that ATP contractions of the preparations are enhanced by permitting the specimens to come nearly to room temperature before they are detached from the applicator sticks and set up for perfusion.

Comment. This model system may be of value in *qualitative* studies of contraction and relaxation phenomena in glycerinated cardiac muscle. Observations concerning mechanisms of relaxation are in progress. The rapidity of the diffusion of solutes around the muscle cells is confirmed by the rapidity of ATP response. The ease of operation of the model system and the simplicity of preparation commend this technic for experimental observations of myocardial contractile proteins.

Summary. A method is described for the study of contraction-relaxation phenomena in glycerol-extracted cardiac muscle. Excised hearts of bullfrogs are mounted and extracted in 50% glycerine; subsequently, perfusion with electrolyte solutions and ATP may be carried out, and the contraction recorded by a mechanical system. The ease of preparation and the dependability of the muscle model may prove to be of value in the study of glycerol-extracted heart muscle.

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Effect of Lipemia and Heparin on Free Fatty Acid Concentration of Serum in Humans.* (21953)

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The discovery that the *in vitro* clearing of lipemia by plasma obtained after injection of heparin involves lipolysis of triglyceride with release of free fatty acid(1-5) has directed attention to the possibility that free fatty acid might be released *in vivo* by the action of post-heparin clearing factor. Furthermore, the possibility that clearing factor may participate in normal fat metabolism and transport, even when heparin has not been injected (6-8), suggests that free fatty acid may play a physiological role.

It was shown(9) that in rats the free fatty acid concentration of the serum rose when heparin was injected or when alimentary lipemia was present. The present study extends these observations to human subjects in whom lipemia was induced both by the alimentary route and by intravenous injection of fat emulsion and in whom heparin was injected intravenously both in the fasting state and during alimentary lipemia.

Subjects, materials and methods. Ten healthy young adult males, conscientious objectors assigned to the Metabolic Research Division of this laboratory, served as subjects. Heparin (Testagar, 10 mg/ml) was injected intravenously at a dose of 0.5 mg/kg body weight. For the production of alimentary lipemia commercial cottonseed oil (Wesson) was used. The fat emulsion used for intravenous injection (Lipomul, Upjohn) had the following composition: cottonseed oil 15%, dextrose 4.2%, soybean phosphatide 1%, and polyethoxy-propylene oxide (Pluronic F-68, Wyandotte) 0.5%. *Blood lipid analyses.* Venous blood samples were taken from the antecubital vein in chilled syringes and placed immediately in an ice water bath.

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TABLE I. Blood Lipid Levels of Human Subjects, Fasting, Lipemic, and after Injection of Heparin.

	Fasting	Lipemic	Lipemic, post-heparin	Fasting, post-heparin
	Optical density			
Whole serum				
Mean	.072	.211	.158	
Stand. dev.	.018	.086	.091	
Infranatant				
Mean	.044	.055	.046	
Stand. dev.	.006	.009	.009	
	Total fatty acid (meq/l)			
Whole serum				
Mean	14.0	17.7	15.8	
Stand. dev.	4.68	6.81	3.35	
Infranatant				
Mean	12.1	13.1	14.4	
Stand. dev.	1.69	2.30	2.73	
	Free fatty acid (meq/l)			
Whole serum				
Mean	.51	.75	1.76	1.00
Stand. dev.	.11	.14	.51	.18
Infranatant				
Mean	.51	.75	2.49	
Stand. dev.	.11	.14	.87	

Refrigeration was maintained during centrifugation and separation of the serum up to the time of analysis. Optical density was determined at 700 mμ in a Coleman Junior spectrophotometer using microcuvettes. Total fatty acid was determined by a modification of the method of Smith and Kik(10), and free fatty acid was determined by a modification(9) of the method of Davis(11). In the study of alimentary lipemia analyses were done on whole serum and on the infranatant obtained by high speed centrifugation. The high speed centrifugation was carried out in a Servall superspeed centrifuge in a room maintained at 5°C. After 30 minutes centrifugation at 14,000 rpm the infranatant was drawn off with a syringe and needle and recentrifuged in a similar fashion.

