minogen, attacked casein at an easily measureable rate. The fact that the hydrolysis had to be carried out at 4° (37° did not produce an active product) suggests that the intermediate chymotryptic activity described by Keller, Cohen and Neurath(4) which appeared during the conversion of procarboxypeptidase to CP at 4° may have been involved. The complexity of the activation of procarboxypeptidase and the varieties of both CP and chymotrypsin which have been described, preclude an answer at this time.

The evidence suggesting that PHP is a partially hydrolyzed product of plasminogen is (1) PHP was soluble at neutral pH. In a control experiment in which plasminogen was repeatedly extracted with phosphate buffer and the extracts pooled and lyophilized, the product obtained was only sparsely soluble at pH 7.4, (2) 10% or more of the plasminogen molecule became TCA soluble during treatment with CP or α-chymotrypsin, (3) the isolated PHP contained 13.8% N as compared with 15.9% found in the plasminogen from which it was derived, (4) in the ultracentrifuge, PHP was homogeneous at pH 3.0 and traveled slower than the equally homogeneous plasminogen from which it had been prepared. Greater diffusion during the ultracentrifuge run indicating a smaller molecule, was also noted for PHP.

Summary. Insoluble plasminogen when treated with a certain carboxypeptidase preparation after destruction of CP activity, or with α-chymotrypsin, produced a soluble, active material with all the biochemical activities of the original substance. The identity of the hydrolyzing enzyme has not been established. Evidence is presented that the product obtained is partially hydrolyzed plasminogen (PHP).

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Transport of Glycerol in Human Blood* (28539)

C. F. BORCHGREVINK† AND R. J. HAVEL (Introduced by Ellen Brown)
Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco

The release of free fatty acids (FFA) from adipose tissue *in vitro* is accompanied by release of glycerol(1). Recent reports of increased plasma levels of glycerol as well as FFA after injection of epinephrine(2) and norepinephrine(3), which increase the rate of hydrolysis of triglycerides in adipose tissue(4), can be considered evidence that such release occurs *in vivo*. The sites of removal of glycerol from the blood have not been studied under physiological conditions. The high activity of glycerokinase in liver and kidney(5,6) suggests that these organs may be important sites of removal. We studied the concentration of glycerol in human blood in various regions. Our observations show that glycerol is released from the area drained by the saphenous vein and is taken up in the splanchnic region and kidney.

Material and methods. We studied 16 women and 14 men, aged 10 to 62 years (mean age 38) during cardiac catheterization. They were not acutely ill and had neither anemia nor polycythemia. They were catheterized in the morning after fasting overnight or between 1 and 2 P.M. after a light breakfast of toast and coffee about 8 A.M. They received no sedatives, nor was heparin injected before the blood samples were withdrawn.

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† Lederle International Fellow, 1962. San Francisco Heart Assn. Senior Fellow, 1963.

TABLE I. Concentrations of Free Glycerol and FFA in Arterial Blood, Mixed Venous Blood, and in Hepatic, Renal and Saphenous Vein. Concentrations Refer to Whole Blood and Not to Plasma.

No. of patients		Glycerol (mmoles/l) Mean	Glycerol (mmoles/l) Range	FFA (mmoles/l) Mean	FFA (mmoles/l) Range	Difference for FFA/difference for glycerol Mean	Difference for FFA/difference for glycerol Range
	Brachial artery	.137	.061 - .220	.51	.24 - .75		
11	Saphenous vein	.367	.127 - .529	.91	.28 - 1.25		
	Difference	−.230	−.050 - −.330	−.40	−.64 - +.07	1.8	.0 - 6.9
	Brachial artery	.103	.066 - .176	.54	.28- .77		
6	Hepatic vein	.046	.016 - .093	.35	.19 - .47		
	Difference	.057	.037 - .083	.19	.05 - .35	3.3	1.5 - 8.2
	Brachial artery	.072	.063 - .084	.36	.18 - .53		
5	Renal vein	.057	.048 - .067	.37	.18 - .54		
	Difference	.015	.011 - .022	−.01	−.02 - .00		
	Pulmonary artery	.102	.043 - .293	.41	.20 - .65		
10	Brachial artery	.102	.046 - .275	.38	.20 - .59		
	Difference	.000	−.011 - +.018	.03	.00 - .07		

Procedure. We took 15 ml of blood simultaneously from the brachial artery and another site (pulmonary artery or hepatic, renal or saphenous vein) and placed it in heparinized tubes. The tubes were chilled in ice water and then centrifuged for 20 minutes at 3°C. The plasma was promptly processed for analysis of glycerol and FFA. Two blood samples were obtained at 10-minute intervals from each of 5 patients. The differences between the concentration of FFA and glycerol in the 2 samples were very small in all patients, suggesting that they were in a steady state with respect to these metabolites.

