DEFECTIVE LIPEMIA CLEARING RESPONSE TO HEPARIN IN IDIOPATHIC HYPERLIPEMIA*

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Previous studies in this laboratory have demonstrated a delay in the elimination of lipids from the blood stream of hyperlipemic patients after intravenous fat infusion (1). These studies suggested a defect in the lipemia clearing mechanism in hyperlipemia. We subsequently found (2) that the serum of patients with hyperlipemia (primary and secondary to diabetes, nephrosis, glycogen storage disease) inhibited the heparin-induced lipemia clearing activity of normal serum. It was further found that the inhibitory activities of hyperlipemic serum were associated with the supernatant fatty layer obtained after high-speed centrifugation (3). Similar inhibitory activities as in hyperlipemic serum are present in human and other mammalian tissue (4). Since normal human and other mammalian tissue, like hyperlipemic serum, inhibit a number of lipase preparations as well as heparin-induced lipemia clearing activity (5, 6), they can be considered to have general antilipolytic action (7, 8, 9).

In our studies of the antilipolytic action of hyperlipemic serum, we found that intravenous heparin produced less clearing activity in patients with hyperlipemia than in normal individuals (2). These studies were extended and are reported here.

METHODS AND MATERIALS

Heparin† (0.7 mg/kg. of body weight) was administered by intravenous injection to 6 patients with idiopathic hyperlipemia and 3 individuals without known disturbance of lipid transport. All subjects had been fasting for at least 12 hours. During the course of the study (24 hours) they were on a fat-free diet. Blood samples were taken immediately prior to and at regular intervals after the administration of heparin. The blood samples were centrifuged at 3,000 g for 30 minutes at 5°C. The supernatant plasma was stored at 5°C. until it was tested.

In order to determine whether a relationship exists between the blood lipid levels and the antilipolytic action of serum, the same patients received 200 cc. of 10 per cent cream orally after they had fasted for 12 hours. For the duration of these studies (24 hours) a fat-free diet was offered. Blood samples were collected prior to the intake of cream and at regular intervals thereafter. The samples were processed as described before.

The optical density of the serum was determined by the method of Geyer *et al.* (10). The total fatty acids (11), the phospholipids (12), and the total cholesterol and cholesterol esters (13) were measured. From these data the neutral fat levels were calculated.

Clearing activity and its inhibition were determined as previously reported (2) in a mixture containing 0.6 cc. of normal human postheparin serum,‡ 0.6 cc. of the sample of serum to be tested, and 0.3 cc. of a standard fat emulsion.§ This mixture was incubated at 37°C. Spectrophotometric determinations were carried out at 5- to 19-minute intervals according to a modification (2) of the method of Grossman (14). The electrophoretic mobilities of the alpha and beta lipoproteins were determined as described by Herbst, Lever and Hurley (15). The evolution of glycerol was measured by Korn's modification (16) of the method of

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† Heparin sodium, Upjohn Co., Kalamazoo, Mich.

‡ Postheparin serum was prepared as previously described (2). It was defibrinated by the addition of thrombin (topical thrombin, Parke, Davis and Co., Detroit, Michigan), 1 cc. of physiological saline containing 1,000 units of thrombin to 20 cc. of serum. The clot was discarded after the fluid had been expressed from it. This procedure, which does not affect the clearing activity of the serum, eliminates the formation of clots which interfere with the spectrophotometric determinations.

§ The standard fat emulsion contained 15 per cent coconut oil, 0.5 per cent pluronic (a nonionic detergent) and 1 per cent polyglycerol oleate. It was generously supplied by Dr. C. Meyer, Dept. of Clinical Investigation, Medical Div., Upjohn Co., Kalamazoo, Mich. It was diluted 30 times for use in these experiments.

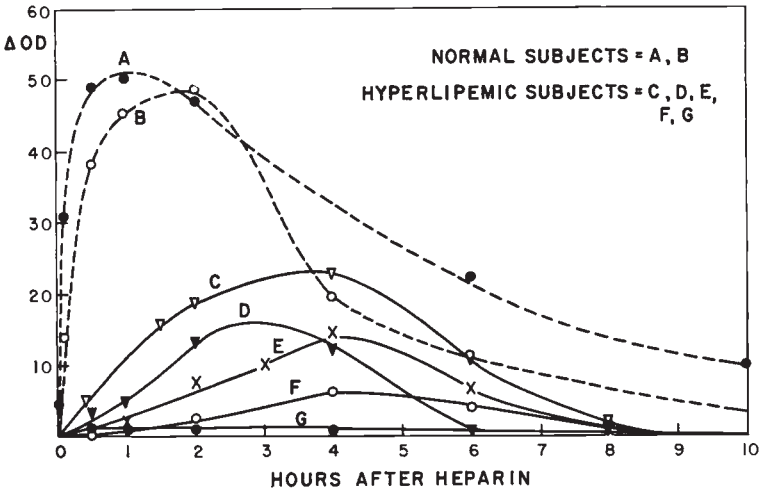

FIG. 1. Development of lipemia clearing activity in the plasma of normal and hyperlipemic subjects following intravenous administration of heparin.

