The Effect of Anoxia and of Muscle Activity on the Lymphatic and Venous Transport of Lactate Dehydrogenase

The concentration of the cytoplasmic enzyme lactate dehydrogenase (LDH) in blood plasma is increased by muscle activity and anoxia[1-3]. The controversial question whether or not extravascular protein molecules are absorbed and carried into the circulation exclusivelly by the lymphatics without the participation of the blood capillaries[4] may be resolved by the study of the lymphatic and venous transport of LDH. The enzyme accumulating in the tissue fluids in consequence of cellular activity or damage could serve as an autologous protein tracer.

Material and methods. In 11 dogs under pentobarbitone-sodium anaesthesia, the thoracic duct was cannulated on the neck and the collateral branches and secondary lymphatics communicating with the veins, were ligated. A cannula was introduced in a crural lymphatic accompanying the saphenous vein. The blood flow to the hind limb was interrupted for $3^1/_2$ h with a tourniquet. Arterial and femoral blood samples and leg lymph were collected before the occlusion of the circulation, immediatally after the release of the tourniquet and 3 h later.

In another group of 13 dogs with thoracic duct fistula, the thigh muscles were stimulated with a square wave impulse generator (frequency 8 imp/sec). In these animals a femoral lymph vessel was cannulated distally from the inguinal lymph node. Arterial and femoral venous blood samples as well as femoral lymph were collected before the stimulation, after 30 and 60 min of muscle activity and finally 1 and 3 h after the end of the second period of muscle stimulation.

LDH activity in plasma and lymph samples was determined according to the method of WROBLEVSKY and LADUE[5].

Results. Muscle anoxia produced, 1 and 3 h after tourniquet release, a 2-to 3-fold increase of the LDH-activity in leg lymph ($p < 0.05$). The serum LDH-level increased immediatally after tourniquet release (Figure 1) and a significant difference could be detected between activities in femoral venous and arterial blood ($p < 0.05$).

In the second group of animals, already 30 min of muscular exercise produced a significant rise of lymphatic LDH. The increase continued during the second stimulation and the postexercise observation periods, culminating in a 5-fold increase ($p < 0.01$). A significant change in serum activity ($p < 0.01$) was first noted after 60 min of muscle activity and 3 h later the serum enzyme level was more than doubled. No significant difference could be detected between arterial and femoral venous blood LDH-activities (Figure 2).

Discussion. The increase of serum LDH-activity after muscle activity and anoxia in the animals with thoracic duct fistula, i.e. where the connection between the lymphatic and venous system was interrupted, can be interpreted as a proof for the direct access of the protein molecules into the blood capillaries. This assumption is supported by the significantly higher than arterial enzyme activity in the venous blood flowing from the anoxic extremity. The lack of arteriovenous difference during electrical stimulation may be possibly explained by the great increase of blood flow in the active muscles.

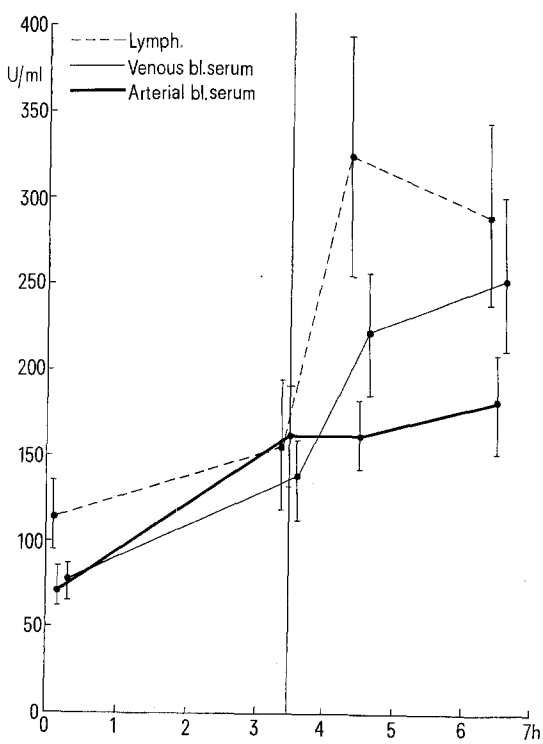

Fig. 1. LDH activities in serum and lymph before and after $3^1/_2$ h occlusion of the blood flow to both hind extremities.

