Enzymatic Activity Related to Human Serum Beta-Liprotein: Histochemical, Immuno-Electrophoretic and Quantitative Studies.* (26824)

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Histochemical technics have been successfully adapted for demonstration of enzymatic activity following starch gel electrophoresis of blood serum(1,2). In the present study these technics for 11 enzymes were applied to human serum following immuno-electrophoresis. A striking localization of activity of all 11 enzymes was found in the beta-lipoprotein precipitin line. The enzymatic activity of beta-lipoprotein fractions separated by an ultracentrifugal procedure was investigated by quantitative methods. The results suggest that beta-lipoprotein may function as a carrier of certain serum enzymes in an inactive state.

Materials and methods. Immuno-electrophoresis of human serum was carried out as described by Grabar(3). Histochemical enzyme stains were then done on the immuno-electrophoresis preparations in the same way as was previously described for starch gel electrophoresis(1,2) for the following 11 enzymes: cholinesterase, esterase, acid phosphatase, alkaline phosphatase, beta-glucuronidase, aminopeptidase, succinic, lactic, beta-hydroxybutyric and glutamic dehydrogenases and cytochrome oxidase.

Standard quantitative procedures were also carried out on whole serum and on purified beta-lipoprotein and other fractions for determination of activity of the following enzymes: transaminase(4), acid and alkaline phosphatases(5), beta-glucuronidase(6), lactic dehydrogenase(7), lipase(8), isocitric dehydrogenase(9), malic dehydrogenase(10), aminopeptidase(11), amylase(12) and cholinesterase(13).

Beta-lipoprotein (low density lipoprotein) was prepared in the Spinco Model E ultracentrifuge after adjusting the specific gravity of plasma to 1.063 with a saturated solution of equal parts of KBr and $NaNO_3$. Outdated bank blood was used as a source of plasma, but all essential findings were checked with serum or plasma freshly drawn from laboratory personnel volunteers. After centrifuging at 40,000 RPM for 12 hours the top ml of milky solution (beta-lipoprotein) was pipetted off. The bottom of the 1.063 tubes was then pooled, specific gravity adjusted to 1.21 and the pool recentrifuged at 40,000 RPM for 24 hours. The 2 ml of milky fluid at the top was carefully collected and designated alpha (high density) lipoprotein (14). Analytical ultracentrifugation was done by the method of De Lalla and Gofman (15).

Results. Histochemical methods. Fig. 1 shows enzymatic activity of the 11 enzymes demonstrated by histochemical methods in immuno-electrophoretic preparations of human serum. In each preparation whole human serum is above the trough and purified human beta-lipoprotein below with anti-human goat serum in the trough.†

The localization in all cases of enzyme activity in the beta-lipoprotein precipitin line is apparent. There is also activity in the area between the origin and the beta-lipoprotein precipitin line. Some enzymes showed activity elsewhere on the slide. Enzymatic activity in the beta-lipoprotein arc was also obtained against anti-whole serum and anti-beta-lipoprotein serum on Ouchterlony plates; examples stained for protein and 2 enzymes are illustrated in Fig. 2.

Hydrolytic enzyme controls containing no substrate or with boiled serum or beta-lipoprotein showed no enzymatic activity. In the DPN-linked enzymes for which the tetrazolium salt Nitro BT was used, minimal staining was seen in the beta-lipoprotein line

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† Hyland Laboratories, Los Angeles, Calif.

