix). Their lipemia could be characterized as a pure "chylomicronemia"; they had pretibial ulcers but no xanthomata; glucose tolerance was normal; up to the present they have shown no evidence of occlusive vascular disease, despite the presence in their plasma of "chylomicron" concentrations in the range of 3,000 to 5,000 mg per 100 ml for as long as 26 years.

SUMMARY

1. Alterations in lipid transport in blood plasma were studied in three siblings with the clinical syndrome of idiopathic hyperlipemia whose plasma contained abnormal concentrations of very low density lipoproteins which had the chemical and physical characteristics of chylomicrons in normal individuals.

2. The concentration of triglycerides in the blood plasma varied directly with the fat content of the diet, and single fat-rich meals given when the subjects' dietary fat intake was low produced marked increases in serum triglyceride concentration which persisted for 48 hours.

3. The blood plasma of these subjects showed little lipoprotein lipase activity *in vitro* after administration of heparin in doses as high as 1 mg per kg of body weight, and intensive administration of heparin to one of the siblings did not lower plasma triglyceride concentration significantly. The deficient enzymatic activity *in vitro* did not result from the presence of inhibitors in the plasma of the affected subjects or from the absence of a plasma cofactor necessary for lipolysis. The triglycerides in the very low density lipoproteins in their plasma were hydrolyzed readily by lipoprotein lipase in post-heparin plasma from healthy individuals.

4. Triglycerides contained in the very low density lipoproteins of the plasma of one of the affected siblings were removed much more rapidly from the circulation of a healthy subject than from that of another sibling. Administration of heparin accelerated and protamine sulfate diminished the rate of removal in the healthy subject but not in the hyperlipemic sibling.

5. Evidence was obtained to suggest that mobilization of fat in the form of free fatty acids from adipose tissue was unimpaired in the affected siblings.

6. In two other subjects with the clinical syndrome of idiopathic hyperlipemia, administration of heparin produced normal levels of lipoprotein lipase activity in their blood plasma. In contrast to the three siblings, the composition of the very low density lipoproteins in the plasma of these subjects differed from that of chylomicrons, xanthomata were present, and, in one, glucose tolerance was impaired.

7. The results of these studies suggest that a genetic deficiency of lipoprotein lipase is responsible for the defective removal of triglycerides from the plasma of the three siblings. They also suggest that more than one defect can result in the syndrome of idiopathic hyperlipemia.

APPENDIX

Clinical data

P. family. This Negro family consists of the parents, 1 daughter and 5 sons; 3 siblings died in infancy. Consanguinity was denied on the basis that the parents were raised in different counties. No other relatives live in the area.

Postabsorptive lipid concentrations (in milligrams per 100 ml) in these subjects at the time of the study (1955) and, when two values are given, in 1959, are as shown in Table VI.

The parents and 3 younger children live on a small farm and raise much of their own food. The animal protein and fat in their diets are obtained largely from chicken and eggs. The father is said to have bronchiectasis. The mother has been obese and hypertensive for many years and had a cerebral vascular accident in 1959. The 3 unaffected children are all well. Data on the hyperlipemic sons are as follows:

J.P. has had pretibial ulcers since early childhood, which are usually more extensive in the summer. He had attacks of upper abdominal pain associated with nausea and vomiting frequently during childhood, but has had none during the past 4 years. At the time of this study, slight lipemia retinalis was present, but no corneal arcus or xanthomata. The skin at the sites of previous ulcerations on both shins was atrophic and depigmented. Peripheral pulses were strong and equal. Results of laboratory tests were as follows: urinalysis, normal; hemoglobin, 17.2 g; sedimentation rate (Wintrobe), 6 mm per hour; prothrombin time, normal. Serum total protein was 6.1 and albumin 3.5 g per 100 ml. Sulfobromophthalein excretion and alkaline phosphatase were

TABLE VI

Postabsorptive lipid concentrations

	Age, time of study	Sickle* trait	Total cholesterol	Free cholesterol	Phospholipids	Triglycerides
	yrs			*mg/100 ml*		
W.P. (father)	59	+	275	70	284	190
			260	69	246	80
D.P. (mother)	48	−	281	72	260	50
			245	71	241	90
F.P. (daughter)	31	+	190	49	245	90
C.P. (son)	23	?	208	54	241	120
J.P. (son)	22	+	384	177	373	3,770
L.P. (son)	17	+	238	116	291	2,980
A.P. (son)	14	−	209	53	232	130
P.P. (son)	7	+	425	208	505	4,800
			335	166	325	3,960

* Demonstrated by paper electrophoresis.

within normal limits. Electrocardiogram and X-ray film of the chest were normal.

