Release of Enzymic Activity from Human Beta-Lipoproteins by Sonic Radiation (36862)

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Human plasma beta-lipoprotein contains several enzymes in a sequestered form (1). Their activities are more demonstrable, that is, their levels of activity are increased, after physical trauma; one of the most effective means of doing this is to subject them to sonic radiation (2, 3). The objectives of this research were to test the possibility that this phenomenon might be an artifact of isolation, and to determine whether the nature of the sequestration could be elucidated by examining the effect of changes in pH and ionic strength upon the increase of enzymic activity after sonic radiation.

Methods and Materials. The beta-lipoprotein (low-density lipoprotein) used in this study was prepared in one of two ways; namely, by selective precipitation as the heparin complex using the method described by Burstein (4), or by centrifugation in the Spinco Model L Ultracentrifuge, following adjustment of the specific gravity of the plasma to a value of 1.063 by using a saturated solution of KCl and KBr (5). Both out-of-date blood bank plasma, or fresh, pooled human plasma were used as sources of the lipoprotein. No significant difference was noted in the observed data. The Beta-L-Immunocrit assay (Hyland Laboratories, Los Angeles, California) was used to determine the concentration and the "nativeness" of the lipoprotein solutions before and after sonication (see Discussion). Glutamic-Oxalacetic transaminase activity (GOT) was determined by the method of Babson as modified by Furno and Sheena (6). Glutamic-Pyruvic transaminase (GPT) was determined by the method of Reitman and Frankel (7). Lactate dehydrogenase (LDH) activity was determined by measuring the reaction velocity of NADH oxidation. This assay procedure measures the reduction in absorbance by NADH at 340 nm over the period of 3 to 5 min at 30-sec intervals in a buffered solution containing pyruvate, sonicated lipoprotein, and NADH (8).

Protein concentrations were determined using the biuret reaction.

All enzymic reactions were measured versus a "zero time" control, an aliquot of the solution added to the cup of the sonic oscillator. Appropriate blanks were utilized to correct for absorbance due to the turbidity of the assay solutions caused by sonation. Exposure to sonic radiation was carried out at 4° in a Raytheon DF 101 Sonic Oscillator (250 watts, 15 watts/cm^2, 10 kc) with about 10 ml of lipoprotein or other solution in the cup. Viscosity measurements were made at 4° using an Ostwald tube calibrated with water at the same temperature. Density determinations were also made at 4° using an analytical balance modified for that purpose. Measurements of pH were made at room temperature.

Results and Discussion. Enzymic activity of the beta-lipoprotein fraction. The purified beta-lipoprotein used in these experiments was isolated from human plasma utilizing the procedure of Burstein. This procedure is performed at a neutral pH, in solutions of low ionic strength, which is in sharp contrast to the high salt concentrations utilized in the centrifugal procedure. Lipoprotein, from the same plasma sample, isolated by both procedures, and subjected to sonic radiation, gave similar results when the levels of LDH were determined. Direct comparison of the levels of enzymic activity, obtained by the two different methods, was not practicable because they were contained in solutions of different ionic strength and composition. The

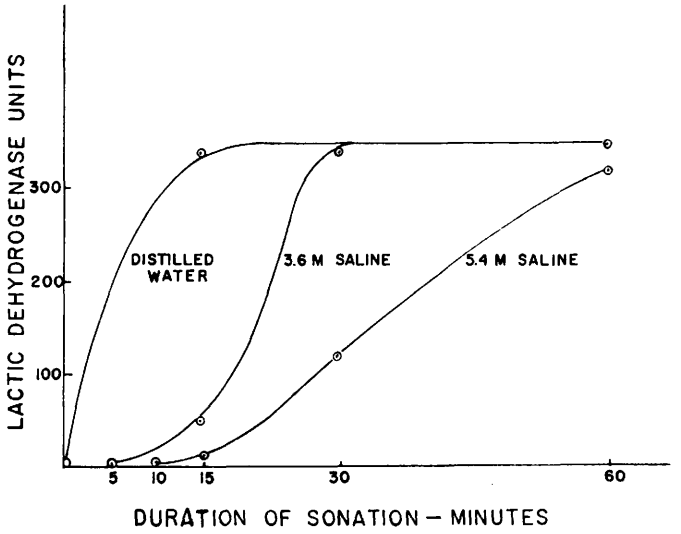

FIG. 1. Effect of NaCl concentration on release of enzymic activity.

effect of ionic strength on the liberation of enzymic activity in unbuffered solutions of beta-lipoprotein is shown in Fig. 1. The time course of the appearance of LDH activity is strongly dependent on salt concentration but the final level is not. It was interesting to observe that the activity appeared earliest in distilled water in which the isolated lipoprotein is relatively insoluble. Similar data were obtained in experiments when GOT and GPT activity were measured.

