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THE ALTERATION OF ENDOTOXIN BY POSTHEPARIN PLASMA AND ITS PURIFIED FRACTIONS

II. Relationship of the Endotoxin Detoxifying Activity of Euglobulin from Postheparin Plasma to Lipoprotein Lipase¹

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Under our experimental conditions, normal guinea pig plasma has little or no ability to detoxify endotoxin. In the preceding paper, we showed that the plasma of guinea pigs which were injected with heparin (postheparin plasma) acquired endotoxin detoxifying activity (1). This finding is evidence for the hypothesis that lipoprotein lipase has endotoxin detoxifying power. We also showed that in parallel with lipoprotein lipase activity, the endotoxin detoxifying activity of postheparin plasma was present in the euglobulin fraction, and it required the presence of serum albumin to exhibit its activity. After delipidization of the euglobulin, both detoxifying and lipoprotein lipase activity were preserved.

This paper describes further experiments designed to further explore the relation between the endotoxin detoxifying behavior of postheparin plasma or its fractions and their lipoprotein lipase activity.

MATERIALS AND METHODS

General procedures. The collection of guinea pig postheparin plasma, the preparation of the euglobulin fractions, the methods utilized for testing endotoxin toxicity for 11-day-old chick embryos, the titration of unesterified fatty acids released on incubation with endotoxin or lipemic guinea pig plasma and measurement of endotoxin turbidity were identical to those described in the preceding publication (1).

The protocol used for testing the effects of

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the starting euglobulin and the fractions obtained by column chromatography on endotoxin was modified from that described previously (1) in the following way: 1.0 ml of the endotoxin from *Escherichia coli* (1.0 mg/ml) and 2.0 ml of 5% bovine serum albumin were incubated with 1.0 ml of the guinea pig postheparin plasma euglobulin fraction, 2.0 ml of the delipidized euglobulin fraction and 3.0 ml of the pooled, concentrated fractions after chromatography, respectively. The incubation mixtures which contained euglobulin or delipidized euglobulin were brought up to 6.0 ml with 0.066 M phosphate buffer, pH 7.8. These mixtures, with controls, were incubated at 37°C for 2 hr. At 0 time and after 2 hr incubation, the unesterified fatty acids and the endotoxin turbidity were measured. The toxicity of endotoxin for 11-day-old chick embryos was also determined with aliquots of these mixtures after 2 hr incubation.

Fractionation procedures

Diethylaminoethyl (DEAE)-cellulose chromatography. Chromatography of the delipidized guinea pig postheparin plasma euglobulin was done in a column (1.0 cm by 42.0 cm) containing washed DEAE-cellulose (Selectacel, Brown Co.) and maintained at 4°C. The column and delipidized euglobulin fraction were equilibrated with a starting 0.01 M phosphate buffer at pH 7.8.

After application of from 5.0 to 7.0 ml of the delipidized euglobulin fraction to the column (6 to 10 mg protein/ml), the effluent was collected with the starting 0.01 M phosphate buffer, pH 7.8. The eluates were collected by using a linear gradient of NaCl in the pH 7.8, 0.01 M phosphate buffer. Three cylinders of a Varigrad apparatus were used for the NaCl gradient. The

