A Simple Method for the Determination of Lipoprotein Lipase

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A rapid method for determining the in-vivo effect of small amounts of intravenous heparin utilizing thin-layer chromatography is presented. A dense band of stainable fatty acids appears and represents nonesterified acids after the fatty acid esters have been hydrolyzed by lipoprotein lipase and determines, therefore, whether the enzyme is activated by heparin.

Since 1943, when Hahn (1) first found that administration of small amounts of heparin cleared lipemic plasma, there have been many reports concerning the determination of lipoprotein lipase in man and animals. The ability to detect those people who have a defect in enzyme activity may prove useful in epidemiologic studies relative to coronary heart disease and specific diseases of lipid dysfunction (2).

Method

Blood is withdrawn from the patient’s vein via a citrate-in-syringe technic utilizing 4% (w/v) sodium citrate as anticoagulant, so that a final dilution will be one part citrate to nine parts blood. This is the standard procedure used in our coagulation laboratory. The blood is centrifuged at 1650 g for 30 min. at 1° and the plasma separated from the cells by means of a pipet. An aliquot of plasma is pipetted into a 3:1 (v/v) solution of chloroform and methanol. Usually, 1 ml. of plasma is added to 7 ml. of the chloroform-methanol mixture, although smaller amounts of plasma may be used.

The emulsion is shaken by hand in a separatory funnel for 2 or 3 min. and allowed to stand for 5 min.; the bottom layer is used for chromatography. Thin-layer chromatographic plates using silica gel G, according to Stahl’s technic (3), are either made or purchased com-

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We wish to greatly acknowledge the help of Rudolph J. Henning, R.B.P., Medical Photographer, St. Vincent’s Hospital and Medical Center of New York.

Received for publication Mar. 4, 1968; accepted for publication Apr. 11, 1968.
mercially. These plates have a gel thickness of from 200 to 500 µ. Samples as small as 10 µl. can be chromatographed satisfactorily. Usually, a 50-µl. sample is placed on the plate. The plate is then placed in a glass jar or other suitable container and ascending chromatography is performed with CHCl₃ (300 ml.):CH₃OH (100 ml.):H₂O (12 ml.):NH₃OH (4 ml.). The solvent front is allowed to ascend 10 cm. from the point of application in a period of approximately 30 min. The plate is then allowed to dry for 1 min. in air at the end of which time it is sprayed with 50% (v/v) sulfuric acid. It is placed in an oven at 150° and kept there for 15 min. or until charring takes place.

Results

In Fig. 1 are five extracts which have been chromatographed. A represents the preheparinized plasma and shows in ascending order: sphingomyelin, lecithin, phosphatidyl ethanolamine, and at the very top, cholesterol. B is a sample of A to which a solution of palmitic acid in chloroform methanol has been added. A dense spot appears just beyond the phosphatidyl ethanolamine. C is the chromatogram of the fatty acid extract. D is an extract of postheparinized plasma, and the dense spot appears in the same area as the control fatty acid and the sample to which fatty acid has been added. In addition, E represents a postheparinized sample to which palmitic acid has been added, and it is seen that the spot appears in the same area. In order to prove further that this is fatty acid, the pre- and postheparinized samples were run on thin-layer chromatography and then the pre- and postchromatograms eluted only in that area where the new band appears. When this eluate is placed on Florsil columns and the eluate from these columns titrated, it is shown that this dark new band represents only free or nonesterified fatty acid (4).

While this method could be quantitated easily by using serially increasing amounts of material, by utilizing a scanning densitometer, or by elution from thin-layer chromatographic plates, it is not intended that the method be made cumbersome in this manner. It is only intended to show the presence or absence of heparin-induced lipolysis in plasma or serum.

References