STUDIES ON LIPEMIA-CLEARING FACTOR IN RELATION TO PYRIDOXAL PHOSPHATE

KOZO YAMADA, HUMIO KUZUYA AND MASATOSHI NODA

*Third Department of Internal Medicine, Nagoya University School of
Medicine, Showa, Nagoya*

(Received February 15, 1960)

Many research workers have shown that lipemia-clearing activity which is detectable in postheparin plasma is due to lipoprotein lipase. Engelberg (1) assumed heparin to be the coenzyme. In this paper, the enzymatical characters of the lipemia-clearing factor purified by Nikkilä's method is reported.

EXPERIMENTAL

Methods

Purification of Clearing Factor of Postheparin Human Plasma — Human blood was collected 10 minutes after heparin injection (0.5 mg per kg body weight) and the plasma was isolated. This postheparin human plasma was purified by Nikkilä's method (2).

Determination of Clearing Activity — As the substrate for the active enzyme the following emulsion was used: 10 ml of *M*/15 phosphate buffer (pH 7.4); 1 drop of 20% sesame-oil emulsion, and 2.5 ml of inactive human plasma. This substrate was used after incubation at 37° for 60 minutes. Inactive human plasma was used after testing its inactivity. To 2 ml of this substrate was added 1 ml of active enzyme solution. This reaction mixture was incubated at 37° for 120 minutes. The clearing activity was measured by reduction in turbidity of this mixture (650 m μ). Protein concentration was determined by biuret reaction.

RESULTS

The absorption spectrum of the purified solution was almost similar to that of pyridoxal phosphate as shown in Fig. 1. The purified solution was then brought to its isoelectric point (pH 5—4.8). 0.05 *M* sodium citrate was added to the precipitate after centrifugation, and the pH was brought back to 7.8. The precipitate thus formed is dissolved and dialyzed against distilled water at 0° after adjusting the pH to 5.4. The absorption maximum of the dialyz-

FIG. 1 Absorption-Spectra of Purified and Dialyzed Clearing Factor

ed solution which had been observed at 410–420 m μ in the purified solution prior to dialysis as shown in Fig. 1, was disappeared.

The clearing activity of two kinds of the purified solutions, one was the dialyzed solution, and the other was the dialyzed solution added

FIG. 2 Optimal pH of Purified Clearing Factor

TABLE I
Purification and Dialysis of Human Plasma

	Protein concentration	Clearing activity	Clearing activity per mg protein
	mg/ml		
A Postheparin human plasma	78	0.264	0.003
B Purified solution before dialysis	2	0.174	0.087
C Purified solution after dialysis	0.5	0	0
D Purified solution after dialysis + pyridoxal phosphate, 10 μ g/ml	0.5	0.059	0.118
E Purified solution after dialysis + heparin, 0.3 μ g/ml	0.5	-0.018	

FIG. 3 Paper Electrophoresis of Purified Clearing Factor

Upper, human plasma
 Lower, purified-clearing factor

with pyridoxal phosphate (final concentration, 10 mg per ml), was compared, and it was found that the clearing activity was reduced by dialysis but it recovered markedly after adding pyridoxal phosphate (Table I).

The optimal pH of the purified enzyme activity was determined using phosphate buffer (M/15) and Ringer's buffer (M/15), and it was found to be pH 8.0 as shown in Fig. 2.

Paper electrophoresis of the purified solution showed that the enzyme was located in albumin fraction (Fig. 3).

FIG. 4 Inhibition of C. F. (purified)

In vitro inhibition of the enzyme activity by various drugs were then studied. Protamine sulfate, quinine sulfate, *p*-chloromercuribenzoate (PCMB), heparin and some inorganic substances inhibited the enzyme activity as shown in Fig. 4. However, inorganic substances inhibited only at high concentrations. Reduced gultathione failed to inhibit.

DISCUSSION

Skorepa *et al.* (3) reported that heparin might be contained in lipoprotein lipase but not in esterase. Engelberg (1) also assumed heparin to be a co-enzyme of clearing factor. Robinson *et al.* (4) noticed the stability of the enzyme system in hapanized plasma to be markedly reduced in the presence of protamine. But Robinson *et al.* (5) suggested in another report that some component necessary for the activity of the lipolytic system induced in blood by the injection of heparin is normally located on the inner surface of the vascular wall and is released from these into the blood stream. Spitzer *et al.* (6) reported the clearing factor to be present in albumin fraction, but Eiber *et al.* (7) recognized in albumin fraction only a trace of the S³⁵ of the heparin injected. The authors also recognized the enzyme to be present in the albumin fraction electrophoretically. From this observation that the lipolytic activity reduced by dialysis was recovered by pyridoxal phosphate, it is concluded that one component necessary for the activity of the lipolytic system is pyridoxal phosphate rather than heparin.

SUMMARY

1. In the absorption spectrum of the purified clearing factor two absorption maxima were recognized, *i.e.*, 270—280 and 410—420 m μ .
2. Lipolytic activity reduced by dialysis was recovered by pyridoxal phosphate, but not by heparin. It is probable that one component necessary for the activity of the lipolytic system is pyridoxal phosphate.
3. Optimal pH of the enzyme activity was 8.0.
4. The enzyme was shown to exist in albumin fraction electrophoretically.
5. The enzyme was inhibited by protamine sulfate, quinine sulfate, pCMB and heparin, but not by glutathione.

REFERENCES

1. Engelberg, H., *Proc. Soc. Exptl. Biol. Med.* **90**, 489 (1958).
2. Nikkilä, E. A., *Bioch. Biophys. Acta* **27**, 612 (1958).
3. Skorepa, J., and Novak, S., *Nature* **182**, 117 (1958).
4. Robinson, D. S., Jeffries, G. H., and French, J. E., *Quart. J. Exptl. Physiol.* **39**, 165 (1964).
5. Robinson, D. S., and French, J. E., *ibid.* **42**, 151 (1957).
6. Spitzer, J. J., *Am. J. Physiol.* **171**, 492 (1952).
7. Eiber, H. B., and Danishefsky, I., *Proc. Soc. Exptl. Biol. Med.* **102**, 18 (1959).