virtually free of ribonucleic acid (RNA). With the medium now described, washed mitochondrial fractions obtained by low-speed centrifugation* (so as to minimize contamination with microsomal material) do contain RNA, in an amount corresponding to at least 3% of the protein present.

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Purification and characterization of the lipemia-clearing factor of postheparin plasma

HAHN[1] first observed that intravenous administration of heparin cleared alimentary lipemia. The active principle of postheparin plasma which possessed clearing ability was named "lipemia-clearing factor" by ANFINSEN and co-workers[2]. Although many of the properties of the clearing factor have been elucidated[2,3,4,5,6,7,8] only very limited purification of this postulated enzyme has been reported. BROWN, BOYLE AND ANFINSEN[9] have achieved a 5-fold purification using ethanol fractionation. NIKKILÄ[10] has reported a 25- to 30-fold purification by repeated precipitation at low ionic strength, pH 5.4 to 5.8. Recently, we have developed a method of purification which yielded a 1480-fold increase in specific activity in the most active fraction, which contained 16% of the original clearing activity.

Method of purification: Postheparin plasma was obtained from dogs which had received 1 mg of sodium heparin* per kg of body weight. The first step in our procedure was similar to that of NIKKILÄ[10]—80 ml of postheparin plasma was diluted 1:15 with cold distilled water; the pH was adjusted to 5.7 by the addition of 0.1 *N* HCl. This mixture was allowed to stand at 4° C for 2 hours and then centrifuged. The precipitate (Fraction I) was made up in 20 ml of *M*/15 phosphate buffer, pH 7.8, and immediately frozen. This fraction is stable and can be kept in the frozen state for several weeks without any loss in clearing activity.

Our next and succeeding steps were carried out using ammonium sulphate saturated at 0° C and pH 5.70. The entire procedure was performed at 0° C. The fractionation was as follows: Fraction I was brought to 25% saturation with ammonium sulphate added dropwise. The mixture was centrifuged and the precipitate (Fraction II) was stored in the deepfreeze at —20° C. Fractionation was continued by bringing the supernatants to 33%, 40%, 50% and 64% saturation with ammonium sulphate. The precipitates at each step were stored as above. The final supernatant was dialyzed in the cold against *M*/15 phosphate buffer for 5 hours. It was then assayed for protein content and clearing ability. The various precipitates were thawed and dissolved in 2.0 ml of *M*/15 phosphate buffer except Fraction II, which required 10 ml of buffer. These were dialyzed as above. Each fraction was assayed for protein content and clearing activity.

It may be pointed out that reprecipitation of Fractions II to V with the corresponding concentrations of ammonium sulphate as described above resulted in a quantitative recovery of their clearing activity in the precipitates obtained. This suggested to us that the clearing factor may be firmly bound to the protein of each particular fraction.

Clearing activity was determined by the method described in an earlier publication[4]. The

* Heparin sodium, 10 mg/ml and 100 units/mg, was furnished through the courtesy of Dr. L. L. COLEMAN, The Upjohn Company, Kalamazoo, Michigan.

amount of each fraction used (*e.g.*, 0.25 ml of Fraction I, 0.026 ml of Fraction VI, etc.) was equivalent to 1.0 ml of the original postheparin plasma. Protein content was determined by the biuret method[11] except in instances where a greater degree of sensitivity was required; then the method of LOWRY *et al.*[12] was used.

Table I summarizes the results in clearing activity, protein content and specific activity of the various fractions of postheparin plasma.

TABLE I

Fraction	*Total ml of clearing factor*	*Total protein in mg/ml*	*Total units**	*Specific activity units* per mg*	*Increase in specific activity*
Postheparin plasma	80.0	52.00	102	0.025	—
Fraction I	20.0	33.92	130	0.19	8
Fraction II (25%)	13.0	14.00	52	0.29	12
Fraction III (33%)	2.6	24.00	43	0.70	28
Fraction IV (40%)	3.5	32.00	47	0.42	17
Fraction V (50%)	3.5	20.75	44	0.60	24
Fraction VI (64%)	2.1	0.50	38	36.00	1480
Supernatant VI	54.0	1.06	14	0.23	9

* 1 unit = the amount of clearing factor which will produce a decrease of 0.100 in optical density in 1 hour. Incubation time = 5 hours.

It can be noted that Fraction I was found to possess greater clearing activity than the original postheparin plasma, suggesting the removal of an inhibitor. This is in agreement with the findings of NIKKILÄ[10]. It may also be seen from the table that during fractionation the number of units acquired greatly exceeds that of Fraction I. This appeared to be further evidence of the presence of an inhibitor in postheparin plasma.

It is of interest to point out that both postheparin plasma and Fraction I exhibited their fastest rate of clearing during the first half hour of incubation. Fraction VI, on the other hand, was markedly slower during the first hour, but clearing continued for a much longer time. Maximum clearing was obtained after 18 hours of incubation.

Properties: (1) The optimum pH for purified clearing factor was found to be 8.50; postheparin plasma has an optimum range between pH 6.4 and 7.5. (2) The purified preparation can be kept in the refrigerator for several days without loss of clearing activity. (3) The enzyme is destroyed by heating to 50° C for 5 minutes. (4) For clearing to take place, albumin or non-heparinized plasma must be added to the system. (5) Fraction III-o of the plasma proteins cannot replace albumin as the fatty acid acceptor during the hydrolysis of synthetic fat emulsion. In the presence of albumin, clearing activity is inhibited by Fraction III-o. (6) Both postheparin plasma and purified clearing factor were inhibited by sodium taurocholate ($4 \cdot 10^{-1} M$). (7) Calcium chloride does not affect the clearing by purified clearing factor. (8) Absorption maximum for purified clearing factor was found to be at 279 mμ.

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