Results. Alimentary lipemia and heparin injection. After a 12-hour overnight fast the 10 subjects received 150 ml of cottonseed oil by fine polyethylene nasogastric tube. Four hours later heparin was given intravenously in a dose of 0.5 mg per kg of body weight. Blood samples were drawn immediately before giving the oil (fasting sample), 4 hours later, just before giving the heparin (lipemic sample), and 15 minutes after the heparin injection (lipemic post-heparin sample). The results are summarized in Table I. The lipemic samples showed an elevation of the values for optical density and free and total fatty acid when compared with the fasting samples. The elevation of free fatty acid concentration was statistically highly significant (Table II). Comparing the values for whole serum and infranatant, it is seen that whereas total fatty acid concentration was significantly reduced by removal of visible turbid lipid, free fatty acid concentration remained unchanged (Table II). Injection of heparin resulted in a decrease in optical density and total fatty acid concentration but a marked elevation of free fatty acid concentration (Table I). The values for free fatty acid concentration of the infranatant samples after heparin injection were even higher than those of the corresponding whole serum. This is attributable to the delay in analysis (about 2 hours) that was required to accomplish the centrifugation. Whole serum post-heparin samples held at 5°C but not centrifuged showed a similar rise in this period of time but pre-heparin samples did not. Thus, in contrast to our previous findings on rat serum(9), refrigeration does not prevent completely *in vitro* lipolysis in human post-heparin serum. For this reason the values for free fatty acid in post-heparin serum given in this paper cannot be taken to represent the true *in vivo* situation since they include an increment that has occurred *in vitro*.

Heparin injection in fasting subjects. Blood samples were taken 15 minutes after intravenous injection of 0.5 mg heparin per kg body weight while the 10 subjects were in the fasting state. Free fatty acid concentration (Table I) was substantially elevated (1.00 meq/l) as compared with the pre-injection control (0.51 meq/l). Because of the possibility that *in vitro* lipolysis was occurring in these post-heparin samples, it cannot be determined how closely this reflects the true *in vivo* situation.

Intravenous injection of fat emulsion. Nine of the test subjects each received 3 separate 500 ml infusions of fat emulsion at 3 different rates on consecutive days. The rates were 30, 60 and 120 drops per minute. The assignment of the order of infusion rates to the subjects

TABLE II. Significance of Differences in Table I.

Difference between	Mean difference	Stand. error of mean difference	t	P
Total fatty acid				
Lipemic, whole serum vs. infranatant	4.58	1.52	3.01	<.01
Free fatty acid				
Fasting, whole serum vs. infranatant	.004	.008	.50	>.05
Lipemic, *Idem*	.008	.007	1.14	>.05
Whole serum, fasting vs. lipemic	.244	.043	5.67	<.01

followed a Latin square design. Venous blood samples were taken at the end of the infusion.

The results are summarized in Fig. 1. Optical density and free and total fatty acid concentrations bore a linear relation to the logarithm of the infusion rate. Analysis of variance showed that the free fatty acid concentrations were significantly different at the various infusion rates and that the regression of free fatty acid concentration on logarithm of infusion rate did not deviate significantly from linearity. In a separate study it was found that injection of the emulsifying agents without fat did not produce any alteration in optical density or free and total fatty acid concentration(18).

