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.

1. McDonald, H. J., Bermes, E. W., *Biochim. Biophys. Acta,* 1955, v17, 290.

2. Solinas, P., Betti, R., Di Leo, E. F., *Clin. Chim. Acta,* 1957, v2, 586.

3. McDonald, H. J., Ribeiro, L. P., *ibid.,* 1959, v4, 458.

4. McDonald, H. J., Kissane, J. Q., *Anal. Biochem.,* 1960, v1, 178.

5. Cornwell, D. G., Kruger, F. A., *Abst. 135th Meet. Am. Chem. Soc.,* 1959, 35c.

6. Light, S., Gurd, F. R. N., *Vox Sanguinis,* 1959, v5, 92.

7. Avigan, J., *J. Biol. Chem.,* 1959, v234, 787.

8. Cornwell, D. G., Kruger, F. A., Hamwi, G. J., Brown, J. B., *Am. J. Clin. Nutrition,* 1961, v9, 24, 41.

9. Berg, G., Scheiffarth, F., Estler, C. J., Schön, H., *Klin. Wochen.,* 1958, v36, 766.

10. Briner, W. W., Riddle, J. W., Cornwell, D. G., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 784.

11. Fredricsson, B., Laurent, T. C., Luning, B., *Stain Techn.,* 1958, v33, 155.

12. Diezel, P. B., Niemanis, G., *Virchow's Archiv.,* 1957, v330, 619.

13. Kutt, H., Lockwood, D., McDowell, F., *Stain Techn.,* 1959, v34, 203.

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

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

ABRAHAM DURY

General Medical Research, Veterans Administration Hospital and Department of Anatomy, School of Medicine, University of Pitssburgh, Pittsburgh, Pa.

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*.

Materials and methods. Female Wistar rats weighing about 200 g were injected intraperitoneally with Reserpine (Serpasil, Ciba), 0.2 mg/100 g, either once—Group A, or once daily for 4 days—Group B. Controls were similarly treated with 0.5 ml of isotonic saline. Food was removed at time of the last injection, and 20 hours later rats were sacrificed under ether anesthesia. The heart was excised, cut open with scissors, quickly rinsed in ice-cold Ringer's solution, and sliced with a razor blade. Tissue, weighing 150-170 mg, from each heart was placed into each of a pair of 25 ml Erlenmeyer flasks containing 3.0 ml of Krebs-Ringer phosphate buffer, pH 7.4. To one flask of a pair was added 0.2 mg (0.2 ml) of heparin (Heparin, Upjohn, diluted with water) while an equal amount of distilled water was added to the other flask. Heart slices were agitated in a Dubnoff metabolic incubator at 37°C, under air, for 45 minutes. Heart slices from control and reserpine pretreated rats were incubated at the same time. The activity of the lipase released into the incubation medium was measured by its ability to produce FFA from an activated coconut oil substrate. The substrate mixture was made up as follows: 1 part of fresh human serum; 1 part of a 5% coconut oil emulsion (Ediol, Schenlabs) prepared by dilution with water; and 8 parts of freshly prepared 10% solution of bovine serum albumin adjusted to pH 7.4. The albumin was a dialyzed and lyophilized stock prepared from BSA powder, Fraction V purchased from Nutritional Biochemicals, Inc. Portions (0.5 ml) of the substrate mixture were pipetted into 15 ml glass-stoppered centrifuge tubes and incubated at 37°C for 30 minutes prior to mixing with 0.5 ml aliquots, taken in duplicate, of the incubation medium from each flask. After incubating the entire reaction mixture for 1 hour at 37°C, the FFA produced was determined by the method of Dole(17) using Nile Blue indicator solution. Substrate blanks were run with each series of incubated heart slices. The activity of the lipase in the medium is expressed as micro-equivalents of FFA produced/g heart/hour.

To demonstrate that the lipolytic enzyme released from heart tissue was similar to lipoprotein lipase, the effects of protamine sulfate and 1M sodium chloride in the incubating medium were tested. Heart slices from 16 normal, 20-hour fasted rats were processed as described above, and to each incubation flask was added 0.2 mg of heparin. For inhibition by protamine, 50 mg of protamine sulfate was added to one flask of a pair at exactly 40 minutes of elapsed incubation. For inhibition by 1M sodium chloride, the medium of one flask of a pair contained 58.5 mg/ml of the salt. The contents of all flasks were incubated for a total of 45 minutes. Aliquots, in duplicate, of each incubation medium were mixed with activated coconut oil substrate, incubated for another hour, and lipase activity determined as described above.