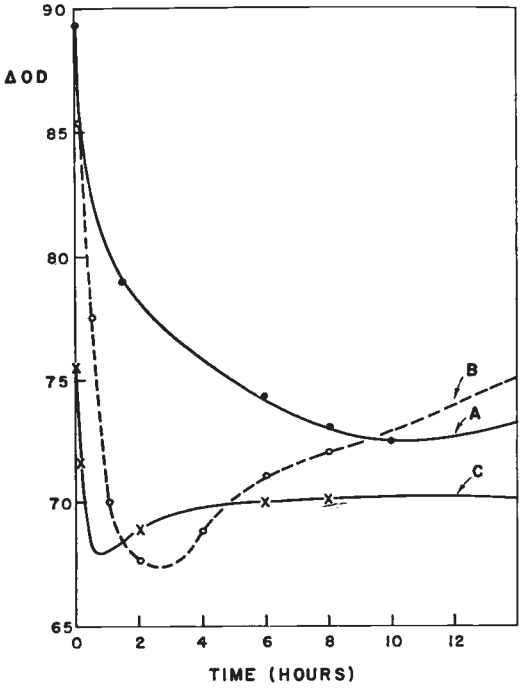

FIG. 2. Turbidity changes in the plasma of normal (lipemic phase) and hyperlipemic individuals following intravenous administration of heparin.

A, B,—hyperlipemic subjects; C,—normal subject.

Lambert and Neish (17); the unmodified method of Lambert and Neish (17) was also used.

RESULTS

Clearing activity following heparin administration appeared considerably later and was less pronounced in the serum of patients with hyperlipemia than in normal individuals (Figure 1). Maximum clearing activity was present in normal individuals within 30 minutes of heparin administration and began to decrease after 2 hours, fell rapidly during the next 3 to 5 hours, and from then on disappeared more gradually over the next 12 to 16 hours. In hyperlipemic patients clearing activity was not noted until 1 hour after the injection, at a time when maximum activity had already been attained in normal serum. The maximum clearing activity in hyperlipemic patients was markedly less than that found in normal individuals and did not appear until 3 to 4 hours after heparin injection (i.e., the time at which the clearing activity in normal individuals had begun to decrease).

The rate of electrophoretic migration of the alpha and beta lipoproteins of normal as well as of hyperlipemic serum began to increase within 15 minutes after the administration of heparin. In the hyperlipemic subjects this increase continued until their clearing activity reached maximum levels and was maintained for 2 to 4 hours while clearing activity was already declining. This phenomenon was similar to that observed in normal individuals with the exception that maximum rates of electrophoretic migration were reached earlier than in hyperlipemic patients. Glycerol levels (in normal and hyperlipemic sera obtained at various intervals after heparin injection) also paralleled the levels of their respective clearing activities.

Turbidity Measurements. As the blood level of clearing activity increased, the turbidity of the

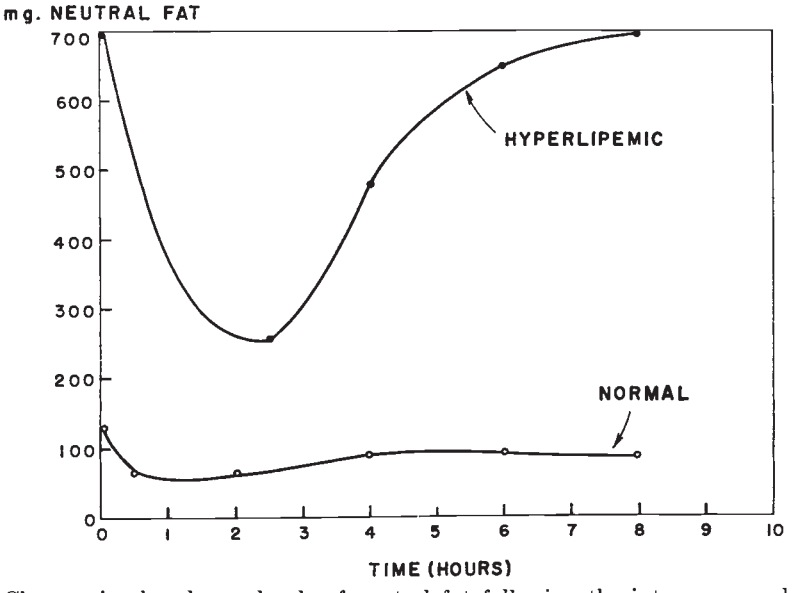

FIG. 3. Changes in the plasma levels of neutral fat following the intravenous administration of heparin.

