Mitchell *et al*(12) proposed that transferrin occurs in two phases: one in the plasma and the other sequestered at cellular sites. Transferrin has also been found attached to immature red cells(13). A longer period of attachment of the transferrin at cellular sites or to the immature red blood cells might explain the decrease in TIBC after endotoxin. Bound iron was shown to be decreased(7) without an increased removal of iron from the plasma(8). It therefore follows that lower than normal amounts of iron were entering the plasma and that less than normal amounts of bound iron would be available to displace transferrin. Jandl and Katz(13) have suggested that transferrin containing iron was selectively attached to cellular sites or immature red blood cells and was then displaced by another molecule containing iron.

If the changes in TIBC after endotoxin were due to an inhibition of protein synthesis the half life of transferrin in the rat must be much shorter than the 6-10 days found in humans(14,15).

Summary. A decrease in total iron-binding capacity of the serum of rats was found to occur after injection of *Escherichia coli* lipopolysaccharide. The decrease was apparently not due to an increased blood volume and was accompanied by other alterations of the serum proteins.

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Incidence of Endogenous Lipoprotein Lipase Activity in Human Plasma.* (29267)

HYMAN ENGELBERG

Division of Laboratories, Cedars of Lebanon Hospital, Los Angeles, Calif.

The mechanisms involved in removal of triglycerides from the bloodstream are being intensively investigated as they are of physiologic interest and also clinically relevant to some lipid abnormalities involved in the genesis of atherosclerosis. There is much evidence, summarized elsewhere(1), that the heparin-activated lipolytic enzyme, lipoprotein lipase, has a major physiologic function in fat transport. This point of view has been disputed, however, and one of the counter-arguments is that the enzyme has only been demonstrable in the plasma of a small percentage of individuals without prior injection of heparin. In a previous study of 482 normal subjects circulating endogenous lipoprotein lipase activity was found in 15-20% of the group(2).

More recent observations indicated that the enzyme was sometimes inactivated by

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TABLE I. Comparison of Plasma Glycerol Values in Platelet-Rich and Platelet-Poor Plasma Aliquots Using Silicone and Non-Silicone Glassware.

	Plasma glycerol in mmole/l			
	Silicone		Non-silicone	
Patient	Platelet rich	Platelet poor	Platelet rich	Platelet poor
EG	.120	.118	.098	.084
IH	.124	.126	.092	.086
AS	.118	.118	.098	.086

contact with glass(3), as well as the previously demonstrated inactivation during the lipolytic process itself(4). It also became evident that lipolysis occurred in the few minutes it took to separate plasma in a refrigerated centrifuge. It therefore seemed possible that failure to appreciate these facts might have obscured recognition of the true frequency of endogenous lipoprotein lipase activity in human plasma. The problem appeared amenable to a simple approach; namely, a comparison of the levels of a product of lipolysis, glycerol, in plasma aliquots in one of which the enzymatic activity had been inhibited as soon as the blood was drawn whereas optimum conditions for lipolysis prevailed for the other aliquot.

Methods. Blood samples were drawn from patients during routine office visits, usually in the afternoon after lunch. Patients who had received heparin in the preceding week were not included. Silicone-treated syringes and needles were used, and 5 ml aliquots of blood placed in oxalated silicone centrifuge tubes one of which contained .01 mg of Polybrene† (5) (a heparin antagonist) or protamine (.1 ml of a .01% solution). After the tubes were inverted several times the plasma was immediately separated in a refrigerated centrifuge at 4°C. Analysis for glycerol was performed in duplicate, usually the following day unless shown otherwise in the tables, by an established method(6,7). Clearing factor studies were done on all samples using a coconut oil substrate as previously described (2). The conditions of special experiments are shown in the tables.

Results. The values shown in Table I, recorded during a study of plasma inhibitors of lipoprotein lipase, led to the present investigation. In 3 consecutive instances glycerol was lower when the plasma sample was not obtained using the silicone technique throughout. This was true in both platelet rich and platelet poor samples. This observation suggested that a lipolytic enzyme in the plasma had been inactivated by contact with glass so that there was more lipolysis of triglyceride in the silicone aliquot.

