Microcirculatory Effects of Emulsified Fat Infusions

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The effects of experimental and clinical hyperlipemia on microcirculation have been considered in discussions and hypotheses regarding the causation of fat embolism, the changes of blood lipids after trauma, and the pathophysiological responses to orally or parenterally administered fat emulsions. The mechanism by which fat embolism develops after traumata, especially fractures, has not been conclusively analyzed. While Adler and Peltier suggested that local fat release from the damaged marrow directly to the circulation is the responsible mechanism, Bergentz in a review postulated that the composition of blood lipids is changed by trauma, leading to microcirculatory disturbances. These are manifested mainly by erythrocyte aggregation.

Parenteral administration of fat emulsions is reported to induce chills, nausea, fever, and intravascular aggregation of erythrocytes. Schubert and Wretlind found that hyperthermia was the main reaction to intravenously infused fat emulsions and emulsifiers in rabbit, dog, and man. The temperature elevations in animals and man were not correlated. An in vitro instability of the fat emulsion in blood and a tendency of the chylomicra to aggregate was observed. This seemed unrelated to the incidence of reactions associated with infusion in man.

Cullen and Swank studied the circulation in the cheek pouch of golden hamsters with alimentary hyperlipemia. They observed inter-

corpuscular adhesiveness of erythrocytes, many of which also stuck to the endothelium. The aggregation tendency increased markedly and in many vessels the rate of blood flow became extremely slow. In some it stopped completely. Some red cells seemed to be covered by a surface film and clumps of platelets, sometimes adhering to red cells, were observed. There was no sign of increased adhesiveness of the white blood cells. The degree of aggregation of red cells did not seem to be related to the degree of lipemia, and aggregation did not depend on length of exposure. Cullen and Swank assumed that the formation of an adhesive envelope around the erythrocytes was the principal cause of the aggregation and of the slowed circulation.

Bergentz et al. observed corpuscular aggregation in the bulbar conjunctival vessels of rabbits after intravenous infusion of fat emulsions. Immediately after infusion there was marked slowing of the capillary circulation and both red and white cells aggregated. The authors concluded that corpuscular aggregation was responsible for the untoward reactions associated with the clinical use of fat emulsions. Swank found a change in shape of erythrocytes in blood samples from rabbits and dogs after a fat meal or after infusion of fat emulsion. The red cells were characterized by rounded protuberances and their diameter was diminished.

An evaluation of the vital microscopic methods used in the investigations discussed above reveals, however, that they probably did not provide adequate degree of resolution and identification of structure to permit analysis of intercorpuscular relations in vivo. Thus the problem remains: do, or do not, the corpuscles of the blood or the chylomicra adhere to each other or to the endothelial wall?
The purpose of the present investigation was to observe the behavior of single blood cells and chylomicra, their relation to each other and to the endothelium by direct microscopy of the living microvascular system in situ. Furthermore, it was intended to obtain some quantitative data on the effect of fat emulsion on the microcirculation.

**Methods**

Microscopic techniques were used in vivo to study preformed connective tissue in ear chambers in ten conscious rabbits and in surgically exposed mesentery in six rabbits under ether anesthesia. These animals, males and females, had been maintained under normal laboratory conditions on a normal diet, had an average age of 12 ± 3 months, and weighed 2.5 ± 0.5 kg. They received infusions of two different fat emulsions,* consisting of (1) cottonseed oil (U.S.P. XVI) 15 g, glucose 4.4 g, purified soybean lecithin 1.2 g, polyoxyethylenoxypropylene 0.3 g, sterile water to make 100 ml or (2) soybean oil 10 g, egg yolk lecithin 1.2 g, glycerol 2.5 g, sterile water to make 100 ml.

The emulsions were infused in a marginal ear vein in a dosage corresponding to 3 g fat/kg body weight, equivalent to 15 ml emulsion/kg, at a rate of 1 to 3 ml/minute. Continuous observations were made up to 5 hours and in the ear chambers the tissues were inspected intermittently up to 48 hours.

The capillary circulation was observed in a

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Leitz intravital microscope in transmitted light, using a modified Berek condenser. Survey views were obtained with 4x (na 0.1) and 10x (na 0.25) objectives and detail views with UO 23 (na 0.55), UO 75 (na 1.0), and UO 100 (na 1.0). Corpuscular flow velocity was determined by an analogue method and microvascular events were recorded by microphotography and microcinematography.

**Results**

I. CONNECTIVE TISSUE IN EAR CHAMBER

In this group nine rabbits received a fat emulsion with 5 μ particles, 3 g/kg body weight being infused at 1 to 3 ml/minute. Six of these animals received emulsion no. 1, and the other three emulsion no. 2. In one rabbit a fat emulsion, differing from the first mainly by containing more 5 μ particles, was infused at 1 ml/minute, a total of 3 g/kg body weight being given.