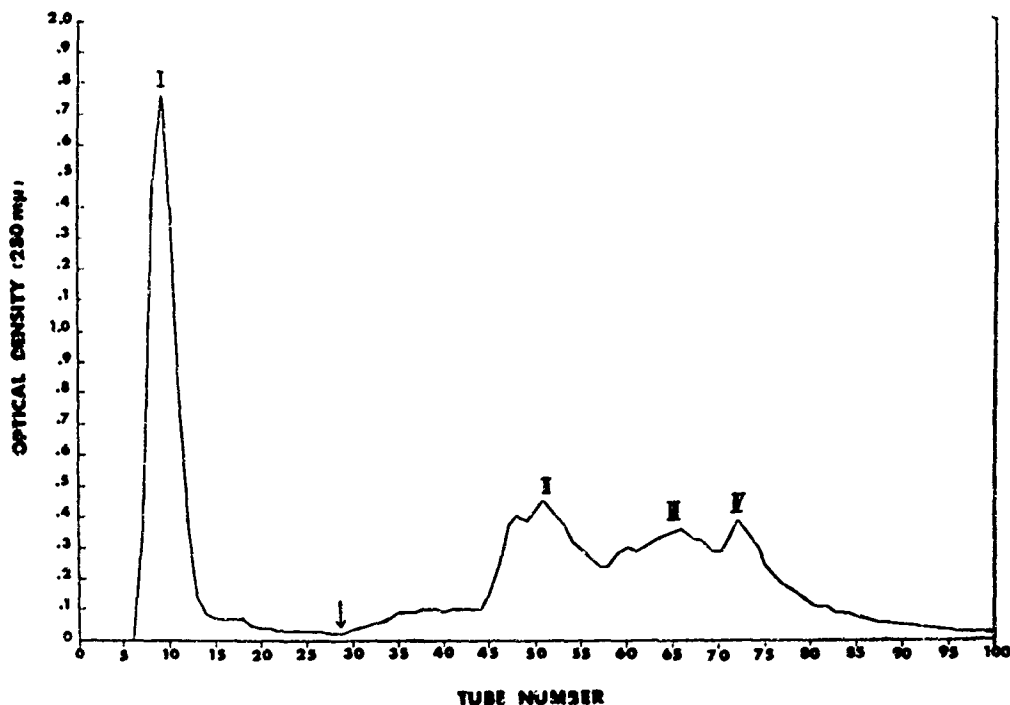


Figure 1. Fractionation of the delipidized euglobulin fraction from guinea pig postheparin plasma by diethylaminoethyl-cellulose chromatography. The arrow shows the beginning of the NaCl-gradient (see text).

first cylinder contained 35.0 ml of the starting phosphate buffer, the second cylinder contained 17.5 ml of the starting buffer and 17.5 ml of starting buffer with 0.25 M NaCl, and the third cylinder contained 35.0 ml of starting buffer with 0.25 M NaCl.

The 3-ml fractions were collected in a fraction collector and the optical densities were determined in a Beckman DU spectrophotometer at 280 m μ .

Except for preliminary experiments, all fractions were collected in tubes which contained heparin in a final concentration of 1.8 μ g/ml. Samples within the protein peaks were pooled immediately, concentrated by ultrafiltration and stored at 0°C for approximately 12 hr until used.

Ultrafiltration. A model 50 Diaplex Ultrafil cell was utilized (Amicon Corporation, 280 Binney Street, Cambridge, Mass.). The UM-1 Diaflo membrane was used which had a flow rate of 0.5 ml/min (2). The ultrafilter cell was enclosed in an ice jacket and maintained at approximately 4°C during the filtration pro-

cedure. The ultrafiltration at 4°C reduced the flow rate by approximately 50% when compared to the same procedure at room temperature.

Immuno-electrophoresis. The fractions were characterized by immuno-electrophoresis using the micro-modification of Scheidegger (3). A pooled antiserum from rabbits which were immunized with the guinea pig postheparin plasma euglobulin fraction was used.

RESULTS

DEAE-cellulose fractionation. The delipidized euglobulin fraction from guinea pig postheparin plasma was fractionated on DEAE-cellulose and the fractions were tested for their ability to protect 11-day old chick embryos against the lethal effects of endotoxin. A total of eight column fractionations were done, using delipidized euglobulin prepared from eight different pools of fresh guinea pig postheparin plasma. Figure 1 is a representative elution diagram.

One peak of protein came through with the 0.01 M phosphate buffer (Fig. 1). On application of the NaCl gradient, two or three further

TABLE I

Effect of euglobulin fractions of guinea pig post-heparin plasma on toxicity of endotoxin, release of unesterified fatty acids, and the endotoxin clearing reaction

Fraction	Endo- toxin Dose per Egg	Mortality (Deaths/ Total Eggs) ^a	Dead	Unester- ified Fatty Acids Released	Endo- toxin Clearing (Δ OD) ^b
	μ g		%	μ moles ^c	
Euglobulin	0.025	12/12	100	0.52	0.11
	0.0125	8/14	57		
Delipidized euglobulin	0.025	10/12	83	0.052	0.04
	0.0125	8/14	57		
Peak I ^d	0.025	11/12	92	0.016	0.025
	0.0125	12/14	86		
Peak II ^d	0.025	11/12	92	0	0.01
	0.0125	10/14	71		
Peak III ^d	0.025	12/12	100	0.013	0
	0.0125	10/14	71		
Buffer control	0.025	11/12	92	0.0065	0
	0.0125	11/12	92		
	0.0063	8/12	67		
	0.0031	0/10	0		

^a Embryos incubated for 24 hr, 37°C.