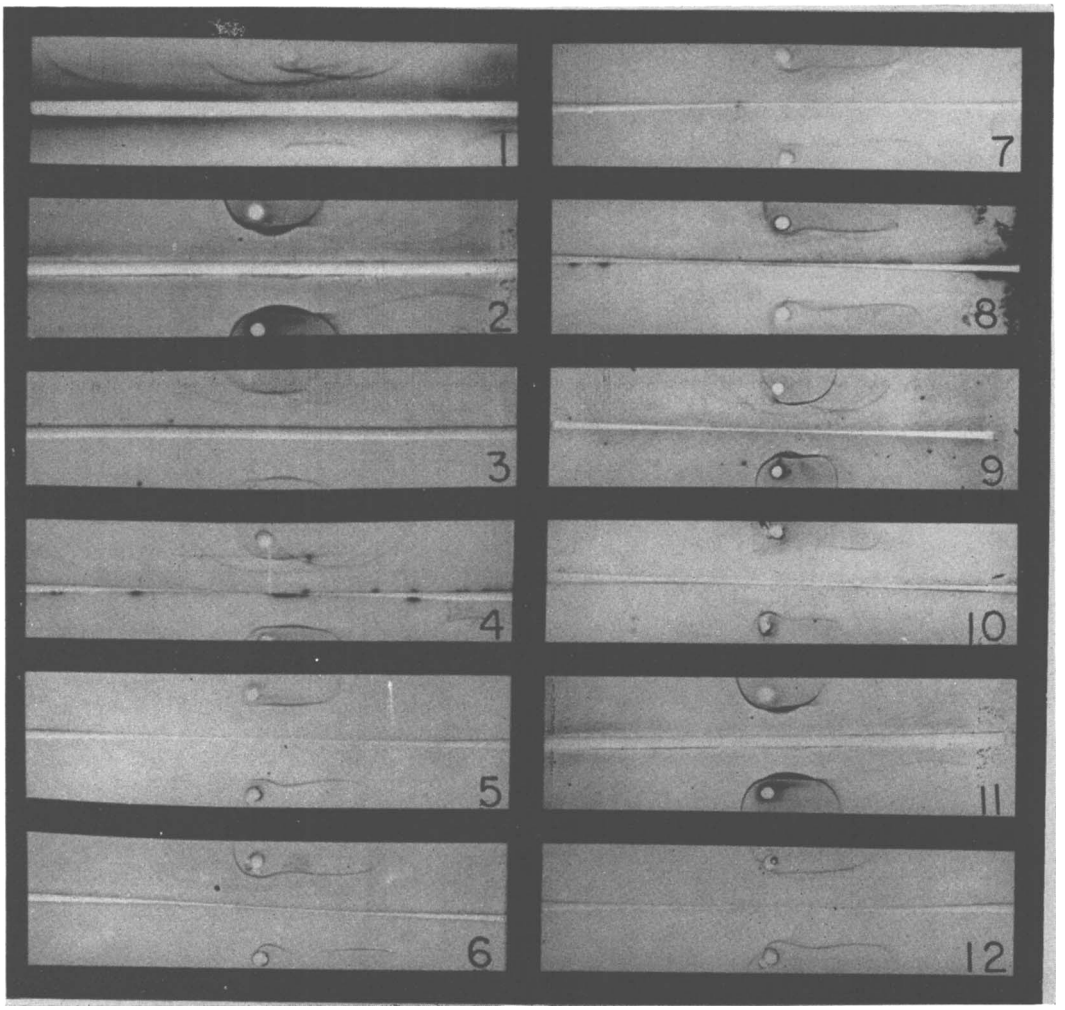

FIG. 1. Immuno-electrophoretic pattern of whole human serum (top) *vs* purified human lipoprotein (bottom). Anti-human goat serum is in trough. 1, Protein stain; 2, succinic dehydrogenase; 3, acid phosphatase; 4, alkaline phosphatase; 5, aminopeptidase; 6, beta-glucuronidase; 7, cholinesterase; 8, esterase; 9, lactic dehydrogenase; 10, glutamic dehydrogenase; 11, cytochrome oxidase; 12, beta-hydroxybutyric dehydrogenase.

in controls containing no substrate. The intensity of the stain was increased by adding substrate. The non-specific staining was entirely eliminated when boiled serum or beta-lipoprotein was used.