L.P. was hospitalized at the age of 8 because of ulcerations over both legs and was found to have milky serum. Despite skin grafting the ulcers have never healed completely. A low fat diet was advised but not followed. At the time of this study the patient was thin but well developed. Ocular examination showed ptosis of the left eye (result of an old injury) and marked lipemia retinalis, but no corneal arcus. Lipemia retinalis was severe when serum total lipid concentrations were above 4 g per 100 ml, slight at 3 to 3.5 g and absent below 3 g. After the lipemia had cleared, optic fundi were found to be normal. Examination of the conjunctival microcirculation by Dr. Robert Akers at a time when serum total lipid concentration was about 5 g per 100 ml showed normal circulation and no tendency toward fragmentation or "sludging" of the cellular elements. There were no xanthomata. The patient had a superficial weeping ulcer, 3 × 4 cm in diameter, over the right pretibial area and a similar ulcer, 6 × 10 cm in diameter, over the left. Peripheral pulses were strong and equal. The liver edge was palpable just below the costal margin; the spleen was palpable 2 cm below the left costal margin. Urinalysis was normal, except for urine urobilinogen (2.1 Ehrlich units in 2 hours). The patient had a mild normocytic anemia; packed cell volume was 32 per cent; sedimentation rate (Wintrobe, uncorrected) was 51 mm per hour. Serum total protein was 7.9 and albumin 3.7 g per 100 ml. Sulfobromophthalein excretion and bilirubin were within normal limits. Electrocardiogram and X-ray film of the chest were normal. For a 5 month period during this study the patient adhered to a low fat diet; the pretibial ulcer on the right leg healed completely and the ulcer on the left decreased to about half its former size. Three years after the study, he had an attack of abdominal pain lasting 3 days.

P.P. was found to have milky serum at age 2 when he was examined because of the findings in his two brothers. At the age of 3, he developed ulcers over both shins and knees, which healed subsequently. He has had no unusual illnesses. At the time of this study he was well developed and nourished. He had old, healed scars over the elbows, knees and shins. Ocular examination showed severe lipemia retinalis, but no corneal arcus. Optic fundi were entirely normal after clearing of lipemia. The tip of the spleen was palpable. Peripheral pulses were strong and equal. Laboratory tests gave the following results: urinalysis, normal; packed cell volume, 32 per cent; sedimentation rate (Wintrobe, uncorrected), 25 mm per hour; serum total protein, 7.4 and albumin 3.8 g per 100 ml; alkaline phosphatase and bilirubin, normal. An electrocardiogram and X-ray film of the chest showed no abnormalities. Since the termination of the study the patient has had a number of attacks of mild upper abdominal pain.

Comment. The borderline serum cholesterol concentrations in both parents might be attributed to a defect in lipid metabolism, but serum triglyceride concentrations were normal. For the present, it may be assumed that the hyperlipemic brothers are homozygous for a trait carried in single dose by the parents, although a gene mutation cannot be excluded. We have no explanation for the constant occurrence of pretibial ulcers in the affected brothers, although possibly the combination of sickle cell trait and marked hyperlipemia results in local hypoxia, making the skin of this area liable to necrosis after slight trauma.

Other hyperlipemic subjects

F.M., a 44 year old white man, has had skin xanthomata for about 20 years. For 3 years he has also had repeated episodes of fever lasting several days, for which no cause has been found. With each attack there is rapid and complete clearing of his milky serum. Treatment has consisted of dietary fat restriction and administration of thyroid extract, ethinyl estradiol and heparin. For 6 months prior to study he received no treatment, except for irregular restriction of fat intake. At the time of the study he was well developed and slightly obese. Blood pressure was 160/92 (right arm, sitting position). No xanthelasmata were noted; optic

fundi were normal. The liver was smooth and not tender to palpation; its edge was 5 cm below the right costal margin. The spleen was palpable 2 cm below the left costal margin. The patient had a mild acneform eruption over the upper part of his body and several tuberous xanthomata over the dorsal surfaces of the hands. The remainder of the examination was within normal limits. Urine was normal, except for trace reduction. A hemogram was normal. Sedimentation rate (Wintrobe) was 49 mm per hour. Fasting blood sugar was 187 mg per 100 ml. An electrocardiogram and X-ray films of the chest showed no abnormalities.

L.W., a 35 year old white man, developed eruptive xanthomata over the buttocks and forearms 2 years before this study. Hyperlipemia was discovered at that time. Needle biopsy of the liver showed fatty infiltration but no fibrosis. The xanthomata disappeared when dietary fat was restricted. The patient drank alcoholic beverages heavily for 10 years, but stopped completely 8 years ago. For 7 years he has had epigastric pain after meals, which is relieved by milk or alkali. X-ray films have shown a deformed duodenum. At the time of the study he was a large, muscular, slightly obese man. Ocular examination showed slight arteriovenous compression of the retinal vessels, but no corneal arcus. No xanthomata were present. Peripheral pulses were strong and equal. The remainder of the examination was unremarkable. Results of urinalysis and hemogram were normal. Sedimentation rate (Wintrobe) was 20 mm per hour. Serum total protein, albumin, alkaline phosphatase, prothrombin time, bilirubin and lipase were within normal limits. No abnormalities were shown by an electrocardiogram, X-ray film of the chest, Master's test and oscillometric studies of leg circulation.

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