^b Δ OD (650 m μ) represents the difference between optical density at time 0 and 2 hr incubation at 37°C minus the buffer control.

^c Represents the difference between unesterified fatty acids at time 0 and 2 hr at 37°C on incubation with endotoxin.

^d Tube contents pooled and concentrated from diethylaminoethyl cellulose column.

peaks of protein appeared. The fractions obtained before the gradient was applied (peak I) usually contained only two proteins, a γ - and β -globulin, detectable by immunoelectrophoretic analysis. The fractions within peak II contained 3 to 4 β -globulins, those within peak III contained 2 β -globulins and 1 α -globulin, and peak IV contained 1 β -globulin and 1 α -globulin. One of the β -globulins was identified as the third component of complement and was usually found in the first peak after the application of the gradient.

Effect of DEAE-cellulose fractions on endotoxin toxicity. Five milliliters of the delipidized euglobulin were fractionated on the DEAE-

cellulose column. The fractions were collected in the absence of heparin. The elution diagram was similar to the one shown in Figure 1 showing one protein peak in the effluent, except that the demarcation between peaks III and IV was not clear. The fractions within each protein peak were pooled and 15.0 ml from peak I, 36.0 ml from peak II and 60.0 ml from peak III were concentrated to 4.5 ml by ultrafiltration (see Materials and Methods). The concentrated preparations were incubated with albumin and endotoxin (1 mg/ml) for 2 hr at 37°C and tested for toxicity for chick embryos.

Table I shows that both the euglobulin and delipidized euglobulin fraction, after incubation with albumin and endotoxin, caused detoxification of the endotoxin when compared to the buffer control. The pooled and concentrated preparations from peaks I, II and III, after incubation with albumin and endotoxin, caused little or no detoxification of the endotoxin.

Unesterified fatty acids were also measured in this experiment. After incubation of the euglobulin or delipidized euglobulin fraction with endotoxin and albumin, 0.52 and 0.052 μ moles of unesterified fatty acid were released, respectively. Essentially, no unesterified fatty acids were released after incubation of the pooled, concentrated fractions from the three peaks with endotoxin and albumin.

In results not shown here, the starting euglobulin, the delipidized euglobulin and the pooled, concentrated fractions within the protein peaks were incubated with lipemic guinea pig plasma (chylomicrons), and albumin, and the unesterified fatty acids were measured. Although 1.2 μ moles of unesterified fatty acid were released from the euglobulin mixture and 0.59 μ moles from the delipidized euglobulin mixture, no unesterified fatty acids were released from the mixtures which contained the concentrated fractions from the three protein peaks.

The change in optical density (Δ OD) is also shown in Table I. A value of 0.11 was obtained with the euglobulin-albumin-endotoxin mixture and 0.04 with the delipidized euglobulin mixture. However, a significant decrease of 0.025 O.D. units was obtained with the concentrated fractions of peak I; no decrease occurred with the other fractions.

The results of this column fractionation indicated that either the DEAE-cellulose had

destroyed the endotoxin detoxifying factor, as well as lipase activity, or an essential co-factor was removed by the cellulose.

Restoration of endotoxin detoxification activity with heparin. It was shown by Robinson (4) and also by Korn (5) that heparin is an essential co-factor for the enzyme, lipoprotein lipase. Robinson (4) demonstrated that on passage of postheparin plasma down an anion-exchange column the lipoprotein lipase lost activity after it was incubated at 37°C for 10 min. He showed that heparin, which is strongly anionic, was partially or totally retained on the anion exchange resin. The inactivation of lipoprotein lipase after incubation at 37°C was prevented by addition of heparin to the fractions from the effluent.

Thus, in two subsequent experiments, heparin was added to each fraction collection tube so that each tube contained a final concentration of 1.8 µg/ml. The delipidized euglobulin (6.3 ml) was placed on a DEAE-cellulose column and fractionated exactly as above, the only difference being the heparin in the collection tubes. The samples within the peaks were pooled and concentrated by ultrafiltration. Twenty-one milliliters from Peak I (Fig. 1), was concentrated to 5.3 ml, 39 ml from peak II was concentrated to 4.4 ml, 30 ml from peak III was concentrated to 4.2 ml and 30 ml from peak IV was concentrated to 4.8 ml. These concentrated preparations were incubated with albumin and endotoxin for 2 hr at 37°C and tested for toxicity for chick embryos.

