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## Serum Lipoproteins in Experimental Diabetes. II. Action of Heparin *in vivo* on Lipoproteins of Depancreatized Dogs.* (21078)

ANGELO FASOLI,† MORTON D. GLASSMAN, EDWARD B. MAGID, AND PIERO P. FOÀ.
*From the Department of Physiology and Pharmacology, Chicago Medical School, Chicago 12.*

Clearing of lipemic serum by heparin was first observed by Hahn(1) and by Weld(2). Further study of the phenomenon demonstrated that heparin combines with a "coprotein" supplied by normal plasma to form a "clearing factor"(3-5), capable of causing profound changes in the lipid and lipoprotein composition of normal human and animal serum and of the serum of patients with atherosclerosis or hypercholesterolemia(6-10).

The object of this investigation was to compare the changes caused by heparin in the serum lipoproteins of normal lipemic dogs with those caused in the serum lipoproteins of diabetic lipemic dogs.

*Materials and methods.* Two normal dogs, kept on a commercial dog food diet, were fasted for about 20 hours and, after taking a control sample of venous blood, fed a meal consisting of 25 g of lard, 2 egg yolks (dog W), and a small amount of commercial dog food. Serum samples were obtained 3 hours after the meal, at which time heparin sodium (2 mg/kg) was injected intravenously;‡ other blood samples were obtained 10 minutes (dog W) and 30 minutes after the injection. Five depancreatized dogs were allowed to develop mild to severe ketosis and lipemia by the withdrawal of insulin. After securing a fasting blood sample, heparin was injected intravenously and another blood sample obtained 30 minutes after the injection. Serum was separated by centrifugation and analyzed for total cholesterol and lipid phosphorus according to the methods of Bloor, Pelkan and Allen (11) and of Horwitt(12), respectively. Serum aliquots were subjected to electrophoresis on filter-paper, following a previously described technic(13,14) and the cholesterol and lipid phosphorus content of the resulting fractions was determined. A semi-quantitative pattern of the total lipid distribution along the strip of filter-paper was obtained by staining the strip with Sudan III(13) and measuring the density of the stain with a spectrophotometer at a wave length of 520 m$\mu$. A curve was obtained by plotting color density against the distance along the strip.

*Results. Normal dogs.* The ingestion of a fatty meal caused a small rise in the total cholesterol and lipid phosphorus content of serum. This was accompanied by a rise in the slow-migrating lipoprotein fractions ($\beta_1$, $\beta_2$), while the cholesterol and lipid P content of the fast-moving components decreased slightly. These changes were not statistically significant. On the other hand, the total lipid content of the non-moving band ($\beta_2\gamma$) increased sharply, probably as a result of neutral fat load. This is indicated also by the intensity of the color in the strips stained with Sudan III (Fig. 1). The administration of heparin

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† Present address: Clinica Medica Università di Milano, Italy.

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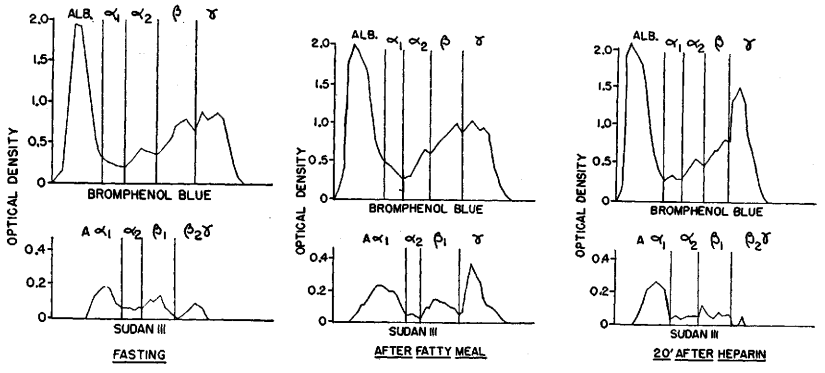


FIG. 1. Paper electrophoresis pattern of proteins (upper curve) and lipids (lower curve) of normal dog serum. Galvanometer readings. Instrument set at zero with unstained portion of strip. A: fasting; B: after fatty meal; C: after heparin.

caused a complete clearing of the lipemia accompanied by a reversal of the changes described above in a matter of minutes. The Sudan III peak in the $\beta_2\gamma$ zone disappeared (Fig. 1); the cholesterol and lipid phosphorus content in the whole serum and in the slow-moving fractions decreased slightly while the lipid content of the albumin-$\alpha_1$ fraction increased slightly. The lipid content of the $\alpha_2$-fraction increased somewhat immediately after the injection of heparin in dog W, but a few minutes later, returned to a level lower than normal. In order to detect possible lipoprotein components migrating faster than albumin, the zone of the strip ahead of the latter was also analyzed; while in one case (dog Y), no lipids were detected in this zone, in the other case (dog W), there appeared small amounts of lipids soon after the injection of heparin.