Glycerol was measured in duplicate by the method of Wieland(7) as modified by Larsen(8). Since we were interested in uptake and release of glycerol, which equilibrates rapidly between plasma and red cells, we calculated the concentration of glycerol in whole blood as the plasma concentration × 0.9(9). FFA were measured in duplicate by the method of Dole(10) as modified by Trout *et al.*(11). Since the volume of packed red cells in our subjects was close to 40% (ranging from 37-44%), the concentration of FFA in whole blood was taken as the plasma concentration × 0.6.

Results. Table I summarizes our data. Both glycerol and FFA were released into the blood in the region drained by the saphenous vein. There was a consistent uptake of both glycerol and FFA in the splanchnic region and of glycerol in the kidney. There was no demonstrable change in concentration of glycerol across the lungs, but removal of FFA was significant, averaging .03 mmoles per liter.

Fig. 1 shows that concentrations of FFA and glycerol in arterial blood are positively correlated.

Discussion. The consistent release of glycerol from the region drained by the saphenous vein and the positive correlation between arterial concentrations of glycerol and FFA strongly suggest that adipose tissue is an important site of entry of glycerol into the blood in man. Similar findings have been reported for the dog by Carlson and Orö (12). It is by no means certain, however, that this is the only tissue that contributes glycerol to the blood. In saphenous vein blood, for example, glycerol could be derived from hydrolysis of triglycerides in other tissues, such as muscle, or of plasma triglycerides by lipoprotein lipase(13).

Our studies also show that glycerol is removed from the blood in the splanchnic region and kidneys. Based on blood flows of

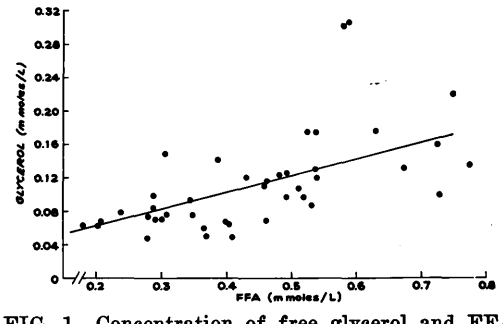

FIG. 1. Concentration of free glycerol and FFA in arterial blood in 30 patients undergoing heart catheterization.

1400 and 1100 ml per minute, respectively (14), net removal of glycerol from the blood was .052 to .11 mmoles per minute in the splanchnic region and .011 to .022 mmoles per minute in the kidneys. These observations agree well with those of Holst(15) and of Larsen(8) in cats. Larsen found that after intravenous injection of glycerol the liver accounted for 80 to 90% of that removed from the blood and the kidneys for 10 to 20%. Removal in other organs must have been negligible. The kinetics of removal were of first order below and of zero order above a concentration of about 100 mg (1.1 mmoles) per liter; this suggested saturation of the removal mechanisms at this level. The reported findings after administration of glycerol to cats are consistent with the limitation of high glycerokinase activities to the liver and kidney of the rat(6). Recently, the intestinal mucosa has been reported to have glycerokinase activity(16), and it may be another additional site of removal.