Table II demonstrates that both the euglobulin and delipidized euglobulin, incubated with albumin and endotoxin, detoxified the endotoxin when compared to the buffer control.

Of the pooled, concentrated fractions from the four protein peaks, only peak I caused significant endotoxin detoxification.

The release of unesterified fatty acids was also measured in these experiments (Table II). Almost as much unesterified fatty acid was released (0.033 µmoles) following incubation of the pooled, concentrated fractions from peak I with endotoxin as when the delipidized euglobulin was used (0.049 µmoles). Almost no fatty acid release was caused by the pooled, concentrated peaks II, III and IV.

In results not shown here, the euglobulin fractions were also incubated with lipemic

TABLE II

Effect of heparin on the euglobulin fractions of guinea pig postheparin plasma on toxicity of endotoxin, release of unesterified fatty acids, and the endotoxin clearing reaction

Fraction	Endotoxin Dose per Egg	Mortality (Deaths/Total Eggs) ^a	Dead	Unesterified Fatty Acids Released	Endotoxin Clearing (ΔOD) ^b
	µg		%	µmoles ^c	
Euglobulin	0.0125	6/12	50	0.50	0.14
	0.0063	3/14	21		
Delipidized euglobulin	0.0125	6/12	50	0.049	0.04
	0.0063	2/14	14		
Peak I (tubes 7-13) ^d	0.0125	7/12	58	0.033	0.03
	0.0063	3/14	21		
Peak II (tubes 45-57) ^d	0.0125	11/12	92	0.0078	0.01
	0.0063	7/14	50		
Peak III (tubes 59-68) ^d	0.0125	9/12	75	0.0078	0.02
	0.0063	3/12	67		
Peak IV (tubes 71-80) ^d	0.0125	11/12	92	0.0033	0.01
	0.0063	10/12	83		
Buffer control	0.0125	11/12	92	0.0033	0
	0.0063	8/12	67		
	0.0031	3/12	25		

^a Embryos incubated for 24 hr, 37°C.

^b ΔOD (650 mµ) represents the difference between optical density at time 0 and 2 hr incubation at 37°C minus the buffer control.

^c Represents the difference between unesterified fatty acids at time 0 and 2 hr at 37°C on incubation with endotoxin.

^d Tube contents collected in heparin (1.8 µg/ml), pooled, and concentrated from diethylaminoethyl cellulose column, Figure 1.

guinea pig plasma (chylomicrons) and albumin, and the unesterified fatty acids were measured after incubation for 2 hr at 37°C. When the euglobulin fraction was incubated with chylomicrons and albumin, 1.5 µmoles of unesterified fatty acid were detected, but this was reduced to 0.27 µmoles with the delipidized euglobulin. Incubation of the pooled, concentrated fractions from peak I with chylomicrons and albumin caused the release of 0.039 µmoles of unesterified fatty acid. This value was slightly less than that obtained from the endotoxin-delipidized euglobu-

TABLE III
Effect of heat on euglobulin fractions of guinea pig postheparin plasma on toxicity of endotoxin

Fraction	Endotoxin Dose per Egg	Mortality (Deaths/Total Eggs) ^a in Experiment		Dead in Experiment	
		1	2	1	2
Euglobulin	0.025	6/15	N.D. ^b	40	
	0.0125	4/15		27	N.D.
	0.0063	1/14		7	
Euglobulin (heated) ^c	0.0125	6/15	N.D.	40	N.D.
Delipidized euglobulin	0.025	10/15	12/15	67	80
	0.0125	3/15	5/15	20	33
	0.0063	2/14	0/15	14	0
Delipidized euglobulin (heated) ^c	0.025	N.D.	12/15	N.D.	80
	0.0125	6/15	7/15	40	47
	0.0063	N.D.	0/14	N.D.	0
Buffer control	0.0125	14/15	9/14	93	64
	0.0063	7/15	6/14	47	43
	0.0031	0/14	0/14	0	0

^a Embryos incubated for 24 hr, 37°C.

^b Not done.

^c Heated 56°C, ½ hr.

lin mixture in Table II (0.049 μ mole), but approximately the same as that obtained from the peak I endotoxin mixture (0.033 μ mole). Essentially, no unesterified fatty acids were released when the pooled concentrated fractions from peaks II, III and IV were incubated with chylomicrons and albumin.

