# HISTOCHEMICAL METHOD OF ELICITING THE ENZYME LIPOPROTEIN LIPASE

(UDC 616.015.14.088.1)

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(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Bylleten' Éksperimental'noi i Meditsiny, No. 7,
pp. 123-125, July 1965
Original article submitted March 3, 1964

Considerable attention has been devoted in recent years to the role of the enzyme lipopretein lipase ("clearing factor") in experimental and clinical investigations into the problem of lipoidoses.

The great interest in this enzyme is explained by the fact that this is the only presently known enzyme participating in hydrolysis (and therefore in synthesis) of lipoproteins, i.e., lipids bound with proteins. The appreciable role of the changes of lipoproteins in the mechanism of the occurrence and development of atherosclerosis [6, 8] is the reason for clinicians, who are studying this problem, also being interested in this enzyme.

In addition to foreign scientists, investigations in this area have also been carried by Soviet authors [1-5, 7].

One of the interesting, but little studied, aspects of this problem is the localization of the enzyme in organs and tissues. The findings available on this problem are based only on a biochemical study of homogenates and perfusates of organs. Localization of the enzyme has not been studied histochemically owing to the absence of an appropriate method.

We attempted to develop a method for determining tissue and cellular localization of lipoprotein lipase.

## METHOD

As a substrate we used a medium which we developed (B. L. Lempert) on the basis of Korn's principle [9] for a biochemical determination of lipoprotein lipase. The medium was prepared in the following manner. In a compression colloid grinder designed by Gol'dshtein and Khotuntsev (gap size 0.2 mm, speed of rotor 45,000 rpm) we prepared a 50% emulsion of pure persic oil; as a stabilizer we used a 10% solution (based on the water phase) of fat-free serum albumin. The emulsion was mixed (1 : 1) with fresh human blood serum and incubated at 37° for 1 h with constant agitation. Then the mixture was centrifuged for 1 h at 1500 rpm. The layer of "oil" that formed on top was dissolved in a physiological salt solution and again centrifuged for 1 h at 1500 rpm. The upper layer, which was a white mass of pastelike consistency, was removed and stored with the addition of 0.5% solution of toluene in a closed vessel in a refrigerator. For the experiment 3 g of this "activated" emulsion was dissolved in 40 ml of the 10% solution of fat-free serum albumin, 2.5 ml of 1 M solution of $NH_4Cl$ and 57.5 ml of water; the pH was brought to 7.4 by 1 N solution of NaOH.

The organs and tissues (heart, liver, lungs, adipose tissue, etc.) investigated for localization of the enzyme were fixed for a short time (12 h) in 10% neutral formalin at 4°, then sliced on a freezing microtome. The sections of the organs, 10$\mu$ thick, were applied to slides and in this form placed in the above-descrived incubation medium. Incubation was carried out for 48 h at 37° (in a thermostat).

Further, it was necessary to determine localization of the enzyme by finding in the tissues free fatty acids which were split off from the substrate under the effect of tissue lipoprotein lipase. For this purpose we used several

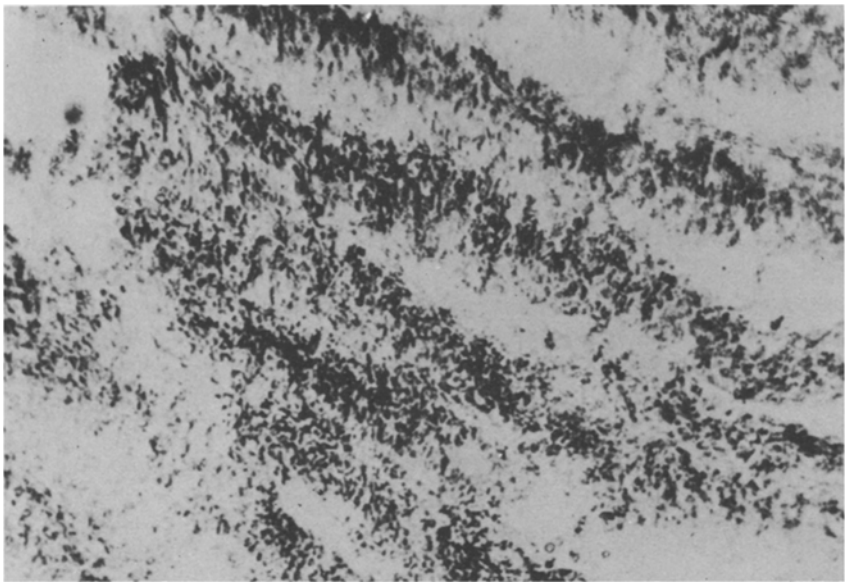

Fig. 1. Lipoprotein lipase (in the form of black granules) in the muscle fibers of a child's heart. Objective 20, ocular 7.

