testinal absorption of coenzyme as compared with that of cyanocobalamin.

Surface counting of intestinal areas after oral administration of labelled coenzyme alone and labelled cyanocobalamin to normal subjects and patients with pernicious anemia shows the radioactivity of the intestine to be much more prolonged after administration of coenzyme than after cyanocobalamin. This may be interpreted as indicating that, of the 2, coenzyme forms the easier and stronger attachment to the intestinal mucosa prior to its absorption, regardless of whether in the presence of intrinsic factor it ultimately passes through the intestinal mucosa or, in the absence of intrinsic factor, returns to the intestinal lumen.

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Received March 10, 1961. P.S.E.B.M., 1961, v107.

Lipoprotein Pre-Staining and Ultracentrifugal Analysis in a Density Gradient.* (26607)

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Pre-staining methods, used for analysis of serum lipoproteins by paper electrophoresis (1-4) and ultracentrifugal flotation(5,6), are not quantitative. Lipids dispersed on celite have been solubilized by agitation with serum lipoproteins(7). A modified procedure, pre-staining with a sudan black B (SBB) - celite slurry, is used here for the ultracentrifugal analysis of serum lipoproteins in a density gradient(8).

Methods. SBB - celite slurry. Two g SBB† was dissolved in 200 ml methyl cellosolve and added to 10 g celite 535. Distilled water, 500 ml, followed by 100 ml 2 *M* NaCl were then added with constant stirring. The suspension was filtered on a sintered glass filter, the cake washed with 300 ml 0.15 *M* NaCl, transferred to a glass stoppered flask, and suspended in 100 ml 0.15 *M* NaCl. *Staining.* Three ml of thoroughly mixed slurry was added to 1 ml of serum in a test-tube and agitated gently on an automatic shaker for 15 hr at room temperature. The tube was then centrifuged for 30 min at 1000 $\times$ *g* and stained diluted serum withdrawn.

Ultracentrifugation. Three ml of the stained serum was placed in a 13.5 ml centrifuge tube containing 1 ml 2 *M* NaCl and mixed. Five ml 0.15 *M* NaCl was layered over this solution and the tube centrifuged

* Supported in part by Research Grants from Nat. Heart Inst., P.H.S.

† Purchased from National Aniline Div., Allied Chem. and Dye Corp., New York.

