Intravascular Effect of Heparin on Plasma Nonesterified Fatty Acid and Triglyceride During Alimentary Lipemia. (25745)

MARTIN A. RIZACK (Introduced by Vincent P. Dole)
The Rockefeller Institute, N. Y. City

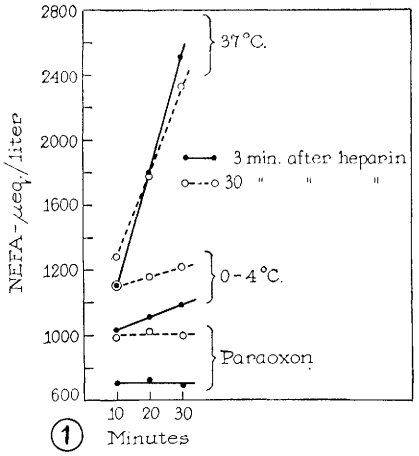
Heparin injected intravenously causes release of lipoprotein lipase into the circulation (1,2), and a decrease in turbidity of lipemic plasma associated with hydrolysis of plasma triglyceride(3). Since this process releases fatty acids(4,5), the level of nonesterified fatty acids (NEFA) has been utilized as index of amount of lipase released by heparin(6,7, 8). It is important to observe that lipolysis, initiated intravascularly, continues in the test tube(6). Lipolysis must be completely abolished after blood is drawn, if intravascular effect of heparin is to be determined. Using a potent lipoprotein lipase inhibitor, p-nitrodiethyl - phenyl - phosphate (paraoxon)*(9), intravascular effect of heparin on plasma turbidity is considerably less than previously observed(10). This inhibitor was utilized in the present study to define changes in plasma NEFA and triglyceride occurring intravascularly in association with the clearing reaction.

Method. Six medical students served as subjects to test methods of eliminating lipase activity from post-heparin plasma. They ingested 240 ml of heavy cream at about 8:00 a. m. Water was allowed *ad lib.* until measurements began 5 hours later. Samples of blood were obtained 3 and 30 minutes after injection of heparin (10 mg intravenously). All samples were collected in tubes containing sodium oxalate as anticoagulant. Each sample was divided into 3 portions: The first portion of plasma was incubated at 37°C after separation in centrifuge at room temperature. The second portion was handled as the first except that paraoxon was added to collecting tubes in sufficient quantity to establish final concentration of 2×10^{-3} molar. The third portion of plasma was separated and maintained at 0-4°C. Aliquots of each of the 3 portions were taken at 10-minute intervals over a 30-minute period and extracted for determination of NEFA. Intravascular effect of heparin was tested in a separate study. Samples of blood were obtained from 12 medical students at 30, 25, 15 and 1 minute before intravenous injection of heparin (10 mg). Additional samples were taken at 5, 15 and 30 minutes after injection. Each sample was divided into 2 aliquots: One was mixed with paraoxon and sodium oxalate, and was processed at room temperature; the other, containing sodium oxalate alone, was kept at 0-4°C. All samples were analysed for NEFA and triglyceride. NEFA was determined by the method of Dole(11), and triglyceride by the method of Van Handel and Zilversmit (12).

Results. Inhibition of lipolysis in vitro. Fig. 1 shows complete inhibition of fatty acid release in post-heparin plasma treated with paraoxon. Cooling caused marked but incomplete reduction in rate of hydrolysis when blood was drawn after injection of heparin, cooled in ice water and separated in refrigerated centrifuge (the whole procedure requiring about 10 minutes). Concentration of NEFA was consistently higher in cooled blood than in aliquot of blood treated immediately with paraoxon and separated at room temperature. The difference between tubes apparently was due entirely to lipolysis in the cooled sample. Paraoxon itself had no effect on determination of pre-existing NEFA; samples of blood, taken before injection of heparin and cooled or treated with paraoxon, showed no significant differences in concentration of NEFA. In contrast to these results, no difference was found in concentrations of triglyceride when samples containing paraoxon were compared with cooled aliquots.