Finally, the Δ OD of each of the euglobulin-endotoxin mixtures was determined. The clearing activity of the concentrated fractions from peak I (0.03) was slightly less than that of the starting delipidized euglobulin (0.04). The concentrated fractions from the remaining peaks had little or no clearing activity.

A second column fractionation experiment was carried out in exactly the same way as described above and identical results were obtained.

A comparison of the results obtained from the column chromatography experiments (Tables I and II) demonstrates the following: 1) The

addition of 1.8 μ g/ml of heparin to the collection tube was essential for maintaining the endotoxin detoxifying activity during the column fractionation. 2) The endotoxin detoxifying factor was found in the effluent (peak I) after the column fractionation, but not in the fractions collected after application of the NaCl-gradient.

The lipoprotein lipase activity, although greatly reduced, was also found in peak I.

Effect of heating euglobulin. Unheated and heated (56°C, ½ hr) aliquots of both the postheparin plasma euglobulin and the delipidized euglobulin were incubated with albumin and endotoxin, and the mixtures were injected into chick embryos. Table III reveals that in Experiment 1 93% of the chick embryos died after injecting 0.0125 μ g of endotoxin from the buffer control. The injection of 0.0125 μ g of endotoxin from the unheated euglobulin mixture caused 27% of the chick embryos to die and 40%, if the euglobulin was heated.

To test the heat stability of the delipidized euglobulin fraction, preparations were made from two different postheparin pools and utilized in Experiments 1 and 2. Only 20% of the embryos died after injecting 0.0125 μ g of endotoxin from the unheated delipidized euglobulin

TABLE IV
Effect on toxicity of endotoxin^a of euglobulin fractions from guinea pig postheparin plasma with incubation time

Fraction	Incubation Time	Mortality (Deaths/Total Eggs) ^b	Dead
Delipidized euglobulin	0	12/14	86
	10	11/14	79
	20	11/14	79
	40	8/14	57
	60	6/14	43
Delipidized euglobulin (heated) ^c	0	12/14	86
	10	8/14	57
	20	8/14	57
	40	8/14	57
	60	5/14	36
Buffer control	0	13/14	93
	60	13/14	93

^a Endotoxin dose per egg was 0.0125 μ g.

^b Embryos incubated for 24 hr, 37°C.

^c Heated 56°C, ½ hr.

mixture (Exp. 1) and 40% died after the delipidized euglobulin fraction was heated. Essentially the same results were obtained in Experiment 2. Experiment 2 also shows that no embryos died when both the heated and unheated fractions were incubated with albumin and endotoxin, and 0.0063 μg of the endotoxin was injected, in comparison to 43% with the buffer control.

Although not shown here, endotoxin clearing and the release of unesterified fatty acids were also measured in these experiments. The ΔOD of the unheated euglobulin-albumin-endotoxin mixture was 0.105. This was reduced to 0.015 after the euglobulin was heated, a reduction of 86%. Similarly, 0.14 μmoles of unesterified fatty acid were released from the unheated euglobulin-albumin-endotoxin mixture, but this was reduced to 0.04 μmoles from the heated mixture.

When the delipidized euglobulin fraction was tested for lipase activity with chylomicrons as the substrate, 0.49 μmoles of unesterified fatty acid were measured from the unheated delipidized

euglobulin mixture. This was reduced to 0.01 μmoles after the heat treatment.

From these data, it is apparent that the endotoxin detoxifying factor found in purified fractions of postheparin plasma was partially heat stable, at least at the concentrations of euglobulin used. The endotoxin clearing and lipase activities were greatly reduced, but not completely destroyed, by this treatment.

Time course of detoxification by heated and unheated euglobulin. Unheated and heated delipidized euglobulin were incubated with albumin and endotoxin for 1 hr at 37°C and aliquots were removed at selected time intervals to measure endotoxin toxicity for chick embryos, unesterified fatty acid release and endotoxin clearing. For each time period, 0.0125 μg of endotoxin was injected per embryo. Incubation of the unheated delipidized euglobulin with albumin and endotoxin showed that 86% of the embryos died at 0 time (Table IV). Endotoxin toxicity diminished until after 1 hr incubation, and only 43% of the embryos were dead. With the heated