Results. Lipolytic activity from normal heart tissue. Release of lipolytic activity was the same with control heart tissue from normal *ad lib.* fed and 20-hour fasted rats. The effect of heparin was to increase similarly the lipolytic activity of heart slices from both types of rats (Fig. 1).

The data presented in Table I demonstrate inhibition of lipolytic activity in the incubating medium when protamine sulfate and 1M sodium chloride, known inhibitors of lipoprotein lipase, were added to the heart slices. Inhibition of enzyme activity by 1M sodium chloride was almost complete while protamine caused a slightly lesser degree of inhibition. These data are taken to indicate that the lipase released into the medium from heart tissue was identical with lipoprotein lipase.

Lipolytic activity from heart slices of reserpine-treated rats. The amounts of lipase released from control heart tissue of Groups

TABLE I. Inhibition of Lipase Liberated by Heart Tissue of Normal, Fasted Rats.

Inhibitor	Inhibition of lipolysis (%)
Protamine sulfate	76.9 (54.9- 87.5)*
1M NaCl	93.6 (87.9-100.0)*

* Mean and range, 8 tissues.
See *Methods* for details.

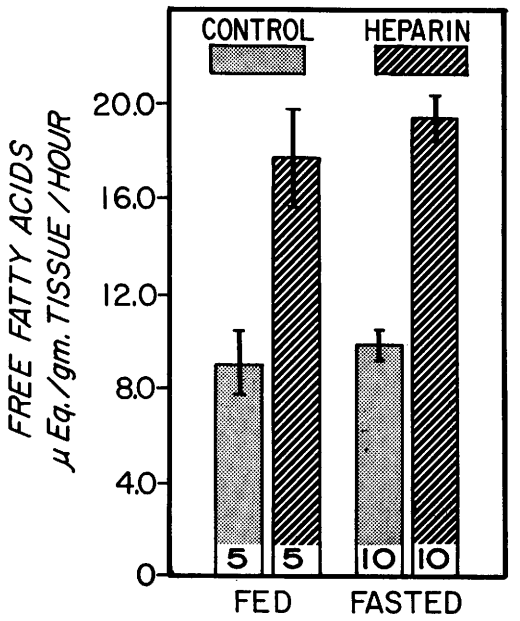

FIG. 1. Release of lipoprotein lipase from incubated heart tissue of normal fed and 20-hour fasted rats. Number at bottom of each column indicates No. of animals in group. Vertical bars equals S.E.

A and B were 63% and 43%, respectively, of the activity released by myocardial slices from normal fasted rats (Table II). The effect of adding heparin to the incubating medium also is shown in Table II. The activity determined in the medium with normal rat heart was 100% greater than without added heparin. In the reserpine pretreated groups, the effect of heparin was an 80% increase in lipolytic activity when heart slices were used from Group A; in experiments with heart tissue from Group B, heparin did not significantly change lipolytic activity of the medium. Furthermore, with heart slices of both reserpine pretreated groups, the absolute amount of activity released by heparin was considerably below that found with normal rat heart. These results suggest that the marked decrease in release of lipolytic activity from heart slices in reserpine-treated rats was due to a primary influence of reserpine on the amount of lipoprotein lipase in myocardial tissue.

TABLE II. Effect of *In Vivo* Administered Reserpine on Lipoprotein Lipase Release by Heart Tissue from Fasted Rats.

Heparin added to medium	Lipase activity released†	
	Controls	Reserpine inj.
None	9.79 ± .62	(A) 6.20 ± .51* (B) 4.28 ± .53*
.2 mg	19.45 ± .82	(A) 11.24 ±1.95* (B) 6.40 ± .79*
	Heparin-induced release of lipase, value of P =	
	.001	(A) .035 (B) NS

Reserpine, I.P., 0.1 mg/100 g of rat. (A) = one injection; (B) = one injection a day for 4 days. All animals fasted 20 hr before sacrificed.

* Difference between control and reserpine tissues occurring by chance alone, P = <0.01.

† Lipolytic activity expressed as μEq. of FFA/g tissue/hr. Controls: 10 tissues; Reserpine: (A) 6 tissues, (B) 4 tissues.

Discussion. The results obtained with normal *ad lib.* fed and 20-hour fasted rat heart slices indicated that such difference in the nutritional state of the donor rat was not important in determining the amount of lipoprotein lipase released without and with heparin during incubation of heart tissue. Hollenberg(10) and Cherkes and Gordon (11) also had observed that a short fasting period did not alter the heparin effect on lipolytic activity of heart slices. On the other hand, with a longer period of starvation (3-4 days) heparin profoundly increased the activity of the enzyme released from heart slices(10). The reason for this result is not clear.