The calculated net removal of glycerol from the blood in the splanchnic region and in the kidney was .06 to .13 mmoles per minute. If we assume that glycerol in the blood is derived chiefly from adipose tissue and not utilized there(4), and is leaving the blood mainly in the splanchnic region and the kidney(8,15), in a steady state this calculated uptake will be equivalent to the amount of glycerol released by adipose tissue. If triglycerides in adipose tissue are completely hydrolyzed and if FFA are not reutilized, 3 moles of FFA should enter the blood for each mole of glycerol. This would mean that .18 to .39 mmoles of FFA was released per minute into the blood from adipose tissue in our subjects. However, we have calculated the removal of FFA in the splanchnic region alone to be .07 to .49, assuming a blood flow of 1400 ml.‡ Since in resting animals only about a third of the FFA transported in blood enter the liver(17,18), it appears likely that the release of FFA from adipose tissue must be higher than our figure based on the calculated glycerol release. In other words, more than 3 moles of FFA leaves adipose tissue for each mole of glycerol. Actually, we did observe that more than 3 moles of FFA was released from the region drained by the saphenous vein for each mole of glycerol in 2 patients (Table I). The lower ratios observed in most of the subjects can of course be explained by utilization of FFA in adipose tissue or other tissues drained by the saphenous vein. FFA could enter the blood from adipose tissue without being accompanied by glycerol if they were derived from partial hydrolysis of triglycerides or from newly synthesized fatty acids or if glycerol is utilized in adipose tissue. Evidence for partial hydrolysis of triglycerides in adipose tissue after injection of epinephrine has been given by Wadström(19).

‡ The amount of both FFA and glycerol removed in the liver may exceed the values calculated for splanchnic uptake because of their release from splanchnic adipose tissue, but this does not affect consideration of relative amounts taken up in this region.

Summary. Glycerol is released from the area drained by the saphenous vein in man and taken up in the splanchnic region and kidneys. The arterial concentrations of glycerol and FFA are positively correlated. In resting subjects, net splanchnic uptake of glycerol was calculated to be .052 to .11 mmoles per minute and renal uptake .011 to .022 mmoles per minute. It seems likely that more than 3 moles of FFA is released from adipose tissue for each mole of glycerol.

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Unilateral Renal Hemodynamic Changes with Hypertonic Mannitol.* (28540)

LEONARD B. BERMAN, KEMAL ÖNEN, AND GEOFFREY CHISHOLM

Kidney Research Laboratory, Loma Linda University Service, Los Angeles County Hospital, Los Angeles, Calif.

The renal hemodynamic effects of mannitol have recently become of interest in prevention of renal ischemia under various clinical circumstances(1,2). Harvey has reported that, in the excised perfused dog kidney, hypertonic mannitol infusions reduce the resistance to blood flow. Hypertonic glucose has a similar effect, while urea does not(3). Lilienfield & Braun(4,5) found that 20% mannitol infused intravenously into anesthetized dogs increased PAH clearance while decreasing both the inulin clearance and the extraction fraction of PAH. They suggested that mannitol had produced dilatation of the efferent arterioles of juxta-medullary glomeruli. The same authors also found, by clearance techniques, that hypertonic mannitol infused during, or after the induction of hemorrhagic shock, prevented or restored the decrease in PAH and inulin clearances observed during the hypotensive period. Teschan and his colleagues(6) confirmed these effects of both hypertonic mannitol and glucose, using direct and electromagnetic flow meter measurements of renal blood flow. A decreased postglomerular resistance was suggested by these authors. Finally, Goldberg and Lilienfield have reported that, while 20% mannitol reduces renal resistance to blood flow, 6.6% mannitol does not(7). They speculate that the effect of the 20% solution is on some extra-renal volume receptor which causes release of a renal vasodilating substance. The present experiments were undertaken to examine this last point.

Materials and methods. Group I. Eight, anesthetized mongrel dogs weighing 18-22 kg were prepared with bilateral ureteral cannulas and a needle in one renal artery. Priming and sustaining infusions of PAH, and creatinine, were begun in the usual manner for estimating renal plasma flow and filtration rate. After a 20-minute waiting period, PAH and creatinine clearances were measured in each kidney (2 clearance periods of about 10 minutes each). Twenty per cent mannitol was then infused into the renal arterial needle by constant infusion pump at the rate of 300 mg/min for 15 minutes. Clearances were again measured in each kidney.

Group II. Five dogs were prepared as in Group I. Control clearances were measured in each kidney. The dogs were then bled from a femoral artery to a total loss of 30-35 cc/kg in 15 minutes. Separate clearances were again measured (single periods of 15 minutes) although urine flows were extremely small. Twenty per cent mannitol was then infused into the renal arterial needle at a rate of 480 mg/min for 10 minutes, followed by a third set of separate clearances (3 pe-

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