*Diabetic dogs.* The following changes were observed in dogs with diabetic lipemia following the injection of heparin (Table I and Fig. 2): 1) varying degrees of clearing of the

TABLE I. Effect of Fatty Meal and of Heparin on Cholesterol and Lipid Phosphorus Fractions in Normal and Diabetic Dogs.

| | Dog | Time | Cholesterol fractions, mg % | | | | | | Lipid P fractions, mg % | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total | F | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ | Total | F | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ |
| Normals | Y | Fasting | 230 | — | 185 | 24 | 11.5 | 9.5 | 11.4 | — | 9.5 | .9 | .75 | .25 |
| | | 3 hr after M* | 234 | — | 180 | 20 | 17 | 17 | 11.5 | — | 9.3 | 1.1 | .6 | .5 |
| | | 20′ after H* | 198 | — | 177 | 6 | 11 | 4 | 11.6 | — | 10.4 | .7 | .4 | .1 |
| | W | Fasting | 201 | 1 | 157 | 11 | 15.5 | 16.5 | 9.9 | — | 8.2 | .5 | .6 | .6 |
| | | 3 hr after M | 202 | 2 | 137 | 15 | 21 | 27 | 10.6 | .1 | 8.0 | 1.0 | .8 | .7 |
| | | 9′ after H | 192 | 10 | 140 | 18 | 10 | 14 | 10 | .7 | 7.1 | 1.2 | .5 | .4 |
| | | 20′ after H | 185 | 5.5 | 148 | 2.5 | 12 | 17 | 10 | .3 | 8.3 | .5 | .55 | .35 |
| Diabetics | P | Before H | 538 | — | 390 | 92 | 32 | 24 | 11.3 | — | 7.7 | 1.9 | 1.1 | .6 |
| | | 30′ after H | 492 | — | 406 | 66 | 11 | 9 | 10.4 | — | 8.2 | 1.5 | .65 | .05 |
| | R | *Idem* | 692 | 20 | 380 | 96 | 126 | 70 | 12.6 | .2 | 6.5 | 1.9 | 3.0 | 1.0 |
| | | | 648 | 243 | 165 | 78 | 82 | 80 | 13.4 | 6.15 | 3.3 | 1.2 | 1.5 | 1.25 |
| | $K_1$ | " | 418 | 40 | 264 | 70 | 27 | 17 | 12.2 | 1.6 | 7.6 | 1.9 | .8 | .3 |
| | | | 420 | 169 | 142 | 54 | 34 | 21 | 12.1 | 5.2 | 4.3 | 1.2 | 1.2 | .2 |
| | Z | " | 319 | 9 | 247 | 18 | 26 | 20 | 7.9 | .2 | 5.5 | .4 | 1.2 | .6 |
| | | | 320 | 21 | 241 | 13 | 21 | 24 | 7.3 | .4 | 5.3 | .35 | .9 | .35 |
| | $K_2$ | " | 354 | 115 | 90 | 78 | 40 | 31 | | | | | | |
| | | | 344 | 163 | 62 | 33 | 37 | 49 | | | | | | |
| | U | " | 444 | 91 | 281 | 29 | 25 | 18 | | | | | | |
| | | | 428 | 250 | 103 | 29 | 21 | 25 | | | | | | |

* M = Fatty meal. H = Heparin I.V.

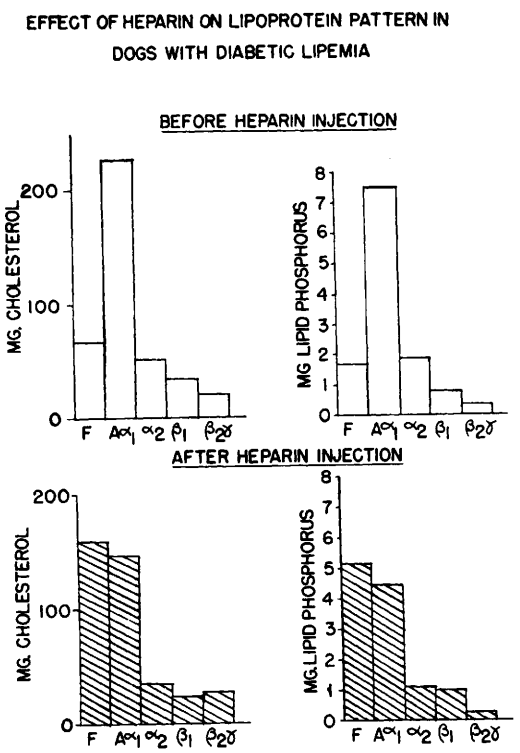


FIG. 2. Distribution of cholesterol and lipid P in main lipoprotein fractions of dogs with diabetic lipemia.