Enzymatic activity of beta-lipoprotein fractions. The purified beta-lipoprotein was examined for activity of the 12 enzymes listed above. Activity in all cases was below the normal serum level and the serum or plasma from which it was made, whether out-dated bank or fresh, showed levels within normal limits. After the beta-lipoprotein fraction had been exposed to sonic oscillation‡ (250 watts, 10 KC) for 40 minutes the enzymatic activity of 8 of the 12 enzymes tested was well above the normal serum level although the lipoprotein fraction represented only the top one ml from a 10 ml sample of normal serum following ultracentrifugation. Cholinesterase and alkaline phosphatase were markedly increased in the beta-lipoprotein fraction by sonic oscillation but the activity did not exceed normal serum levels. Aminopeptidase

‡ Raytheon Mfg. Co., Waltham, Mass. Sonic oscillator.

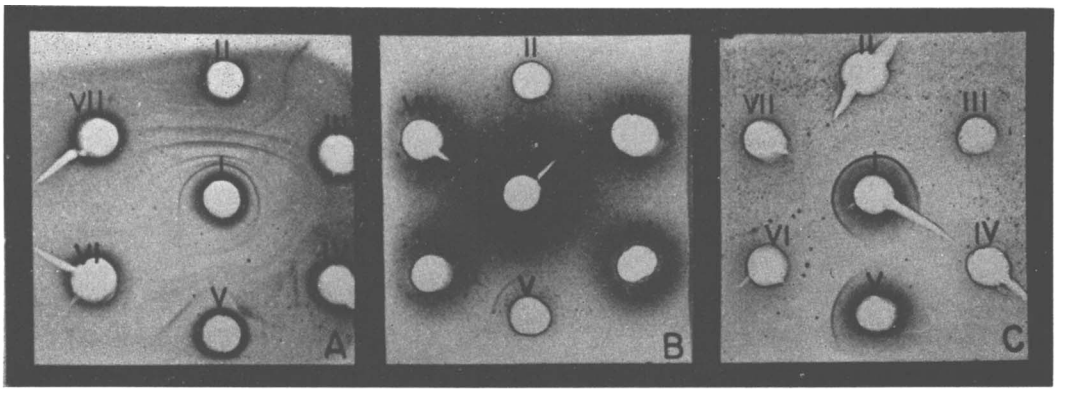

FIG. 2. Ouchterlony plates. A, protein stain; B, cholinesterase stain; C, succinic dehydrogenase stain. I, human serum; II, anti-human goat serum; III, anti-human beta-lipoprotein goat serum; IV, anti-human beta-2 macroglobulin (Waldenström) goat serum; V, human beta-lipoprotein + II; VI, II + IV; VII, III + IV.

activity was not affected by sonic treatment and no amylase activity was found either before or after treatment (Table I). Analytical ultracentrifugal analysis of the beta-lipoprotein fraction before and after sonic treatment is shown in Table II.

The effect of sonic oscillation is illustrated by the transaminase results (Fig. 3). Purified beta-lipoprotein showed activities varying from 3 to 10 units per ml in various preparations. After 40 minutes in the sonic oscillator this activity had increased to over 50 units per ml. After storing for several days the activity was again reduced, and could be restimulated to even higher levels by reexposure to sonic frequencies.

Freezing and thawing, addition of dinitrophenol, ether extraction, and bubbling oxygen through the mixture were also effective to varying degrees in liberating enzyme activity from the purified lipoprotein.

High density lipoprotein (s.g.>1.063) showed no activity either by quantitative technics or immuno-electrophoresis.

Discussion. The sequestration of enzymatic activity, as well as its liberation by physical methods, in beta-lipoprotein, is similar to observations on the enzymatic activity of mitochondria(16). Mitochondria are also known to contain lipoprotein(17). Apparently the relationship of beta-lipoprotein to mitochondria has never been investigated.