TABLE III. Effect of Polybrene Added *in vitro* on Glycerol Level of Pooled Plasma.

	Plasma glycerol in mmole/l
Control	.122
With polybrene .01 mg/5 ml	.123

Table II shows the effect of various concentrations of polybrene on the lipolytic and clearing activity of post-heparin plasma. It is apparent that smaller quantities of polybrene inhibit the enzyme. Table III is a control study which demonstrates that the addition of polybrene to plasma *in vitro* does

TABLE II. Effect of Polybrene on Lipolytic and Clearing Activity of Post-Heparin Plasma.

		Incubation time at 37°C		
		0	30′	60′
Control (1.6 ml Hep. plasma, 1.6 ml N.S., .3 ml 1% Ediol)	Glycerol in mmole/l	.077	.098	.112
	Optical density	.480	.290	.155
Control + .01 mg polybrene	Glycerol	.078	.080	.080
	O. D.	.480	.460	.440
Control + .1 mg polybrene	Glycerol	.078	.088	.088
	O. D.	.480	.340	.260

† Supplied by Dr. G. Berryman, Abbott Laboratories, N. Chicago. Polybrene is hexadimethrine bromide, poly-(1,5-dimethyl-1,5 diazo undecamethylene-metho-bromide).

TABLE IV. Glycerol in Plasma Aliquots (Silicone Technique), With and Without Polybrene, Immediately and 24 Hours After Separation of Plasma.

Contents of tube	Time of analysis	Glycerol in mmole/l*
Plasma + polybrene	Immediately after separation of plasma	.113
Plasma alone	*Idem*	.118
Plasma + polybrene	24 hr later	.122
Plasma alone	*Idem*	.145

* All glassware chilled. Plasma separation in refrigerated centrifuge at 0°C.

not affect the determination of glycerol.

The results presented in Table IV show a higher plasma glycerol level when polybrene was not added to the blood even when the analysis was done immediately after the plasma was separated. This indicates that triglyceride lipolysis resulting from endogenous lipoprotein lipase activity may proceed during the separation of the plasma in refrigerated centrifuges. Further lipolysis occurred during the next 24 hours (plasma kept refrigerated), although it was partially inhibited by polybrene.

Table V shows that in 91 of 121 subjects less glycerol was found in plasma aliquots where polybrene or protamine had been added when the blood was drawn, whereas the plasma glycerol content was higher in only 4 instances. This difference (91:4) is statistically highly significant ($p > .001$). Thus the presence *in vitro* of an inhibitor of lipoprotein lipase prevented triglyceride lipolysis indicating that the enzyme was present and active in the plasma of those 91 individuals. The average glycerol values in plasma aliquots with and without polybrene or protamine are presented in Table VI. The values in the tubes containing polybrene or protamine were adjusted for the slight dilution (2%) introduced by addition of the .1 ml of the inhibitor to the 5 ml of blood. The difference between the average of 2 plasma aliquots is .008 mmole/liter, which is 4 times the standard deviation of the glycerol method (.002 mmole/l) as determined in this laboratory by 20 replicate analyses.

TABLE V. Effect of Polybrene or Protamine on Plasma Glycerol Values in 121 Subjects.

	No. of cases
Glycerol decreased in tube with inhibitor	91
Glycerol unchanged	26
Glycerol increased in tube with inhibitor	4

Table VII shows that the clearing of a coconut oil substrate by a plasma aliquot was demonstrable in only 30 of the 91 instances where lipolysis occurred. Conversely, an effect on Ediol was found in some instances where lipolysis of endogenous triglyceride had not taken place.

Discussion. The results demonstrate the presence of lipolytic activity, inhibited by known inhibitors of lipoprotein lipase, in the plasma of 75% of normal individuals. This adds support to the concept that lipoprotein lipase functions intravascularly in the removal of triglyceride from the bloodstream in man. The present data should not be interpreted as an argument against the functioning of the enzyme at the capillary wall as well as in the circulating blood. Both sites, in the plasma and at the capillary wall, are intravascular as the triglyceride-bearing lipoproteins had not penetrated the vascular barrier prior to hydrolysis.