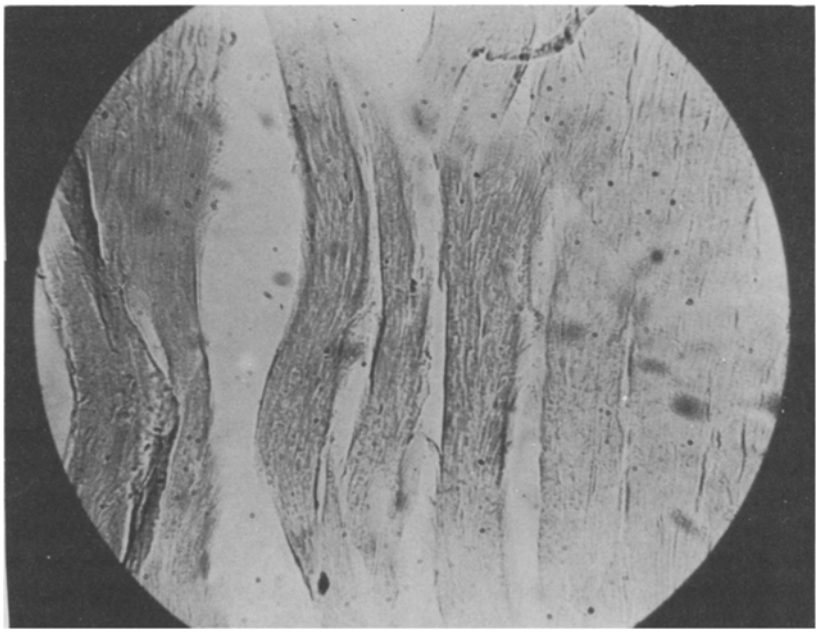

Fig. 2. Loss of enzyme granulation after incubation of the same section in a substrate with the addition of a specific inhibitor of lipoprotein lipase—1 M solution of NaCl.

modified methods of Gomori, who histochemically studied the distribution of pancreatic lipase in tissues. According to this method the fatty acids liberated from the substrate were transferred to calcium soaps when the sections were acted on by a 10% solution of $CaCl_2$. Further placement of the sections in a 1% solution of lead acetate led to substitution of a lead ion for the calcium ion in the calcium soaps. The sections were treated with a freshly prepared

0,5% solution of sodium sulfide, as a result of which a brown precipitate of lead sulfide formed in the tissues where the fatty acids were distributed. The distribution of lipoprotein lipase was judged by the localization of this precipitate in the cell elements. Preparations treated in this manner were washed and embedded in glycerin-gelatin.

Although the substrate we used was developed especially for demonstrating lipoprotein lipase and was activated by an appropriate method (see above), nevertheless we considered it necessary to use a control which would make it possible to differentiate lipoprotein lipase and lipase-esterase. Therefore, paralled to the main experiments we carried out control experiments with the same medium to which we added 1 M solution of NaCl (synthetic inhibitor of lipoprotein lipase which does not affect pancreatic lipase). The organ and tissue sections were placed simultaneoulsy into the "pure" substrate and that containing 1 M solution of NaCl and later were simultaneously treated to determine lipoprotein lipase (activated by a 1 M solution of NaCl) and ordinary lipase. In addition to this, similar sections were treated for the lipase by Gomori-Mark's method (with Tween-80 as a substrate) and for nonspecific esterase by Nachlas-Seligman's method (with $\alpha$-napthyl acetate as a substrate). Cholinesterase and pseudocholinesterase were differentiated by means of $2 \cdot 10^{-6}$M solution of esterine.

## RESULTS

Using the indicated method, we were able to trace the location of lipoprotein lipase in human organs and tissues and in various laboratory animals (rats, rabbits, dogs). As an illustration we will show a picture of the distribution of the enzyme (in the form of bright granulations) in the myocardium of a child (Fig. 1). Enzyme granulation is absent in Fig. 2.

The specificity of reaction is proved by the disappearance of the stain after preliminary incubation of the sections in a medium with the addition of the inhibitor—1 M solution of NaCl.

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**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.**