Intravascular effect of heparin. Following injection of heparin concentration of NEFA in plasma (treated with paraoxon) rose 327 $\pm$ 219 μeq/l (Fig. 2). This value is significantly less than the 618 $\pm$ 419 μeq/l rise observed in cooled specimens ($P<0.001$). Con-

* Obtained through courtesy of Dr. Schrader, Farbenfabriken Bayer, A. G., Wuppertal-Elberfeld, West Germany.



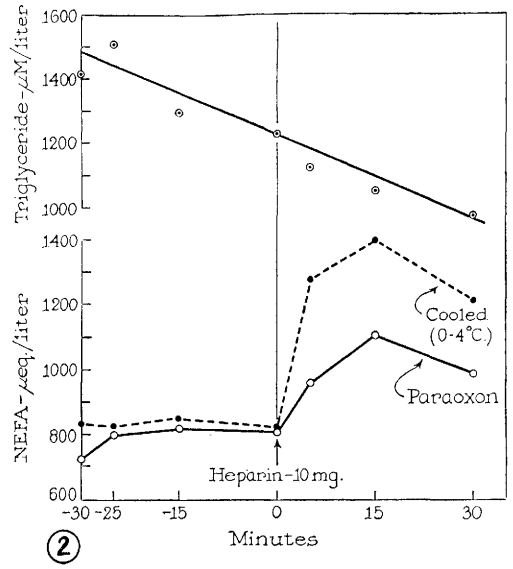


FIG. 1. NEFA concentration of plasma incubated *in vitro*.

FIG. 2. Effect of heparin on NEFA and triglyceride concentration of plasma.

centration of triglyceride in plasma fell throughout period of observation (Fig. 2). Mean rate of decrease before heparin was 9.3 ± 3.2 μM/l min and afterwards was 6.3 ± 2.5 μM/l min. The difference in rates was not statistically significant (P>50).

Discussion. Amount of NEFA released *in vitro* when post-heparin blood was cooled, but is not otherwise treated, corresponded to hydrolysis of less than 100 μM/l of triglyceride, a quantity at borderline of sensitivity of the analytical method. This is sufficient to account for lack of significant difference in triglyceride concentration between cooled and paraoxon-containing specimens.

It is also theoretically possible that hydrolysis of triglyceride might be underestimated. Lipoprotein lipase acts preferentially on the alpha position of triglyceride to form diglycerides(13), and the analytical method used to determine triglycerides does not distinguish between diglyceride and triglyceride. However, post-heparin plasma has been analysed for lower glycerides by silicic acid chromatography(14) and contained less than 2% of total glycerides as diglyceride or monoglyceride even though no precaution was taken to inhibit lipolysis. It seems likely, therefore, that lower glycerides did not constitute a significant fraction of plasma glycerides in the present study.

From data obtained by incubating post-heparin plasma *in vitro,* rate of release of NEFA after injection of heparin was calculated to be 72.8 ± 18.3 μeq/l 3 min after injection and 53.3 ± 12.1 μeq/l 30 min after; these rates are equivalent to complete hydrolyses of triglyceride at rates of 14.6 and 26.1 μM/l min. Since triglyceride appears to be removed from circulation at a rate of 125 μM/l min(15), heparin, at dosage used, would not be expected to have a significant effect on triglyceride concentration of plasma.

The above findings do not support the opinion that heparin significantly accelerates clearance of circulating triglyceride. Earlier studies, seeming to indicate marked reduction in circulating triglyceride after heparin, appear to have been complicated by incomplete inhibition of lipoprotein lipase.

Summary. Clearance of triglyceride from plasma during alimentary lipemia was not significantly influenced by intravenous injection of heparin. Injection of heparin in lipemic subjects caused a small rise in NEFA concentration of plasma, but quantity of triglyceride hydrolysed was insignificant in relation to its large turnover rate.