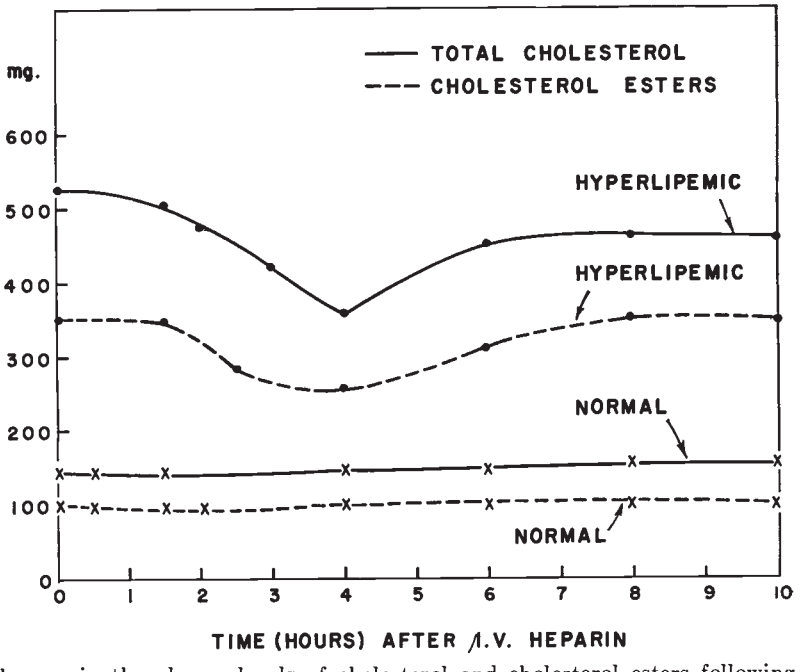

FIG. 4. Changes in the plasma levels of cholesterol and cholesterol esters following intravenous administration of heparin.

serum decreased (Figure 2). In hyperlipemic individuals the decrease in turbidity preceded the onset of maximum clearing activity. Simultaneously with the increase in turbidity and the onset of clearing activity, the blood levels of the neutral fats (Figure 3), of the total cholesterol and cholesterol esters (Figure 4), and of the phospholipids (Figure 5) declined. In hyperlipemic individuals, the decrease in the concentration of the lipids was pronounced because of their high initial levels. The maximum rate of decline of the neutral fats preceded maximum clearing

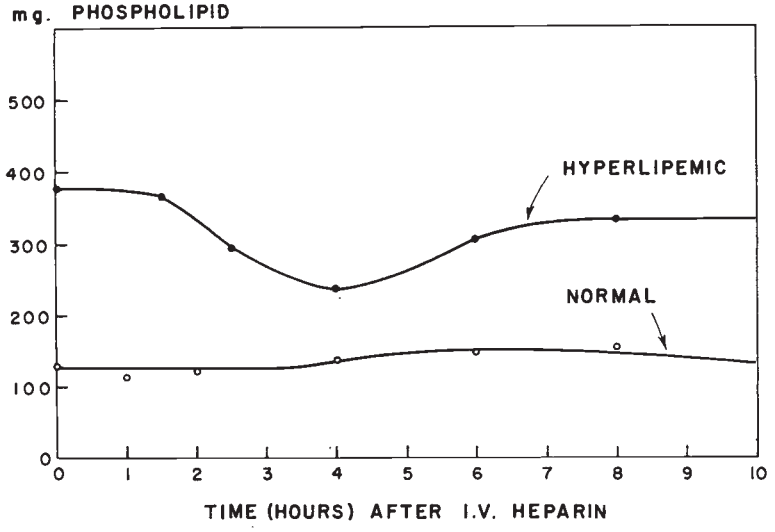

FIG. 5. Changes in the plasma phospholipid levels following intravenous administration of heparin.