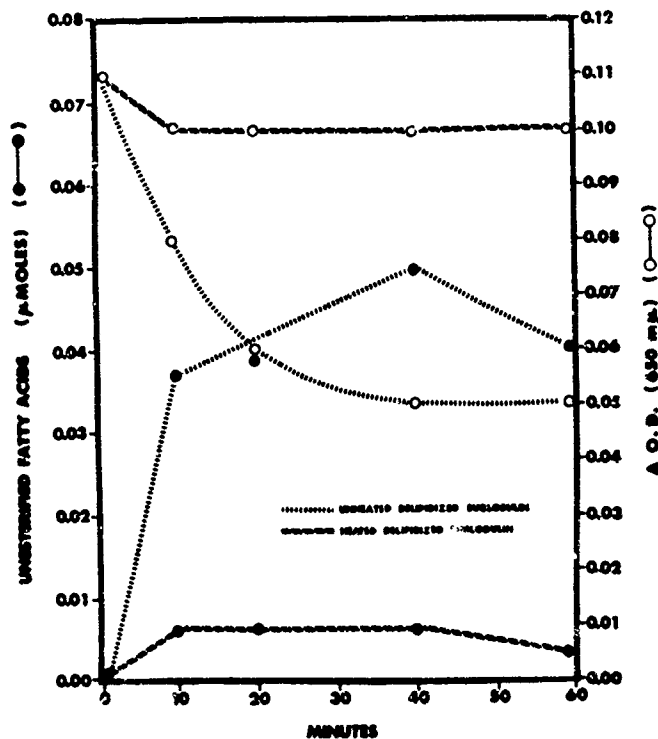


Figure 2. Release of unesterified fatty acids, and endotoxin clearing, after the heated (56°C, ½ hr) and unheated delipidized guinea pig euglobulin fraction were incubated with *Escherichia coli* endotoxin.

preparation, a similar diminution in endotoxin toxicity occurred until, after 60 min, only 33% of the embryos died. These observations with the unheated and heated fractions differed significantly from the buffer control, where 93% of the embryos died.

In Figure 2, the results of the unesterified fatty acid measurement and the endotoxin clearing reaction are shown. When the unheated delipidized euglobulin was incubated with albumin and endotoxin, an increasing quantity of unesterified fatty acids was measured with time. More unesterified fatty acids were measured at the 40-min interval than after 60 min which may be due to oxidation of the liberated fatty acids. The reduction in titratable unesterified fatty acids was also observed when the experiment was repeated.

It is also apparent that the unheated delipidized euglobulin caused a progressive decrease in endotoxin turbidity with time. By heating the fraction, both the clearing reaction and the lipase activity were practically abolished.

DISCUSSION

In the preceding paper, a parallel was found between the endotoxin detoxifying action of guinea pig postheparin plasma or its euglobulin fraction and the presence of lipoprotein lipase (1). Only after guinea pigs were injected with heparin was their plasma capable of detoxifying endotoxin and only then did they show lipoprotein lipase action. Both the endotoxin detoxifying factor and lipoprotein lipase were found in the euglobulin fraction and the lipid-rich portion of the euglobulin fraction after delipidization. The endotoxin detoxifying and lipoprotein lipase activities of the euglobulin both required the presence of serum albumin for their demonstration.

We concluded from these observations that even though all the evidence showed that the endotoxin detoxifying factor and lipoprotein lipase are the same, the evidence was indirect and insufficient. In this investigation, we attempted to add more evidence to the hypothesis that lipoprotein lipase can detoxify endotoxin.

Initial attempts to purify the endotoxin detoxifying factor and lipoprotein lipase by DEAE-cellulose chromatography without the addition of heparin were unsuccessful (Table I). As Robinson (4) did for lipoprotein lipase, heparin

was added to the collection tubes prior to chromatography of the delipidized euglobulin fraction. It was found, after collecting the fractions in tubes containing heparin, that both the endotoxin detoxifying factor and lipoprotein lipase were active and both activities were found only in the fractions from the effluent (Table II, peak I). The results indicate that heparin is required not only for the lipoprotein lipase activity of postheparin guinea pig plasma, but also for the endotoxin detoxifying activity as well. This is a further similarity and a remarkably close one between the two activities. The use of heparin also allowed the demonstration in the same experiments that, after DEAE-cellulose chromatography, lipoprotein lipase and endotoxin detoxifying activity were present in the same fraction. Here, also, the two activities behaved the same.