serum which, in the case of dog P, appeared to be nearly complete; 2) a moderate decrease in total serum cholesterol and lipid phosphorus; 3) a slight change in total serum lipids; 4) a marked change in the lipid distribution characterized by a sharp increase in the fraction moving faster than albumin. Small amounts of this fraction (which, for the sake of brevity, will be called fraction "F") are sometimes present in the serum of diabetic dogs, however, after the injection of heparin, it may account for as much as 40% of the total serum cholesterol and lipid phosphorus (Table I). Fraction F can be seen also in the free-electrophoresis pattern of the serum of one dog studied by this method (Fig. 3);§ in this case (Dog U), fraction F represented 11% of the total serum protein area. The cholesterol/lipid P ratio of fraction F closely resembles that of the albumin-$\alpha_1$ fraction. Though a complete analysis of its composition was not made, the high lipid content of fraction F is noteworthy; 5) the albumin-$\alpha_1$ fraction decreased in amounts which appear to be equivalent to the rise in fraction F; and 6) the slow-moving $\beta_2\gamma$ fraction changed very little or not at all, most often increasing slightly rather than decreasing.

*Discussion.* The mechanism of action of the clearing factor produced after injection of heparin is not fully understood. It is now generally agreed that the phenomenon of clearing is accompanied by a conversion of lipoproteins of low density, low electrophoretic mobility and large molecular size into smaller, faster-migrating ones(9,15): The results obtained in normal dogs with alimentary lipemia which are very similar to those obtained in man(10,15,16), are consistent with this conversion theory. Still matter for speculation is how this conversion is brought about (17-19). It is possible that the highly mobile fraction F may play a role in the clearing phenomenon, perhaps as an albumin-like co-factor(5). This is suggested by: 1) the sharp increase in fraction F after heparin;

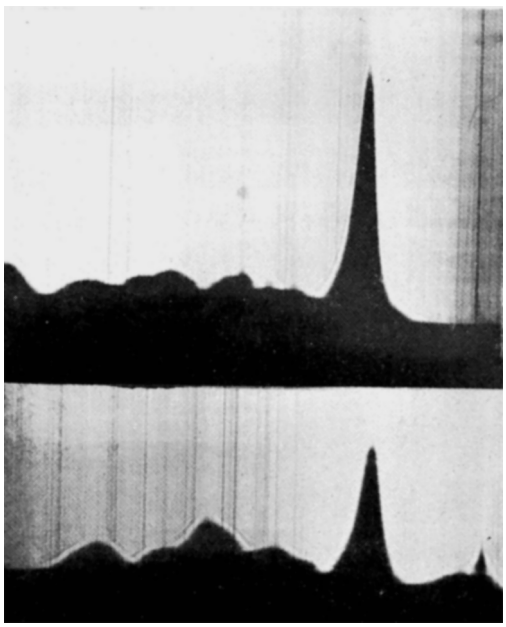

FIG. 3. Free electrophoresis pattern (ascending limb) of normal dog serum before (A) and after (B) heparin injection. Note fraction F at the right of the albumin peak. Left to right. Veronal buffer pH 8.6. Ionic strength 0.1.

§ Free electrophoretic determinations were performed through the courtesy of Doctor Steelman of Armour Laboratories.

2) its high lipid content; and 3) its high mobility indicating small molecular size with a net increase in electrical charge and, most likely, increased solubility. The appearance of an extra-boundary fraction comparable to our fraction F was observed recently by Lever and co-workers(20) studying human sera by means of free electrophoresis. The authors suggest that the new fraction might be the results of a combination of heparin with a lipoprotein, or of the splitting of large lipoprotein molecules caused by the lipolytic action of the clearing factor. The presence of fraction F in diabetic dogs untreated with heparin and its appearance in normal dogs after a fatty meal as well as after heparin suggests the alternate possibility that, at least in the dog, fraction F might be a normal serum component, and that the differences between normal and diabetic animals in their response to heparin might be a quantitative rather than a qualitative one. Fraction F appears to be related to fraction $a_1$, as indicated by the reciprocal relationship of their lipid content. It is possible that the partial clearing observed in the serum of diabetic lipemic dogs after heparin may be due, at least in part, to an increase in this lipid carrier of relatively high solubility. The residual turbidity is probably due to fraction $\beta_2\gamma$ which, unlike in the normal animal, does not appear to be affected by heparin, suggesting a derangement of the clearing phenomenon in most diabetic decompensated dogs.

*Summary.* Heparin causes a clearing of the lipemic serum of normal and, less, of depancreatized dogs. In normal animals with alimentary hyperlipemia, this clearing is associated with a decrease in the lipid content of the slow-moving lipoprotein fractions, while in the diabetic lipemic dogs, the outstanding change is the appearance of, or sharp increase in, the amount of a fraction "F" migrating faster than albumin and containing large quantities of lipids. The possible significance of these results is discussed.

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