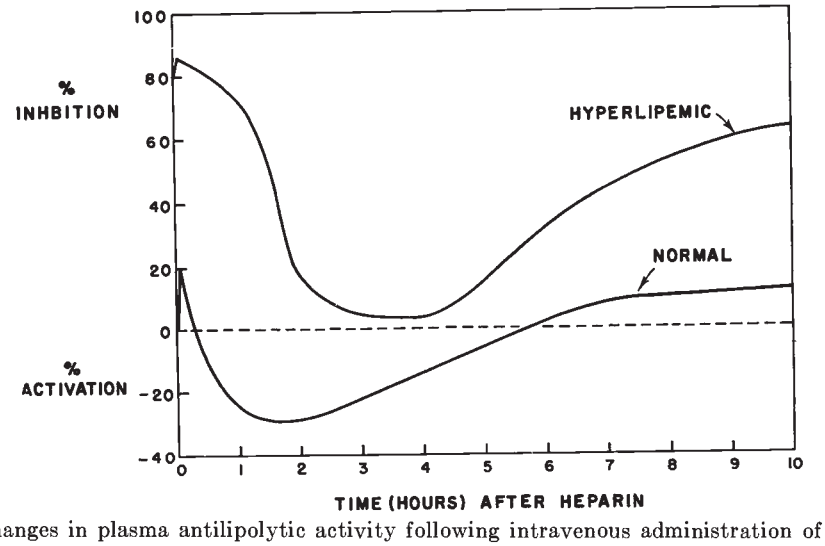

FIG. 6. Changes in plasma antilipolytic activity following intravenous administration of heparin.

activity. The lowest levels of neutral fat were reached prior to those of cholesterol and phospholipids and occurred at the time of maximum clearing activity.

Within one minute following the administration of intravenous heparin the serum of normal individuals was found to inhibit clearing activity and the serum of hyperlipemic patients which was inhibitory even before the injection of heparin became more markedly inhibitory. The inhibitory activity disappeared rapidly from the serum of normals, while it persisted in the serum of patients with hyperlipemia (Figure 6). The inhibitory activity in the serum of hyperlipemic patients diminished over the next 3 to 4 hours as their clearing activity increased; inhibition reached a minimum when clearing activity was at its highest level. The serum of the hyperlipemic patients then gradually regained its inhibitory activity. Antilipolytic activity returned to its preheparin levels within 16 to 24 hours.

A questionable antilipolytic activity appeared in the serum of normal individuals after oral fat intake (Figure 7). The levels of antilipolytic activity in the serum of hyperlipemic patients

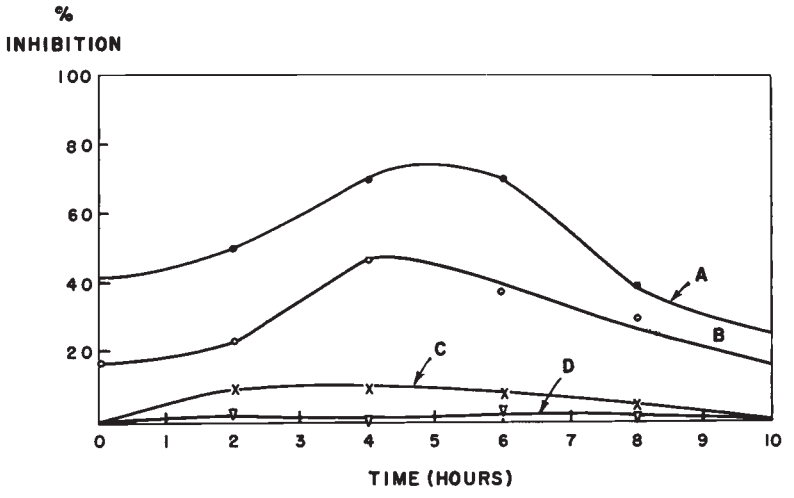

FIG. 7. Changes in plasma antilipolytic activity following ingestion of a fatty meal. A, B,—hyperlipemic subjects; C, D,—normal subjects.

increased considerably after oral fat administration.

DISCUSSION

The data presented here indicate considerable differences both *in vivo* and *in vitro* in the effects of heparin on the formation of lipemia clearing activity in patients with hyperlipemia and in normal subjects.

The previously reported defect (2) in the lipemia clearing response of hyperlipemic patients to heparin has been confirmed by the studies presented here. The clearing activity induced by heparin in patients with hyperlipemia is considerably less than in normal individuals. Current studies suggest that the hyperlipemic patient produces more clearing factor than the normal individual in response to an equivalent amount of heparin (7). The relatively high levels of antilipolytic activity in hyperlipemic serum, however, neutralize the action of the clearing factor, resulting in a low net clearing activity. Thus it appears that the delay in the appearance of heparin-induced clearing activity in hyperlipemic patients is associated with a time-consuming compensatory increase in the evolution of clearing factor.