The appearance of enzymic activity also depends upon the pH of the medium in which the lipoprotein is suspended (Fig. 2). The buffer used was a solution of citrate, phosphate, and histidine; 0.01 *M* with respect to each. Although the buffering ion was different at each pH, the solution was essentially the same at the pH of the assay solutions, thus eliminating the possibility of interferences in the assay procedure being responsible for the observed difference. These data do not distinguish between the possibilities of an anion effect and that of hydrogen-ion concen-

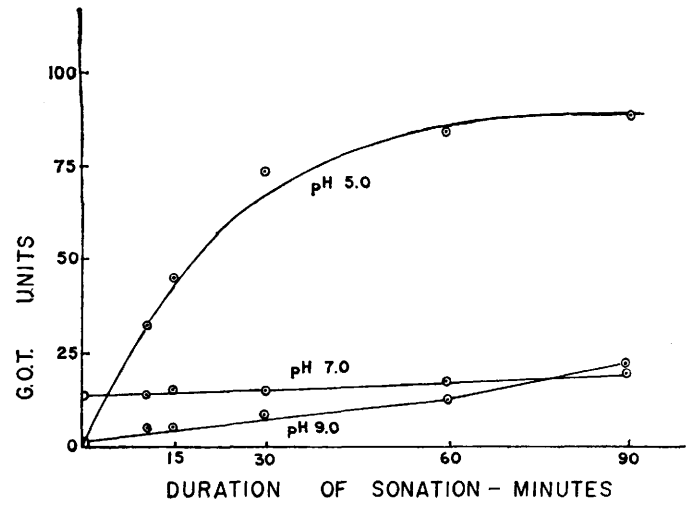

FIG. 2. Effect of pH on release of enzymic activity.

tration but other data implicate the latter. When the lipoprotein was dissolved in weakly buffered (0.01 *M*) solutions, exposure to sonic radiation was generally accompanied by a reduction in the pH and an increase in the viscosity. This is not a general response of solutions subjected to the same radiation (9) (Table I).

Liberation of enzymic activity was delayed by increasing the concentrations of sodium chloride and was inhibited by alkaline solutions. Sonic radiation of beta-lipoprotein solutions disrupts the structural integrity of the molecule as evidenced by a change from a clear yellow solution to a milky white one, an increase in the specific viscosity, and a reduced ability to react in the precipitin reaction. The change in appearance occurred in every instance but the change in viscosity was greatest in the more acidic solutions. If radiation was carried out for a very long time (more than one hour), the fat globules came out of solution and formed small spheres which were easily observed in the capillary while performing the viscosity measurements in the Ostwald tube.

Other effects of sonic radiation. The effect of sonic radiation on beta-lipoprotein was to reduce its reactivity with the anti-serum supplied in the Beta-L-Immunocrit assay. After 60 min of exposure, the amount of precipitate formed with the anti-serum was less than one-third that from the original sample (Table I). Beta-lipoprotein which had been "delipidized" (beta-protein) by ether extraction in the presence of sodium dodecyl sulphate did not form a precipitate with the anti-serum under the conditions of the immunocrit assay.

Enzymic activity of beta protein. The glutamic-oxalacetic transaminase activity of the beta-protein was determined using the method of Furno and Sheena. The level of activity was low but was still about four times that of plasma in terms of the original lipoprotein concentration. Sonation of the beta-protein did not result in any detectable increase in enzymic activity (GOT and LDH).

Discussion. The report of several enzymes sequestered in the beta-lipoprotein fraction of human plasma suggested the possibility of

TABLE I. Changes Occurring After 60 Minutes of Sonation.

	pH room temp.		Specific viscosity seconds, 4°		Density 4°		Beta-L-Immunocrit mm of ppt.		Activity; GOT	
	Before	After	Before	After	Before	After	Before	After	Before	After
β-Lipoprotein	3.6	3.7	191.8	226.9	1.0025	1.0025	—	—	0	22
β-Lipoprotein	6.6	5.5	193.6	196.6	1.0017	1.0012	1.6	0.5	0	17
Albumin (0.1%)	5.2	6.9	186.3	183.8	1.0063	1.0055	0	—	—	—
Water	6.2	7.1	183.2	183.2	1.0000	1.0000	—	—	—	—

Specific viscosity: average of five trials, the mean deviation being ± 0.2 seconds.

a role hitherto unsuspected in the biological functions of lipoproteins (2). The nature of the sequestered enzymes and the manner in which their activity was released suggested that it might be possible to gain some insight into the nature of binding of enzymes to lipoprotein. In doing so, it might also be possible to explore the structure and function of beta-lipoprotein molecules as they relate to these enzymic activities.