1. Brown, R. K., Boyle, E., Anfinsen, C. B., *J. Biol. Chem.,* 1953, v204, 423.
2. Korn, E. D., *ibid.,* 1955, v215, 15.
3. Hahn, P. F., *Science,* 1943, v98, 19.
4. Shore, B., Chowere, I., Rose, G., PROC. SOC.

EXP. BIOL. AND MED., 1953, v83, 216.
5. Robinson, D. A., French, J. E., *J. Exp. Physiol.*, 1953, v38, 233.
6. Grossman, M. I., Moeller, H. C., Palm, L., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 106.
7. Spitzer, J., Miller, H. I., *ibid.*, 1956, v92, 124.
8. Gordon, R. S., Cherkes, J., *J. Clin. Invest.*, 1956, v35, 210.
9. Korn, E. D., Quigley, T. W., Jr., *J. Biol. Chem.*, 1957, v226, 837.
10. Felt, V., Reichl, D., *Lancet*, 1957, v1, 664.
11. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.
12. Van Handel, E., Zilversmit, D. B., *J. Lab. Clin. Med.*, 1957, v50, 152.
13. Borgstrom, B., Carlson, L. S., *B. and B. Acta*, 1957, v24, 638.
14. Hirsch, J., Ahrens, E. H., *J. Biol. Chem.*, 1959, v233, 311.
15. Havel, R. J., Fredrickson, D. S., *J. Clin. Invest.*, 1956, v35, 1025.

Received March 1, 1960. P.S.E.B.M., 1960, v104.

Successful Replacement Therapy in Lactating Thyro-parathyroidectomized. Rats.* (25746)

R. VON BERSWORDT-WALLRABE† AND C. W. TURNER
Dept. of Dairy Husbandry, University of Missouri, Columbia

Successful replacement therapy in thyro-parathyroidectomized (TPEC) rats requires optimal amounts of thyroxine (T4) as well as parathyroid hormone (PTH). Study of T4 secretion rate in lactating rats indicated 3 μg/100 g body weight (BW) was upper normal range(1). When this amount was injected into lactating rats from days 7-13 postpartum, milk yield increased 37% and when maximum removal was insured with oxytocin (OXT) milk yield increased 63.2%(2). With T4 and PTH at levels of 10, 2 x 20 and 3 x 20 USP parathyroid units/day partial replacement therapy of TPEC lactating rats was effected(3). Completely successful replacement therapy is here reported using both litter weight and milk yield on day 14 as indices. Technics are described by which animals with accessory parathyroid tissue (APTT) may be detected.

Material and methods. Primiparous lactating rats of Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, fed Purina Lab Chow and water *ad lib.* Animal room was maintained at uniform temperature of 78 ± 1°F. Shortly after delivery, each litter was reduced to 6 young. TPEC was performed under ether anesthesia in less than 20 minutes on day 7 postpartum (PP). Replacement therapy was started shortly after operation: 3 μg T4/100 g/day and 2 x 40 USP units PTH‡/day were given subcutaneously. Parameters of lactational performance were mean litter weight and milk yield. Amount of milk removed by litter of 6 on day 14 under the influence of 1 USP unit oxytocin (OXT)§ was estimated by method described previously(4). To repeat this test at 24 hour intervals litters were weighed at end of 10 hours isolation, immediately replaced and allowed to nurse for 30 minutes. Litters were weighed again to nearest 0.5 g. Difference in litter BW before and after each nursing was used to represent amount of milk secreted during previous 10 hours. After this test, litters were allowed to stay over-night with mothers. To prevent feed intake other than mother's milk by young, mothers were allowed to eat only during 10 hour separation period from day 15 on. PTH was discontinued 5 hours prior to nursing period in 22 animals on day 14, in 12 animals morning of day 13.

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2133. Approved by Director. This investigation supported in part by research grants from U.S.P.H.S.

†Research Fellow, Dept. of Animal Physiol. and Animal Nutrition, Univ. of Goettingen, West Germany and Medical Fellow of Population Council.

‡ Parathyroid hormone kindly supplied by Eli Lilly & Co., Indianapolis, Ind.

§ Oxytocin supplied by Armour Lab., Chicago.