Discussion. The results of the present study confirm the findings of the previous study in rats(9) in showing that an increase in free fatty acid concentration of the serum occurs during lipemia. In addition it was found that the free fatty acid resides in the non-particulate portion of the serum since its concentration was not reduced by removal of the turbid lipid particles by high speed centrifugation. Serum albumin is known to have a high binding capacity for fatty acid(12-16) and it is probable that most of it is so bound in serum. It has been demonstrated in this Laboratory that when C^{14}-labeled triglyceride is incubated with post-heparin plasma *in vitro* incorporation of activity into the albumin fraction, as detected by filter paper electrophoresis, occurs(17). The highest concentration of free fatty acid encountered in this study (excluding the heparin studies where *in vitro* lipolysis occurred) was 1.46 meq/l in one of the subjects after rapid intravenous injection of fat emulsion. Assuming a molar concentration of albumin in serum of 0.7 mM/l. this would represent a 2 to 1 molar ratio of fatty acid to albumin. *In vitro* studies (12) have shown that the binding capacity of albumin for fatty acid may go as high as 6 to 1. Although the present study was complicated by the occurrence of *in vitro* lipolysis in the post-heparin samples, it is reasonable to assume that the entire elevation was not due to this factor and that heparin injection in human subjects produces an elevation of *in vivo* free fatty acid concentration comparable to that which has previously been demonstrated in rats(9). Much higher concentrations of total fatty acid were achieved in the studies with intravenous infusion of fat emulsion than in those on alimentary lipemia. It was found that the concentration of free fatty acid rose as the concentration of total fatty acid rose. This suggests that the rate of

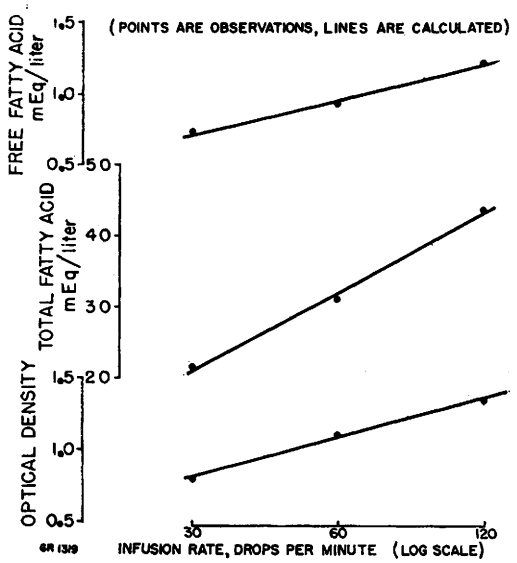

FIG. 1. Relation between rate of infusion of fat emulsion and blood lipid levels at end of infusion in human subjects.

lipolysis is augmented by mass action as the concentration of substrate (triglyceride fat) increases. The findings of this study are consonant with the hypothesis that lipolysis plays a physiological role in removal of turbid fat particles from the blood and that unesterified fatty acid bound to protein may be a mechanism for transporting fat from blood to tissues. Heparin probably accelerates the activity of the lipolytic mechanism(5).

Summary. 1. During alimentary lipemia and lipemia produced by intravenous injection of fat emulsions there was a rise in the concentration of free fatty acid in serum. The free fatty acid was found in the non-turbid portion of serum (infranatant of high speed centrifugation). Injection of heparin produced an elevation of free fatty acid concentration which was greater in lipemic than in fasting subjects. A portion of this elevation after heparin was attributable to *in vitro* lipolysis. 2. It is suggested that lipolysis with free fatty acid formation may play a role in normal fat metabolism and transport.

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Effect of Triiodothyronine on Oxygen Consumption of Tissues Not Responsive to Thyroxine.* (21954)

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The most prominent effect of thyroxine injection in experimental animals is an increase in metabolic rate. Corresponding increases in oxygen consumption have been obtained with such tissues as heart, liver, kidney, diaphragm, skeletal muscle and salivary gland removed from thyroxine-injected animals. In contrast, many other tissues do not participate in the metabolic stimulation, notably brain, spleen, testis, prostate, seminal vesicle, ovary, uterus, thymus and lymph node(1,2).

Since the discovery of triiodothyronine (TRIT) as a form of thyroid hormone even more potent than thyroxine(3), it has been proposed that the latter may be active only after partial deiodination to triiodothyronine (4). Evidence is accumulating that such a transformation does occur(5,6), although its obligatory character is far from established (7). It seemed possible that the lack of response of the tissues mentioned above, as far as thyroxine was concerned, might be due to

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