CHANGES IN SERUM LIPOPROTEINS AFTER PARENTERAL FEEDING WITH EMULSIFIED FATS

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After intravenous injection of a 10% emulsion of sunflower oil into dogs a considerable increase in the electrophoretic mobility of both serum lipoprotein fractions was found at the height of the lipemia. During the first few hours after intravenous injection of emulsified fats, mass degranulation of the mast cells (heparinocytes) takes place. This is evidence of increased secretion of heparin by the tissues during parenteral lipemia.

During parenteral feeding of animals with emulsified fats [1, 4-6] high lipid concentrations considerably in excess of the upper normal physiological limits are created in the blood (parenteral hyperlipemia). There is good reason to suppose that lipoproteins as a transportable form of blood lipids must play an important role in the removal of the fat injected parenterally as "synthetic chylomicrons" from the bloodstream and, on this account, they must undergo specific functional changes.

The few investigations into this problem which have been published [11, 12, 15, 16] give an incomplete picture of the character and dynamics of the changes in lipoproteins during parenteral lipemia, and some of the facts described require confirmation. The present investigation was carried out for this purpose.

TABLE 1. Changes in Content of Lipoprotein Fractions in Serum of Dogs after Intravenous Infusions of Emulsified Fats (n=30)

Time of investigation	Statistical index	Content of fractions (%)			Coefficient β/α	Wt. of densitogram fragments (mg)			
		α	β	lipid residue		α+β	α	β	lipid residue
Before infusion	M $\pm m$	60,2 1,6	19,6 0,7	17,7 1,3	0,34 0,02	55,3 2,4	40,3 1,2	13,4 0,7	12,6 1,2
At end of infusion	M $\pm m$ P	37,3 1,7 <0,001	20,9 0,8 >0,2	40,8 2,1 <0,001	0,59 0,04 <0,001	49,8 2,9 >0,1	31,4 2,0 <0,001	17,3 1,1 <0,001	34,1 2,1 <0,001
2 h after infusion	M $\pm m$ P	49,1 2,2 <0,001	24 1 <0,001	25,2 2,4 <0,01	0,52 0,04 <0,001	55,4 3,2 >0,5	36,3 2,4 >0,1	18 1,3 <0,01	18,6 2 <0,02

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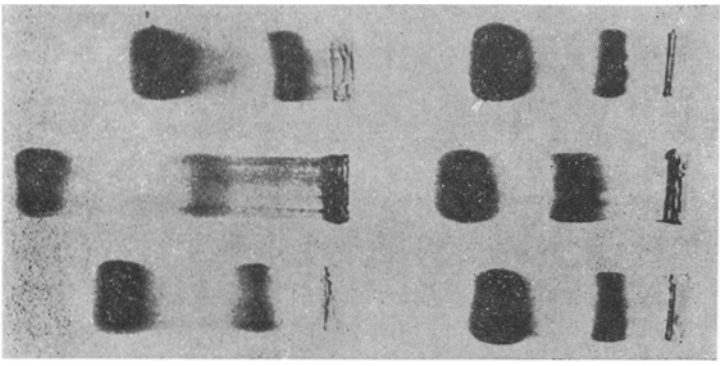

Fig. 1. Electrophoresis of serum lipoproteins of two dogs after a single infusion of emulsified fats. Top row of band – before infusion; middle row – at end of infusion; bottom row – 2 h after infusion.

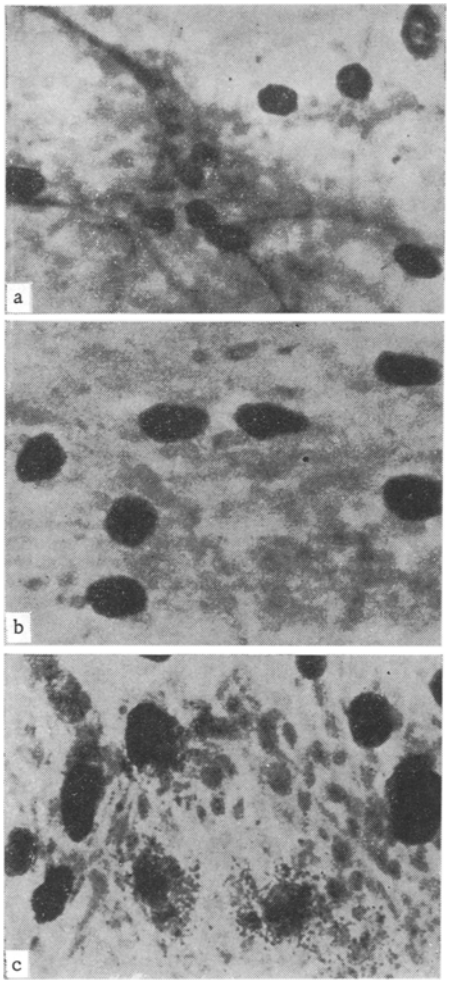

Fig. 2. Morphological changes in mast cells (heparinocytes) of omental lymph glands of rats after intravenous injection of emulsified fats: A) control – compact forms of cells; B) 1 h later – increase in size and granulation of cytoplasm of mast cells; C) 4 h later – marked degranulation of most mast cells. Toluidine blue, 400×.

EXPERIMENTAL METHOD

A highly dispersed nontoxic sterile emulsion of fats containing 10% specially purified sunflower oil, 1.2% lecithin extracted from brain tissue, 5% glucose, and 83.8% pyrogen-free water, prepared in the writer's laboratory, was used. Most of the fat in the emulsion was emulsified to a particle size below 1.5 μ. The emulsion was stable and on keeping at 4-8° it did not separate into layers over a period of several months.