A typical sequence of events was consistently repeated in the first group (fig. 1). As early as a few minutes after commencement of the infusion stellate cells began to form (fig. 1c). In animals infused at high rates this process had, after some five minutes, progressed to the point where practically all the erythrocytes were stellate. This phenomenon was accompanied by the appearance of spherical chylomicra with a diameter of 1 to 2μ in rapidly increasing numbers, as estimated from consecutive photomicrographs (fig. 1a). After 15 minutes microscopic pictures of the capillary content of chylomicra in situ resembled a dense cloud of mosquitoes (figs. 1a, 2, and 3).

At this stage the flow velocities of the corpuscular elements were markedly retarded in all parts of the capillary bed down to one-fifth of the original value (fig. 1f). Simultaneously the granulocytes began to adhere in increasing numbers to the vascular wall (figs. 1d and 4) and gradually formed a characteristically cobbled layer, particularly in venules. Special attention was paid to intercorpuscular relations between erythrocytes and platelets. These two types of corpuscle moved freely in relation to one another and to the endothelial wall (figs. 1b, e, 2, and 4). In other words, they did not exhibit any tendency toward adhesion that could have reduced their ability to pass along the capillary bed.

After some 30 minutes the chylomicra began to adhere to the endothelial wall (fig. 1a). They then passed through the endothelial membranes out towards neighboring histiocytes, or in some cases lay free in the ground substance. But even before extravasation of chylomicra had begun, some granulocytes had been observed engulfing fat particles while still within the blood vessels.
The adherent granulocytes constituted a partial impediment to blood flow. It was, however, remarkable how easily (and without stickiness) not only erythrocytes and platelets but also free granulocytes passed along the meandering, labyrinthine canal which, at this stage, the interior of many venules resembled.

The rate of corpuscular flow began to increase gradually about an hour and a half after completion of infusion. Another hour later it had returned to approximately the initial speed (fig. 1f). The plasma, however, still contained, after three hours, a comparatively large number of chylomicra of the original size. No coalescence of chylomicra was observed either within blood vessels, or within endothelial cells during passage through the endothelial membranes or outside the endothelium.

The increase in velocity of corpuscular flow was accompanied by detachment of a large proportion of the granulocytes adhering to the endothelium. The released cells seemed entirely normal in shape and size. No alterations of the endothelial wall and no extravasation of granulocytes were observed.

The same picture, in principle, was still present 24 hours after the infusion (figs. 1a, c, d, e), when occasional granulocytes still could be seen adhering to the endothelium and chylomicra were sparse in the plasma but relatively abundant and of unchanged size in endothelial cells, periendothelial cells, and the ground substance. The rate of capillary circulation was, on the whole, normal. Erythrocytes, granulocytes, and platelets moved freely in relation to one another without showing any signs of adhesiveness.

In the single case of an emulsion with more 5 μ particles, the circulating chylomicra at an early stage blocked numerous capillaries completely and permanently; these fat microemboli were markedly rigid (fig. 5). Indeed, in contradistinction to what usually happened when vascular occlusion was due to granulocytes adhering to the endothelium, neither erythrocytes nor platelets could insinuate themselves between the endothelial wall and the obstructing chylomicron. In due course the velocity of corpuscular flow again increased in basically the same manner as in previous experiments, except in the capillary branches completely blocked as described above. It is interesting that not even in such blocked capillary loops, with their almost stagnant content of plasma charged with chylomicra and corpuscles, did the erythrocytes, granulocytes, and platelets display any observable tendency to adhere to one another or to the endothelial wall. It is, however, under stagnant conditions, extremely difficult to evaluate intercorpuscular relations.
II. MESENTERY

In this group five rabbits received a fat emulsion with less than 5 μ particles, 3 g/kg body weight being infused at 1 to 3 ml/minute. Four of these animals received emulsion no. 1, and one animal emulsion no. 2. The circulatory changes and corpuscular behavior observed in this tissue followed the same sequence of events as under section 1. Thus increased stickiness of individual blood corpuscles was not visible in this tissue. Corpuscular flow velocity changes were similar to those described in section 1.

In one rabbit a fat emulsion containing more 5 μ particles, 3 g/kg body weight, was infused at 1 ml/minute. In the case of larger particles some capillaries were, within 15 minutes after the infusion had commenced, totally blocked by chylomicra having a diameter of 10 to 15 μ (fig. 6). The circulation in such vascular loops was completely arrested throughout the observation period of five hours.

**Discussion**

The aim of the present investigation was to study capillary function after intravenous infusion of emulsified fats. The outstanding vital microscopic features (fig. 1) included appearance of stellate erythrocytes, adhesion to the endothelial wall of granulocytes and, simultaneously with appearance of chylomicra in the plasma, a comparatively short period of slowing down of the rate of capillary blood flow. No tendency toward aggregation of erythrocytes or platelets was seen despite a very diligent and systematic search for such signs. In our opinion a twofold mechanism underlies such a decrease in flow velocity: (1) changes in flow properties of plasma due to suspension of chylomicra therein, and (2) mechanical obstruction by granulocytes adhering to the endothelial wall.