Lawrence and Melnick had reported the presence of enzymes in the beta-lipoprotein fraction isolated by preparative ultracentrifugation (2). Most of the lipoprotein used in our research was isolated as a heparin-lipoprotein complex under conditions quite different from those of the ultracentrifugal method. The finding of enzymic activity associated with lipoprotein when isolated by both procedures, and in the "beta-protein," is strong evidence against the possibility that this activity is the result of an isolation artifact. Such a possibility is suggested by the report of Levitova (10), who found LDH and GOT in a fraction associated with the ultracentrifugally isolated beta-lipoprotein fraction of rabbit plasma.

The data accumulated in this research are consistent with the physical-complex model proposed by Lawrence but the data do not rule out the possibility that some of the weaker chemical bonds found in proteins may play a role. This is suggested by the pH and ionic strength-dependence of the enzyme activation.

The structural degradation of the lipoprotein and the increased acidity of lipoprotein solutions subjected to sonic radiation may or may not be related to the release of enzymic activity although the pH dependence of the release seems to support this view. A simple interpretation of these observations is that acid catalysis is involved in the scission of bonds contributing to the structural integrity of the "native" lipoprotein molecule and its ability to sequester enzymes. Those scissions may or may not contribute specifically to the increase in H^+, but it is noteworthy that an increase in H^+ is not generally observed in solutions subjected to sonic radiation. In summary, then, the bonds binding the sequestered enzymes are most stable in an alkaline medium, labile in an acidic medium, and their cleavage is accompanied by an increase in hydrogen ion concentration. Liberation of enzymic activity by sonic radiation is accompanied by a gross disruption of the structural integrity of the lipoprotein molecule. Solutions turn from a clear yellow to a milky white, there is an increase in the specific viscosity, and decreased reactivity with the specific anti-serum is noted. Whether the reduction in the amount of precipitate in the immunological assay reflects an overall disruption of all of the lipoprotein molecules or simply a reduction in the total number of native (reactive) molecules was not determined.

Lawrence suggested that lipoproteins may serve as carriers for active molecules which are made inactive by sequestering them within the lipoprotein, but they may also serve other functions. Results of this research indicate that the free enzyme (GOT, as Versatol E, Warner-Chilcott Labs., Morris Plains, NY) is much less resistant to denaturation by sonic radiation than is the form which is bound to the lipoprotein (Fig. 3). This would seem to indicate that the sequestered complex may serve to protect as well as to inactivate. The resistance of intact beta-lipoproteins to proteolysis is well known (11) although they are also known to undergo marked changes due to oxidation, in the presence of traces of various metal ions, especially cupric ions (12). Furthermore, sequestration within lipoprotein moieties would allow the enzyme molecules to pass hydrophobic barriers normally impermeable to large polar molecules. It is possible that the presence of high concentrations of beta-lipoproteins in atherosclerotic plaques is a manifestation of this proposed function.

Summary. Glutamic-oxalacetic and lactic dehydrogenase activity associated with human beta-lipoprotein can be increased by exposure to sonic radiation. Maximum activity is reached earliest in solutions having a low salt concentration and an acidic pH. The increase in activity occurs concomitantly with an increase in hydrogen ion concentration and specific viscosity. Disruption of the

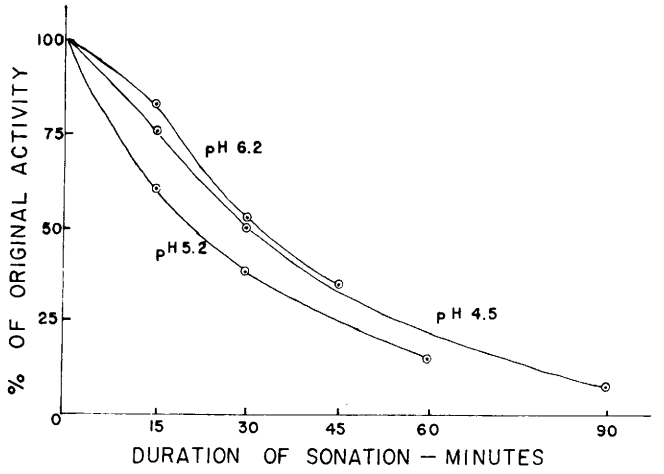

FIG. 3. Effect of sonation on the GOT activity of "Versatol-E."

lipoprotein molecule is evidenced by increased viscosity, increased turbidity, and decreased immunologic reactivity toward a specific antiserum. Enzymic activity found in beta-lipoprotein isolated by using two radically different techniques gave equivalent results when exposed to radiation. This strongly mitigates against the possibility of an isolation artifact.

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