Heating the euglobulin or delipidized euglobulin fractions at 56° for ½ hr was seemingly successful in separating the two activities. There was little or no change in endotoxin detoxifying activity after heating, but the lipoprotein lipase activity was reduced to negligible amounts. The interpretation of the differential effect of heat on the two activities is uncertain. The simplest interpretation and one which cannot be contradicted by presently available evidence is that the two activities are the property of two different molecular species with different heat stabilities. An alternative explanation is that the apparent differences in heat stability reflect differences in the steepness of the dose-response curves given by the two activities; the endotoxin detoxifying activity giving a very shallow, and the lipoprotein lipase activity a very steep one. That this is not wholly an hypothesis of desperation is seen in Table V, Experiment 2, of the preceding paper. In this experiment, there was no difference detectable in the endotoxin detoxifying activity of 0.5 ml, 0.25 or 0.125 ml of a euglobulin preparation. In addition, Skarnes *et al.* (6) found that when relatively large amounts of the endotoxin detoxifying component in human plasma were tested, after heating at 56° for an hour, the activity appeared heat stable. However, when minimal amounts were compared the heat lability of the endotoxin detoxifying activity was clearly evident.

It is obvious that despite the several and detailed similarities in behavior of lipoprotein

lipase and endotoxin detoxifying activity, under widely varying conditions, there is no clear-cut evidence that they are two activities of the same molecule. Neither is the apparent difference in heat stability of sufficient assurance that the two activities are properties of different molecules. Further purification of both activities is indicated before any conclusion should be drawn. The results described here and in the preceding paper (1) indicate that such purification should be worthwhile.

Initially, it was thought that lipoprotein lipase was responsible for the endotoxin-clearing reaction. This was based on observations that, under our experimental conditions, the euglobulin fraction from either normal guinea pig serum or plasma did not clear endotoxin, but the postheparin plasma euglobulin fraction did. Furthermore, both the endotoxin-clearing reaction and lipoprotein lipase activity were abolished by heat. It was thought that lipoprotein lipase may clear endotoxin by disaggregating the macromolecules in a manner similar to that described for sodium dodecyl sulfate (7) or sodium deoxycholate (8). The observation that the peak I fractions collected without the addition of heparin cleared endotoxin, but had no detoxifying or lipase activity suggests that lipoprotein lipase is not involved in the endotoxin-clearing reaction. Extension of the same reasoning also suggests the clearing and detoxifying reactions are independent.

Endotoxin is a complex molecule whose biologic properties can probably be modified in a number of different ways. Comparison of the work reported here with some of the published work of others is in accord with this view. Skarnes (9) has reported that the disaggregation of endotoxin was related to the presence of a heat-stable α_1 -lipoprotein esterase. After disaggregation, a heat-labile α_1 -globulin esterase was thought to detoxify endotoxin. Because of many notable differences between the endotoxin disaggregating and detoxifying factors of Skarnes and the postheparin plasma factor, they are probably different entities. However, it is interesting that Skarnes mentioned a β -lipoprotein fraction of human serum which degraded endotoxin.

A hypothesis has been presented by Rudbach *et al.* (8) to explain the detoxification of endotoxin by plasma: endotoxin is first depolymerized

by substances in plasma with surfactant properties into smaller molecular weight units, which are then bound by plasma proteins. By using the proteolytic enzyme pronase, the endotoxin-bound protein was digested off, followed by ethanol precipitation (10). This treatment restored the endotoxin activity. There seem to be clear-cut differences between our results and those reported by Rudbach *et al.* (8)

SUMMARY

Fractionation of the delipidized euglobulin from guinea pig postheparin plasma on DEAE-cellulose resulted in one protein peak in the effluent and two to three protein peaks following an NaCl-gradient. Both endotoxin detoxifying action and lipoprotein lipase activity were found in the fractions prior to the application of the NaCl-gradient, if the fractions were collected in heparin. Without added heparin, neither of the two activities was obtained. Associated with both detoxifying and lipase activities was the release of unesterified fatty acids and the endotoxin-clearing reaction.

Heating the postheparin plasma euglobulin fraction either had no effect or partially inactivated the endotoxin-detoxifying factor, but destroyed lipoprotein lipase activity. Both unesterified fatty acid release from the endotoxin-albumin-euglobulin mixtures and the endotoxin-clearing reaction were destroyed by heat.

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