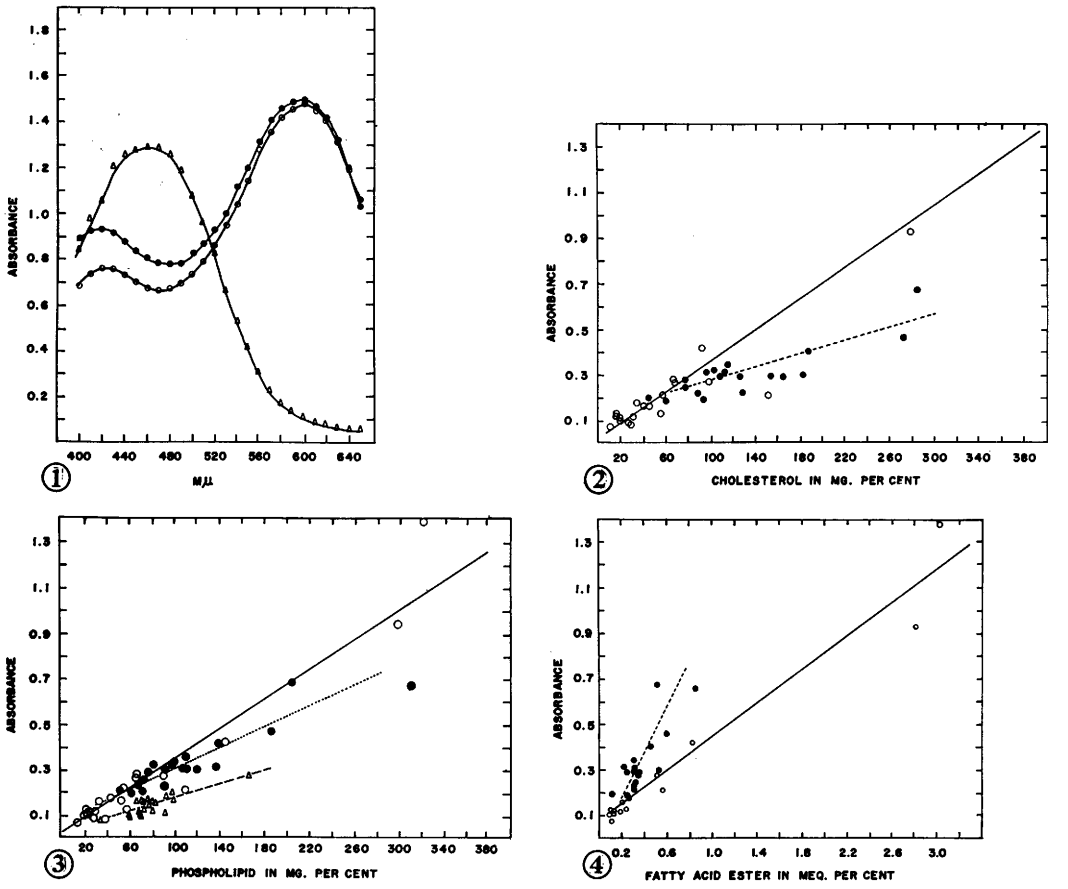

FIG. 1. Absorbance of dye solutions in ethanol: SBB, ○; SBB from slurry, ●; polar dye from filtrate, △.

FIG. 2. SBB absorbance of lipoprotein fractions as a function of cholesterol content: S_f 10-400, ○; S_f 0-10, ●.

FIG. 3. SBB absorbance of lipoprotein fractions as a function of phospholipid content: S_f 10-400, ○; S_f 0-10, ●; high density, △.

FIG. 4. SBB absorbance of lipoprotein fractions as a function of FAE content: S_f 10-400, ○; S_f 0-10, ●.

at 9,300 × *g* for 30 min in a Spinco Model L ultracentrifuge. The top chylomicron layer was removed with a tube cutter and infranatant serum layered over 5.5 ml 2.5 *M* NaCl in a centrifuge tube. Traces of SBB-celite adhering to the tube were not transferred. The gradient tube was filled with 0.15 *M* NaCl and centrifuged at 100,000 × *g* for 20 hr. Three stained lipoprotein fractions—the top S_f 10-400, middle S_f 0-10, and bottom high density lipoproteins—were transferred to 25 ml volumetric flasks. Ethanol was added, the flasks heated to boiling, cooled and diluted to volume with ethanol. The contents were filtered through Whatman No. 42 paper and SBB absorbance read at 600 mμ in a Beckman Model B spectrophotometer. In control experiments, lipoproteins were isolated by density gradient ultracentrifugation and analyzed for cholesterol, phospholipid, and fatty acid ester (FAE) content(8).

Results. Absorbancies in ethanol of the original SBB, SBB precipitated on celite, and dye from a celite cake filtrate are recorded in Fig. 1. A water soluble dye with an absorbance maximum at 460 mμ is found in the filtrate. The absorbance maximum for SBB is at 600 mμ. Reproducible stain uptake was obtained with typical replicate

TABLE I. Cholesterol and Sudan Black B Distribution in Serum and Serum Lipoproteins of a Hyperlipemic Diabetic Subject on Insulin and Unsaturated Fatty Acid Therapy.

Fraction and analysis	Date 1/11/60	1/15/60	2/1/60
Serum			
Cholesterol, mg %	508	465	268
SBB absorbance*	1.810	1.485	.779
Chylomicron			
Cholesterol, mg %	19.1	54.4	10.9
SBB absorbance	.097	.211	.054
S_f 10-400			
Cholesterol, mg %	371	279	92.5
SBB absorbance	1.380	.928	.420
S_f 0-10			
Cholesterol, mg %	45.0	77.5	90.0
SBB absorbance	.200	.249	.222
High density			
Cholesterol, mg %	28.0	27.0	30.4
SBB absorbance	.132	.099	.084

* Sum of SBB absorbancies in different fractions.

analyses of 0.574 ± 0.033, 0.441 ± 0.019, and 0.481 ± 0.014 for different serum pools. Stain intensity varied with the slurry preparation. One ml aliquots from a serum pool were stored at −20°C for several months with no change in SBB uptake from a given slurry and were used to standardize different slurry preparations. Stain intensity increased with agitation time. Reproducibility and proportional stain uptake improved when agitation was continued for 15 hr.

In Fig. 2-4, SBB uptake for lipoproteins isolated from subjects with widely varying lipoprotein concentrations is compared with their cholesterol, phospholipid, and FAE content. Table I illustrates the usefulness as well as the shortcomings of the SBB staining technic in following total serum lipids and individual lipoprotein concentrations in hyperlipemic subjects during therapy. Total cholesterol, as well as chylomicron and S_f 10-400 lipoprotein cholesterol, correlate well with staining intensity whereas S_f 0-10 and high density lipoprotein cholesterol do not.