Experiments were carried out on 10 dogs weighing 12-15 kg which received intravenous injections of the emulsified fats by the drip method daily for 3 weeks in a dose of 20 ml/kg body weight at the rate of 3 drops/kg/min. The infusion lasted on the average 2 h. Electrophoretic analysis of the serum lipoproteins was carried out on the 1st, 8th, and 21st days of the experiment. Blood for testing was taken before, at the end of, and 2 h after the infusion.

Electrophoretic fractionation of the lipoproteins was carried out on agar gel by the method suggested for electrophoretic investigation of proteins [13, 9, 8]. The serum was first stained with Sudan black B [7]. The three samples of serum obtained in connection with each infusion of the emulsified fats (before, at the end of, and 2 h after the infusion) were subjected to electrophoresis in parallel tests of the same agar block, so that changes in the electrophoretic mobility of the individual fractions could be clearly seen. The results of electrophoresis were assessed microphotometrically. The relative percentages of the fractions were determined from the weight of the areas of the densitograms corresponding to these fractions cut out of paper.

In view of the known disadvantages of expressing quantities of lipoprotein fractions as percentages, so that the true nature of the changes taking place could be determined their content was also expressed as a conventional gravimetric value. This conventional value was the weight of the fragments of the densitograms cut out from paper as mentioned above. The total content of lipids in the blood was determined gravimetrically.

To study the changes in the heparinocytes experiments were carried out on 28 albino rats. The rats received an intravenous injection of the emulsified fats in a dose of 10 ml/kg, and 1, 2, 3, 4, 5, 6, and 24 h after the injection four rats were sacrificed at each time. Histological films were prepared from the mesentery of the small intestine and the omentum, and after fixation in 10% neutral formalin these were stained with a 0.5% aqueous solution of toluidine blue made up in Michaelis buffer.

EXPERIMENTAL RESULTS

Each isolated infusion of the emulsified fats led to a marked increase in the blood lipid level. Immediately after the end of the infusion the blood serum was white in color (lactemia) and the total lipid content of the serum was more than doubled. In the period after the infusion the lipemia dropped very quickly, and tests made 2 h after the end of the infusion showed that the total blood lipid level had fallen close to its original value.

At the end of infusion (Table 1), i.e., at the height of the lipemia, the content of lipid residue was considerably increased. The α-lipoprotein content was reduced and, as comparison of the conventional weight values shows, this was a true decrease and not due to a redistribution of the relative percentages. An increase in the coefficient β/α was observed and was due, first, to a decrease in the α-lipoprotein content and, second, to an increase in the β-lipoprotein content. The combined content of $(\alpha+\beta)$lipoproteins, as comparison of the conventional weight values shows, was unchanged. This is a paradoxical finding because with an increase in the total lipid content in the blood the lipoprotein content should also have increased.

The most demonstrative and characteristic change in the lipoproteins at the height of the lipemia was an increase in the electrophoretic mobility of the two fractions. The electrophoretic bands at the end of the infusion were between $\frac{1}{4}$ and $\frac{1}{3}$ longer than the electrophoretic bands obtained before injection of the preparation (Fig. 1).

Parallel with the steep drop in the lipemia 2 h after the infusion, the normal electrophoretic picture was restored: the content of lipid residue fell to its initial level, the lipoproteins no longer migrated more rapidly in the electric field, and as a result, the electrophoretic bands were almost indistinguishable in length from those obtained before infusion. The value of the coefficient β/α still remained high, mainly because of an increased content of β-lipoproteins.

Daily infusions of the emulsion for 3 weeks did not give rise to cumulative effects, and on quantitative comparison of the lipoprotein fractions on the first and last days of the experiment no significant differences were found.

From the standpoint of explanation of the pathogenesis of the changes in lipoproteins following parenteral injections of emulsified fats as described above, an interesting paper has been published by Herbst and Hurley [14], who found a similar phenomenon in man after injection of heparin during a high alimentary lipemia. In these cases they observed rapid clearing of the lipemic plasma accompanied by a marked increase in the electrophoretic mobility of both lipoprotein fractions. They postulated that this phenomenon was attributable to activation of the blood lipoprotein lipase, an enzyme reducing the size of lipoprotein molecules, by heparin.

During the clearing of lipemic plasma the consumption of endogenous heparin proceeds at a high rate, as is shown by degranulation of the blood basophils [2]. Investigations of the blood heparin content after intravenous injection of emulsified fats for parenteral feeding have shown [12] that in most people its level is raised. The workers who carried out these investigations concluded that the entry of fat into the blood stream stimulates the secretion of endogenous heparin.

Experiments were carried out on albino rats in an attempt to discover how tissue heparinocytes (mast cells) react to intravenous injection of emulsified fats. Microscopic examination of intestinal mesenteric and omental film from these animals showed that between 2 and 5 h after infusion of the emulsified fats mass degranulation of the heparinocytes (Fig. 2) takes place and that the process does not begin to be reversed until after 6 h.

The changes in the serum lipoproteins found after intravenous infusion of emulsified fats, and, in particular, the phenomenon of their increased electrophoretic mobility, are most probably associated with increased activity of heparin-lipoprotein-lipase (the "clearing factor" of the blood). They indicate the high intensity of the physiological processes leading to evacuation of fat from the bloodstream in parenteral hyperlipemia.

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