Inhibition of Lipemia Clearing Activity by Serum of Patients with Hyperlipemia. (23292)

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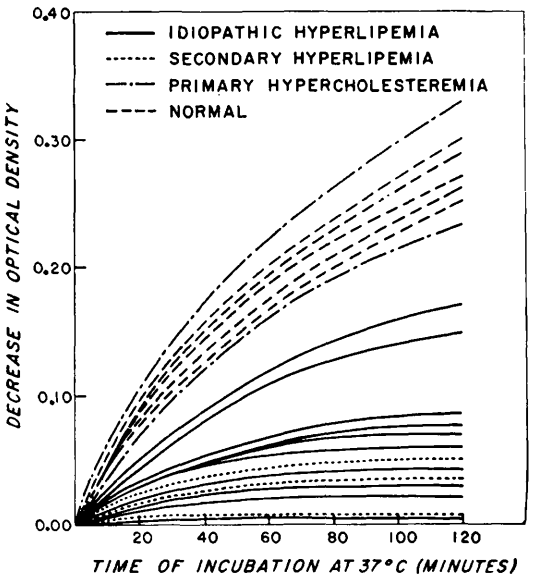
A defective lipemia clearing system in patients with idiopathic hyperlipemia was demonstrated in the studies of Lever and Waddell(1) who showed a delayed elimination of lipids from the blood stream after an intravenous fat infusion. Inhibition of lipemia clearing by the sera of patients with idiopathic and secondary hyperlipemia is reported. So far, a relation between an inhibitor of the lipemia clearing system and disturbances of lipid transport in humans has not been reported. Seifter and Baeder(2), however, have shown that a dialyzable inhibitor of the lipemic clearing system is present in the blood of rats, particularly following experimental production of a nephrotic syndrome, or following administration of cortisone. Hollett and Meng(3) have separated a nondialyzable component of normal human plasma, which inhibited purified clearing factor completely, while clearing activity in post-heparin plasma was only partially inhibited.

Materials and methods. The degree of inhibition of lipemia clearing activity was tested on sera obtained from 10 patients with idiopathic hyperlipemia, 2 patients with hyperlipemia secondary to glycogen storage disease, 1 patient with hyperlipemia secondary to nephrosis, 2 patients with primary hypercholesteremic xanthomatosis and 10 control subjects. These sera were mixed with normal human or canine serum exhibiting lipemic clearing activity (active serum). Clearing activity had been induced by administering heparin intravenously (0.75 mg of heparin/kg body weight to dogs). No difference was observed whether human or dog serum was used. Equal amounts (0.6 cc) of serum to be tested and of active serum were mixed and 0.3 cc of a standard fat emulsion* was added. As an indication of clearing activity, the optical density was measured spectrophotometrically at intervals, as previously described by Grossman(4). In addition varying amounts of hyperlipemic sera were used in a total volume of 1.2 cc to establish at which level complete inhibition of clearing was obtained. Paper electrophoretic analysis was carried out, according to a previously outlined method(5), on mixtures containing hyperlipemic serum, active serum and standard fat emulsion. For comparison of the degree of clearing activity produced by heparin in normal and hyperlipemic subjects 100 mg of heparin were administered intravenously to 4 normal human subjects and to 4 patients with idiopathic hyperlipemia. To 1.2 cc of the respective post-heparin sera, 0.3 cc of standard fat emulsion was added *in vitro* and the optical density of the mixture was measured at intervals as described previously(4).

Results. In contrast to normal sera and those obtained from patients with primary hypercholesteremic xanthomatosis, the sera of all 13 patients with idiopathic and secondary hyperlipemia inhibited to varying degrees the clearing activity which is present in normal sera after the intravenous administration of heparin. When equal amounts of serum to be tested and active serum were used, the decrease in optical density produced in a standard fat emulsion within 120 minutes, varied for the normal and hypercholesteremic sera between 0.23 and 0.33, averaging 0.27 unit; and for the hyperlipemic sera between 0 and 0.17, averaging 0.07 unit (Fig. 1). At sufficiently high concentrations of hyperlipemic serum complete inhibition of clearing activity was observed in all 10 cases. The amounts of hyperlipemic serum necessary to produce complete inhibition of clearing activity varied from 1 part of hyperlipemic to 19 parts of active serum in the most strongly inhibiting serum, to 5 parts of hyperlipemic to

* The standard fat emulsion contained 15% coconut oil, 0.5% Pluronic (a nonionic detergent) and 1% polyglycerol oleate. It was supplied by Upjohn Co., Kalamazoo, Mich.



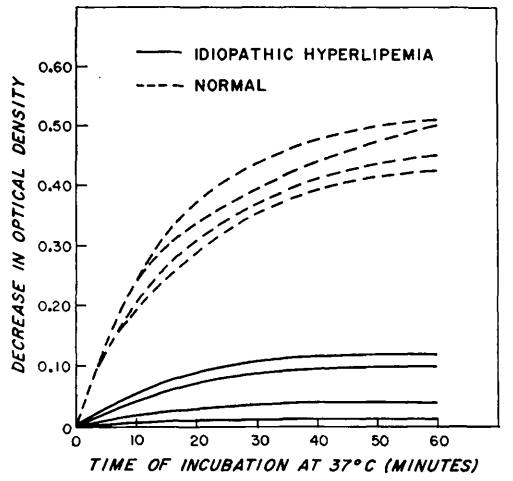


FIG. 1 (top). *In vitro* clearing activity of mixtures of equal amounts of normal post-heparin serum with either normal fasting serum or serum from patients with primary or secondary hyperlipemia or primary hypercholesteremia. (Only 5 of the 10 curves for normal sera are shown. The other 5 curves fell within the same range.)