Discussion. Stain intensity and reproducibility are difficult to control when SBB dissolved in organic solvents is added to serum (5,6). Total dye uptake is often similar for sera of widely varying lipid content. Staining with SBB-slurry more nearly reflects total lipid concentration. However, SBB uptake varies with different lipoproteins and is not a simple measure of total lipid. Individual lipid components differ considerably in their affinity for stain. Berg *et al.*(9) have shown that although staining of any given lipid by SBB on paper is proportional to the amount of lipid, individual lipids differ widely in their capacity to retain dye. Triglycerides stain most intensely, while equal weights of cholesterol, phospholipid, and cholesterol ester take up considerably less SBB. Since lipoproteins differ markedly in lipid content, those containing relatively large amounts of triglyceride (elevated FAE), the chylomicrons and S_f 10-400 lipoproteins, stain proportionately more for a given amount of lipid and correlate with lipoprotein concentration (Table I). Since S_f 0-10 lipoproteins contain relatively less triglyceride, and since the relative FAE content of this fraction is enhanced in hyperlipemia when the absolute concentration of the fraction is suppressed(8), staining correlates poorly with concentration of S_f 0-10 lipoproteins in hyperlipemia (Table I). Elevations in S_f 0-10 lipoproteins with a normal lipid composition, as found in myxedema or idiopathic hypercholesterolemia, may be satisfactorily estimated by this procedure. Prestaining is a useful adjunct in flotation studies with other animal sera as well as lipoproteins from other sources such as egg yolk since the lipid containing fractions are readily visualized in appropriate density gradients.

Celite slurry increases surface area between non-polar dye and lipoproteins in solution. Precipitation on celite by increasing solvent ionic strength results in highly dispersed SBB-celite, enhancing staining more than celite dispersions prepared by solvent evaporation(7). Serum aliquots may be stored at −20°C without major changes in lipoprotein properties(10) and used as standards for different slurry preparations. The polar dye found in filtrates during slurry preparations is probably the SBB decomposition product(11) capable of staining

more polar lipids(12) and proteins(13). SBB may be purified by repeated solvent precipitation from a methyl cellosolve solution with saline.

Summary. The preparation and standardization of a SBB - celite slurry for lipoprotein pre-staining is described. Dye uptake is proportional to lipid concentration and may be used to estimate chylomicrons, S_f 10-400 and S_f 0-10 lipoproteins isolated by density gradient ultracentrifugation. Precipitation on celite by altering solvent polarity is an effective means for preparing highly dispersed SBB and other lipid soluble substances. A polar dye contaminant is removed from SBB by solvent precipitation.

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Received March 14, 1961. P.S.E.B.M., 1961, v107

Effect of Reserpine on Lipoprotein Lipase Activity of Rat Heart. (26608)

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The clearing factor lipase in post-heparin plasma and in extracts of adipose and heart tissue was shown to be similar and named lipoprotein lipase(1-3). It generally is believed(4,5) that lipoprotein lipase plays a role in transfer of fat from the circulating blood into tissues and mobilization of fat from adipose tissue. Although heparin-activated release of lipoprotein lipase from peripheral tissues is well documented(5-8), the physiologic control of activation and release of lipoprotein lipase is relatively unknown. Studies by Hollenberg(9,10) and Cherkes and Gordon(11) have indicated that activity of lipoprotein lipase in heart and adipose tissue is related to nutritional state of the rat. However, fasting induced opposite effects on the activity of lipoprotein lipase in heart and fat tissue suggesting the involvement of other factors. Another mode of control, possibly more direct, of the activity of lipoprotein lipase is by hormonal or neurohumoral mechanisms. Several types of hormones markedly influence the level of plasma free fatty acids (FFA) and effect release of FFA from adipose tissue(4,12). In this connection, Wadstrom(13) and White and Engel(14) have shown that hydrolysis of triglycerides in adipose tissue was stimulated by epinephrine and norepinephrine *in vivo* and *in vitro*.

The work reported here was done on rat myocardial tissue because it normally contains a relative abundance of norepinephrine and epinephrine(15) which are rapidly depleted following injection of reserpine(16). Also, the activity of lipoprotein lipase in rat heart has been shown responsive to a physiological stimulus(10). This paper reports the effect, in rats, of reserpine pretreatment on the amount of lipoprotein lipase and its release by heparin from heart tissue *in vitro*.