TABLE VI. Average Glycerol Values in Aliquots of Plasma and Plasma + Polybrene or Protamine in 121 Subjects.

	Plasma glycerol in mmole/l
Plasma alone	.098
Plasma + polybrene	.090

TABLE VII. Comparison of Evidence of Lipolysis and of Clearing of Ediol in 121 Subjects.

	Clearing of Ediol	No clearing of Ediol
Evidence of lipolysis 91 cases	30	61
No evidence of lipolysis 30 cases	5	25

Summary. 1. Endogenous lipoprotein lipase activity was found in the plasma in 75% of normal subjects. 2. *In vitro* clearing of a coconut oil substrate is a much less reliable indicator of the presence of the endogenous enzyme.

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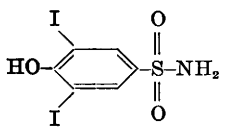
Antiviral Activity of 3,5 Diiodo-4-Hydroxybenzenesulfonamide in Chick Embryo Lung Tissue Culture.* (29268)

W. D. KUNDIN,† MARY LOUISE ROBBINS AND PAUL K. SMITH‡
Departments of Pharmacology and Microbiology, George Washington University School of Medicine, Washington, D. C. and Section of Virus Research, Dept. of Pediatrics and Dept. of Microbiology, University of Kansas School of Medicine, Kansas City, Kan.

Analogues of thyroxine, as well as thyroxine itself, have been tested with varying success for possible chemotherapeutic activity against the growth of viruses(1-6). While several such derivatives have been shown to be ineffective against the influenza virus in tissue culture(1), 3,5 diiodo-4-hydroxybenzenesulfonamide§ (DHS) was found to possess virus-inhibiting properties.



Methods. For the present experiments growth of the WS strain of influenza A virus was determined by means of hemagglutination titrations. The chick embryo lung tissue culture technique, preparation and administration of test compounds, hemagglutination procedures, as well as tests for the effect of the compound on virus inhibition, virus inactivation and virus growth in eggs and mice, have been described(1).

Results. DHS was found to be effective in inhibiting the growth of influenza A virus in chick embryo lung roller tube tissue culture. Using a virus inoculum of 10^3 50% egg-infectious doses (EID_{50}) DHS was non-toxic to tissue in concentrations up to 0.5 mg/ml, yet it inhibited virus growth in concentrations down to 0.03 mg/ml, giving a therapeutic index of 16.

To determine whether the antiviral activity of DHS was due to its structural resemblance to normal metabolites, several compounds were tested for their ability to reverse the DHS-induced inhibition. Thyroxine, tyrosine, phenylalanine, p-aminobenzoic acid, 3,5 diiodo-1-tyrosine and 3,5 diiodo-4-hydroxybenzoic acid were ineffective in this regard.

Of some 28 other sulfonamides, sulfones or other sulfur bearing compounds, and 100 benzoyl and phenyl compounds, only 4‖ were effective in inhibiting the growth of influenza virus, implying that those structural moieties were not related to DHS viral inhibition. These 4 compounds were not as inhibitory as DHS (therapeutic indices of 8,4,4,4 respectively).

The influence of DHS at 0.12 mg/ml on the rate of virus growth in tissue culture at different time intervals was ascertained (Fig. 1). From the twelfth hour onward DHS depressed the virus infectivity titer about 100-fold until hour 44 when, perhaps because of exhaustion of DHS, the titer began to approach that of the control tubes. At no time did the virus titer reach that of the controls.

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† Present address: Section of Virus Research, Dept. of Pediatrics, Univ. of Kansas School of Medicine, Kansas City, Kan.

‡ Deceased October 6, 1960.

§ Supplied by Schering Corp., Bloomfield, N. J.

‖ Aniline, benzal dicarboxylic acid, 2-acetoxy-5-bromobenzoic acid and p-nitrobenzoic acid.