FIG. 2 (bottom). *In vitro* clearing activity of serum from 4 normal human subjects and from 4 patients with idiopathic hyperlipemia following an intrav. inj. of 100 mg of heparin.

1 part of active serum in the least strongly inhibiting serum. A consistent relationship between the degree of inhibition and severity of the hyperlipemia was not noted. The sera of the 3 patients with secondary hyperlipemia inhibited clearing at ratios of 1 part of hyperlipemic to 9 parts of active serum.

Paper electrophoretic analysis revealed that even in those mixtures of hyperlipemic serum and active serum, in which spectrophotometric measurement showed complete inhibition of clearing, the electrophoretic pattern still revealed accelerated migration of the alpha and beta lipoproteins, typical of the presence of clearing activity.

Determination of the *in vitro* clearing activity, evoked by an intravenous injection of 100 mg of heparin in normal subjects and patients with idiopathic hyperlipemia, revealed much less decrease in optical density and thus less clearing activity in the hyperlipemic patients than in the control subjects (Fig. 2). The decrease in optical density after 1 hour of incubation varied in the 4 normal control persons between 0.38 and 0.49, averaging 0.45 unit; and in the hyperlipemic sera between 0 and 0.11, averaging 0.06 unit.

Discussion. The inhibition of clearing exerted by the serum of patients with idiopathic and secondary hyperlipemia provides an explanation for the high serum lipid levels in these patients. It would be expected that a correlation exists between degree of inhibition and severity of the hyperlipemia. We have not found such a relationship, however. Since dietary intake of fat has a pronounced influence on the lipid levels in these patients, it is likely that in our group of patients who were on varying diets, some on a strict low-fat diet and others not, variations existed in the lipid levels independent of the severity of the inhibition. In order to establish whether a relation exists between degree of inhibition and severity of the hyperlipemia, studies, under standardized conditions, are being carried out.

The observation that the speed of electrophoretic migration of the lipoproteins was increased even when there was complete suppression of clearing and of a fat emulsion, suggests that electrophoretic measurements represent a much more sensitive indicator of clearing activity than optical density measurements.

The greatly reduced post-heparin clearing activity in the serum of patients with hyperlipemia, as compared to normal serum, is further indication of a defect in the clearing mechanism. It is consistent with the obser-

vation of Gitlin (personal communication) that intravenous administration of heparin caused much less clearing activity in the serum of patients with secondary hyperlipemia due to nephrosis, than in normal individuals. He had found that increasing the albumin concentration in these patients who had low serum albumin levels, had no direct effect on degree of clearing. In view of our observation of inadequate clearing in patients with idiopathic hyperlipemia who have normal serum albumin levels, it seems that low values for serum albumin are not a major factor in producing inhibition of lipemia clearing.

Our finding of inhibition of clearing by serum of patients with primary as well as secondary hyperlipemia suggests the possibility of a common basis for the high blood lipid levels in these disorders.

Summary. 1. Serum from patients with primary and secondary hyperlipemia was found to inhibit the clearing activity present in normal serum after intravenous administration of heparin. 2. The degree of inhibition exerted by the hyperlipemic sera varied among the patients, but at appropriate concentrations of hyperlipemic serum complete inhibition could be attained in every case. 3. Intravenous administration of heparin caused considerably less clearing activity in hyperlipemic patients than in the control subjects.

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Anti-AcG: Specific Circulating Inhibitor of the Labile Clotting Factor.* (23293)

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With recent knowledge that a number of protein factors normally participate in the complex mechanisms of blood clotting, it is understandable that inhibitors might be developed, in the body, to act as specific antibodies against one or other of these protein factors. For instance, anti-AHF or anti-PTC have been demonstrated in occasional hemophiliacs or Christmas disease patients, respectively, particularly after transfusions (1). The present data are from the case of an elderly white male who developed hematuria following an uneventful gall-bladder operation. This bleeding tendency seemed to get worse after transfusions. The following tests establish the presence in his blood of a powerful inhibitor, specific for AcG (syn. (pro)accelerin, labile factor, factor V, etc.).

Methods are established routines(2), with the modifications indicated. A summary of the routine test findings is shown in Table I (see discussion). The significance of the several abnormal test results will emerge as the experimental analysis proceeds.

Prothrombin time tests. A major initial discovery was the prolongation of the plasma prothrombin time test (*cf.* Quick, 3). This was true also in mixtures of equal vols, of patient's (Y) and normal (N) plasma, immediately suggesting an inhibitor, since normal plasma might be expected to correct the test if it were merely a matter of factor deficiency. Partial, but not complete, correction of the prothrombin time could be obtained by (a) dilution of Y, (b) addition of AcG ($BaCO_3$-

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