A possible interpretation of these findings is that beta-lipoprotein may form a relatively unstable complex with certain molecules whose activity is normally intracellular thereby carrying them in the circulatory system in an inactive state. The complex is probably a

TABLE I. A Comparison of Activity of Purified and Activated Beta-Lipoprotein with Whole Serum.

Enzyme	Activity: Serum units	Activity: Purified beta-lipoprotein†	Activity: Activated beta-lipoprotein†	Units	Ref.
Transaminase SGO-T	8 - 40/ 1 ml	5	150	Karmen	4
" SGP-T	5 - 35/ 1 ml	7	130	"	4
Acid phosphatase	0.5 - 1.5/100 ml	0	6	Bodanski	5
Beta-glucuronidase	42 - 319/ 1 ml	20	450	Fishman	6
Lactic dehydrogenase	100 - 350/ 1 ml	50	500	Sigma	7
Lipase	0 - 1/ 1 ml	0	3	Tietz, *et al.*	8
Isocitric dehydrogenase	47 - 264/ 1 ml	20	350	W-WA	9
Malic "	42 - 195/ 1 ml	35	300	Siegel, *et al.*	10
L-Aminopeptidase	75 - 230/ 1 ml	75	75	G - R	11
Cholinesterase	40 - 80/ 1 ml	±	25	Rappaport	13
Alkaline phosphatase	2 - 4.5/100 ml	0	2	Bodanski	5
Amylase	80 - 150/100 ml	0	0	mg glucose liberated	12

† Avg of 2 or more determinations.

TABLE II. Ultracentrifugal analysis (mg/100 ml) of Beta-Lipoprotein Fractions.

	Sf° 0-12	Sf° 12-20	Sf° 20-100	Sf° 100-400
A	145.4	0	4.8	5
A*	37.7	0	14.9	25
B	134.5	4.8	4.8	5
B*	32.3	0	9.9	10

A. Prepared from plasma from outdated bank blood.

B. Prepared from fresh plasma.

* After 40 min. in sonic generator.

physical rather than a chemical one, inasmuch as it can be easily dissociated by physical means. Reinactivation of the enzyme-lipoprotein mixture on standing and liberation of the enzyme activity again on reexposure to sonic frequencies would seem to support this as would the fact that certain antigen-antibody reactions are potentiated by freezing and thawing(18). The term "sequestration" is proposed for this property of beta-lipoprotein.

Summary. Activity of 17 different enzymes was found to be present in human serum beta-lipoprotein by either standard quantitative or histochemical-immunoelectrophoretic technics. By the former methods the activity could be increased by several physical procedures which apparently affected the beta-lipoprotein molecular structure. The histochemical technics were annulled by boiling and were substrate specific except for slight reactions of the DPN dependent enzymes without substrate. Staining which occurred in these cases was more intense, however, when substrate was added. These observations suggest a possible new theory of beta-lipoprotein function: namely, that it may form a complex with enzymes as well as certain other molecules whose activity is normally intracellular thereby inactivating them while in the circulatory system.

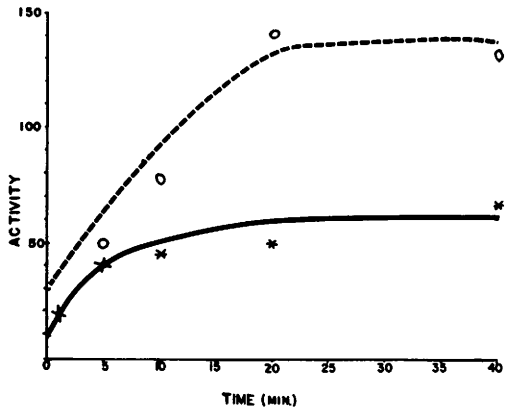

FIG. 3. Effect of sonic oscillation on transaminase (SGO-T) activity of beta-lipoprotein. Solid line, first exposure; broken line, re-exposure after 1 wk in cold room.

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