It is evident that the decrease in optical density following heparin administration is due only in part to the removal of neutral fat from the blood stream; previous ultracentrifugal studies (18), as well as the present *in vitro* studies indicate that transformation of the lipids and the lipoproteins is, to a considerable extent, responsible for the optical density changes since the concentration of the total fatty acids as well as that of cholesterol and phospholipids obviously remains unaltered in the *in vitro* test system. This may explain why the optical density decreases prior to the decline in the neutral fat levels of the blood and why it remains low even after they have returned to their pre-injection levels.

Changes in the lipoproteins were further indicated by the electrophoretic studies. Electrophoresis disclosed an increase in the mobilities of the alpha and beta lipoproteins in hyperlipemic serum prior to the appearance of optical density changes or evolution of glycerol. This suggests that electrophoresis, as previously noted (2), is a more sensitive method for the demonstration of lipemia clearing activity than the other methods employed here.

The glycerol levels increased as the optical density changes and the rate of electrophoretic migration of the lipoproteins increased and those of the lipid levels declined. These studies, therefore, indicate that lipolysis and transformation of the lipoproteins (18) are interrelated with the elimination of fats from the blood stream.

Increased antilipolytic activity appears in the serum of patients with hyperlipemia after oral fat intake, whereas in normal serum with no measurable antilipolytic activity before fat intake, little or no inhibition is found afterwards. This difference between hyperlipemic patients and normal individuals indicates that elevation

of the lipid level alone does not result in increased antilipolytic activity.

The lipemia clearing response to heparin of hyperlipemic patients differs from that of normal individuals primarily in degree. Hollett and Meng (19) have demonstrated the presence of an inhibitor of lipemia clearing in normal human serum. The present as well as previous studies (2, 3) indicate, however, that the levels of inhibitory activity in hyperlipemic serum greatly exceed those in normal serum.

SUMMARY

1. Equivalent amounts of heparin induce less clearing activity in patients with hyperlipemia than in normal individuals. Development of maximum levels of clearing activity is considerably delayed in hyperlipemia.

2. The rate of electrophoretic migration of the alpha and beta lipoproteins is increased immediately after the administration of heparin in hyperlipemic patients as well as in normals. This suggests that even in hyperlipemic individuals some immediate clearing activity is present.

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DISCUSSION

Dr. Theodore Cornbleet (Chicago, Illinois): In their earlier experiments on the clearing response to heparin, the essayists did not have the opportunity to observe the effects of toluidine blue, a heparin antagonist. Does toluidine blue intensify lipemia in idiopathic hyperlipemia? One may conceive of the toluidine blue fixing the heparin and preventing the latter's escape from the mast cells. If, on the other hand, Idiopathic Hyperlipemia is based on the relative absence of heparin in the mast cells, then it would not be expected that toluidine blue would intensify the lipemia.

Dr. Earl G. McNall (Los Angeles, Calif.): I would like to congratulate Dr. Klein for a most interesting presentation. I am very interested in

the various interactions of heparin because heparin has been reported by other workers and our own group to bind properdin.

There are quite a number of polysaccharides derived from mammalian tissues and from plant and bacterial sources which have been studied in their infection-promoting and properdin binding activities. Some of these polysaccharides also have heparin-like activity. The most active polysaccharides which have heparin activity are sulfated derivatives. Carrageenin which is a sulfated polygalacturonic acid derivative, sulfated dextran, sulfated amylose, and a sulfated polysaccharide from seaweed-laminarin, all prolong blood clotting and have lipemia clearing activity. It is apparent that the clearing activities of heparinoids depend on their ultimately causing a release of esterases into serum. And these esterases, by hydrolyzing chylomicrons, produce clearing of lipemic sera. It is to be hoped that clinically suitable, chemically descript polysaccharides may someday replace heparin which must be purified from animal tissues since varying molecular weight classes of this polymer as well as its chemical make up vary slightly depending upon the organ and species from which the heparin is prepared.

Dr. Edmund Klein (in closing): In answer to Dr. Cornbleet's question, there is no evidence that toluidine blue inhibits lipemia clearing by direct complex formation with clearing factor.

With regard to Dr. McNall's point, the activating effects on the system are not confined to polysaccharides, and can be demonstrated with a number of other materials.