The evidence presented here indicates that heart lipoprotein lipase activity is reduced following one or more injections of reserpine into rats. Moreover, heparin failed to elicit a significant increase of the activity when heart tissue was used from rats pretreated with reserpine for 4 days, suggesting a primary influence of reserpine on amount of lipoprotein lipase in myocardial tissue.

The mode of action of reserpine is not indicated in this study. However, one possibility is that the reduced lipoprotein lipase activity in myocardial tissue is related to reserpine-induced depletion of stores of heart catecholamines. Paasonen and Krayer (16) have shown that the effect of reserpine on heart tissue catecholamines was remarkably similar to the disappearance of neuro-

hormones from the myocardium due to degeneration of adrenergic nerves. A working hypothesis is that the activity of lipoprotein lipase is stimulated by free (or "released") norepinephrine and/or epinephrine in heart tissue. In this connection, it was found (studies in progress) that pretreatment of rats with monoamine oxidase inhibitors (Marplan and Iproniazid) increased lipoprotein lipase activity released from heart tissue and the effect of heparin on enzyme activity was increased 2-fold.

Summary. The lipoprotein lipase activity released from heart slices of normal *ad lib.* fed and 20-hour fasted rats was the same. Heparin elevated equally the activity of tissues from both types of rats. Pretreatment with reserpine reduced the amount of activity of lipoprotein lipase released by incubated heart slices. The effect of heparin was negligible when heart tissues were used from rats treated with reserpine once daily for 4 days. The possibility of a relationship between reserpine-induced decrease in activity of lipoprotein lipase and depletion of catecholamines from heart tissue was discussed.

1. Korn, E. D., *J. Biol. Chem.*, 1955, v215, 1.
2. ———, *ibid.*, 15.
3. Korn, E. D., Quigley, T. W., Jr., *Biochem et Biophys Acta*, 1955, v18, 143.
4. Frederickson, D. S., Gordon, R. S., Jr., *Physiol. Revs.*, 1958, v38, 585.
5. Robinson, D. S., *Am. J. Clin. Nutrition*, 1960, v8, 7.
6. Shore, B., Nichols, A. V., Freeman, N. K., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 216.
7. Jeffries, G. H., *Quart. J. Exp. Physiol.*, 1954, v39, 261.
8. Robinson, D. S., Harris, P. M., *ibid.*, 1959, v44, 80.
9. Hollenberg, C. H., *Am. J. Physiol.*, 1959, v197, 667.
10. ———, *J. Clin. Invest.*, 1960, v39, 1282.
11. Cherkes, A., Gordon, R. S., Jr., *J. Lipid Res.*, 1959, v1, 97.
12. Engel, F. L., White, J. E., *Am. J. Clin. Nutrition*, 1960, v8, 691.
13. Wadstrom, L. B., *Nature*, 1957, v179, 259.
14. White, J. E., Engel, F. L., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 375.
15. Von Euler, U. S., *Acta Physiol. Scand.*, 1946, v11, 168.
16. Paasonen, M. K., Krayer, O., *J. Pharm. & Exp. Therap.*, 1958, v123, 153.
17. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.

Received March 15, 1961. P.S.E.B.M., 1961, v107.

Nucleic Acid Synthesis by Leukocytes in Presence of Anti-Leukocyte Factors.* (26609)

ANNA MARIA WILLIAMS AND ROBERT F. SCHILLING
Department of Medicine, University of Wisconsin Medical School, Madison

A variety of evidence(1) indicates reactions between antinuclear substances in sera from patients with systemic lupus erythematosus and cell nuclei, nucleoprotein, and DNA.† There appear to be at least 2 factors: one which reacts with cell nuclei and is responsible for the "L.E. phenomenon," and another, present only in certain sera, which reacts with purified DNA from different sources and species. Both factors migrate with the gamma globulins on zone electrophoresis, and it has been hypothesized that the described interactions are antigen-antibody reactions.

Calabresi, Edwards, and Schilling(2), using a fluorescent antibody technic, have detected antinuclear globulins in the sera of all patients with SLE or Felty's syndrome of rheumatoid arthritis which they studied. They also obtained evidence, by plasma transfusions, that a leukopenic factor was present in the plasma from 2 patients with

* This research was supported by a grant from U.S.P.H.S.

† The following abbreviations are used: DNA, deoxyribonucleic acid; SLE, systemic lupus erythematosus; RNA, ribonucleic acid; CGL, chronic granulocytic leukemia.