Only two specimens, namely one preformed connective tissue in an ear chamber and one mesentery, from rabbits given equal doses of the same type of fat emulsion, displayed blocking of some capillary loops by chylomicra measuring approximately 10 to 15 μ. These fat emulsions which produced blocking were later analyzed, special attention being paid to their stability and particle size. As shown in table 1 they contained more particles measuring 5 μ than were contained by the normal emulsion but did not otherwise differ appreciably. Their contents of 15 μ particles were not increased. Thus this additional study in vitro did not explain the appearance of the blocking 10 to 15 μ chylomicra. After a lapse of three weeks the same rabbit and ear chamber were used for a similar experiment with a different emulsion and on that occasion no such blocking chylomicra were seen. It appears, therefore, that the explanation should be looked for in the oil or its emulsifier rather than in specific susceptibility of the rabbits in question.

After infusion of the normal emulsion (fig. 1a-f) the microcirculation contained at an

<table>
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<tr>
<th>Particle size</th>
<th>&quot;Blocking&quot; emulsion</th>
<th>Normal emulsion</th>
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<tbody>
<tr>
<td>μ</td>
<td>number/mm³</td>
<td>number/mm³</td>
</tr>
<tr>
<td>15</td>
<td>2.3</td>
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<td>5</td>
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<td>3.2</td>
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<td>500</td>
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<tr>
<td>1.6</td>
<td>6100</td>
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* ml 0.1 NaOH required to neutralize free fatty acids in 50 ml emulsion.
early stage numerous chylomicra circulating freely in the plasma. Their size resembled that of the particles in the emulsion. Within approximately 15 minutes after the infusion was started, granulocytes had appeared more or less abundantly. A high proportion of the latter also adhered more or less firmly to the endothelial wall. The freely circulating white corpuscles did not display any increased tendency to adhere to one another, to those corpuscles which were attached to the wall, or to the vascular wall itself. This behavior by the granulocytes may be part of a pyrogenic reaction.4

The number of stellate cells varied with the rate of infusion. This is explained presumably by rapidly reversible osmotic effects ascribable perhaps to the emulsifier. Similar deformation has been observed after local application of hypertonic saline.11 In all experiments the platelets circulated freely. They displayed no interconnections or adhesiveness to one another, to other corpuscles or to the endothelial wall.

Reduction of corpuscular flow velocity was accompanied by an increased ratio of corpuscles/plasma. But however densely packed, erythrocytes should not be considered to be adherent to one another unless observations are made with microscopic resolution adequate to visualize relations between individual corpuscles. Our experiments indicated that the erythrocytes were separate without signs of sticking and also indicated that they slipped past one another in the capillaries without adhering.

These findings differ from those reported by Bergentz et al.6 in conjunctival vessels of rabbits, as well as from Cullen and Swank's5 findings in hamsters with alimentary hyperlipemia. As far as the first of these investigations is concerned, the mode of administration was the same as in our studies and cannot, therefore, be responsible for the difference between the results. There is, however, a decided difference between the resolving powers of the microscopic systems used, as indicated also in the microphotographs published. This may be a suitable explanation for the difference in results.

Concerning Cullen and Swank's investigation, it should first be emphasized that the different modes of fat administration, alimentary and intravenous, are most unlikely to be associated with such fundamental variation in intracapillary behavior of the blood corpuscles. Even in their work it seems as if the degree of structural identification and differentiation in the microscopic picture might not have been quite adequate for the intercorpuscular analysis performed. Indeed, a quotation of these authors' remarks seems justified. "It should be realized that the presence of 'sludged' blood in man has been determined under conditions which do not permit clear visualization of the blood elements because of the limited magnification possible, and that the classical rouleaux formation of Fähraeus has been confused with the 'sludged' blood concept by many investigators." It is justified because it undoubtedly applies even to experimental studies. Our findings agree with recently reported studies by Frayser12 on the pulmonary circulation and alterations in blood gas exchange following intravenous injection of 15% cottonseed oil.

Summary

Changes of flow through minute vessels during hyperlipemia after intravenous infusion of fat emulsion have been analyzed by high resolution vital microscopy of connective tissue in ear chambers and of mesentery in rabbits. The shape of erythrocytes was characteristically changed. Granulocytes often stuck to the endothelium. The velocity of capillary blood flow diminished at first and then reverted fairly quickly to normal. Some chylomicra passed through the capillary walls. No intercorpuscular aggregation, no adhesion of erythrocytes and no adhesion of platelets were observed.

References

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