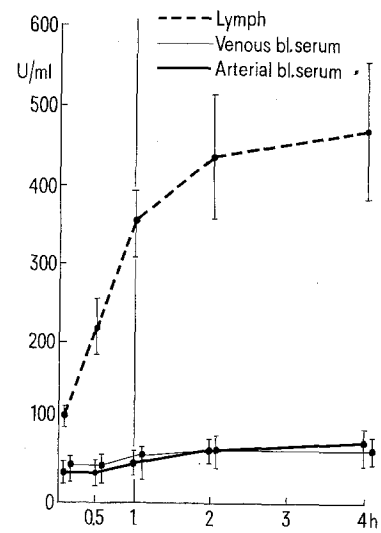

Fig. 2. LDH activities in serum and lymph before, during and after muscle stimulation.

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Zusammenfassung. Nach $3^1/_2$stündiger Muskelischämie wurde bei Hunden mit Ductus thoracicus-Fistel eine signifikante Zunahme der Milchsäuredehydrogenase-Aktivität im Blutserum und in der Lymphe der anoxischen Extremität bei gleichzeitiger signifikanter arterovenöser Aktivitätsdifferenz beobachtet. Auch Muskelarbeit verursachte eine Erhöhung der Enzymaktivität im Serum und in der Lymphe. Durch Unterbrechen der Verbindung zwischen Venen und Lymphsystem konnte das direkte Eindringen des Enzymproteins in die Blutkapillaren bewiesen werden.

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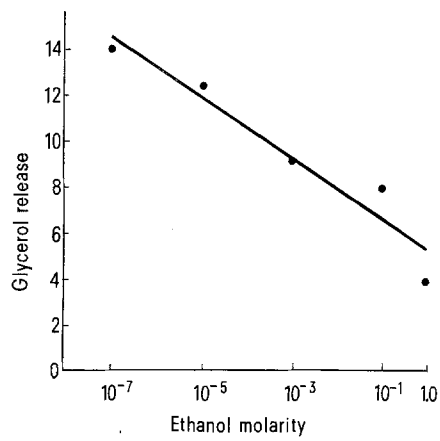
Ethanol Inhibition of Serum Stimulated Lipolysis in Isolated Fat Cells of the Rat

The effects of ethanol on lipid metabolism in liver and adipose tissue in vivo, have been investigated by a number of workers[1–4]. However, there is little information available on the acute effects of ethanol on isolated adipose tissue in vitro. BIZZI and CARLSON[5] showed that when pieces of epididymal adipose tissue, obtained from fed rats, were incubated with increasing concentrations of ethanol (2–20 mg/ml), there was a significant increase in basal glycerol release, but no effect on noradrenaline stimulated release of glycerol. When epididymal adipose tissue from fasted rats was used, there was no change in the basal release of glycerol with increase in ethanol concentration. SCHIEG[6] has shown that ethanol diminished glyceride-glycerol formation, from glucose, in isolated lipocytes, and suggested that ethanol may, itself, be utilized as a substrate for fatty acid synthesis.

Recent studies have demonstrated the capacity of fasted rat serum to mobilize intracellular triglyceride from free fat cells of the rat, in vitro[7–11]. The aim of this study was to investigate the effects of increasing concentrations of ethanol on serum stimulated lipolysis in isolated fat cells obtained from fasted rats.

Materials and methods. Blood, for serum, and epididymal adipose tissue, were obtained from overnight fasted male albino Wistar rats, and a suspension of isolated rat fat cells prepared by a modified RODBELL[12] scheme previously described[7]. Fat cells were dispensed into polypropylene incubation vials containing Krebs-Ringer bicarbonate buffer (with 3.5 g/100 ml human albumen and 45 mg/100 ml glucose), ethanol at concentrations ranged between 10^{-7} *M* and 1.0 *M*, and 1.10 ml of fasted rat serum. (It was shown previously[7–10] that when serum doses ranged in arithmetic progression from 0.10 ml 1.50 ml were employed in stimulating lipolysis, the maximally effective dose was 1.10 ml. Further increases in serum dose did not result in corresponding increases in response.) The effect of ethanol on fat cells in the absence of serum, was also investigated. Incubation vials were gassed with 75% nitrogen, 20% oxygen and 5% carbon dioxide, capped and incubated for 90 min in a shaking water bath at 37°C.

Glycerol release was employed as an index of lipolysis. Glycerol was estimated by a modified WIELAND[13] technique described previously[7], and related to the weight of adipose cell lipid present. Lipid content of an aliquot of isolated fat cells was estimated by a modified FOLCH[14] technique previously described[7,15].



Regression line for ethanol inhibition of glycerol release from isolated fat cells obtained from fasted rats. Release (nanomoles of glycerol/mg lipid/90 min) on the vertical axis was plotted against ethanol molarity on the horizontal axis $n = 5$, $r = 0.966$, $P < 0.001$.

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Effects of ethanol on basal lipolysis in isolated fat cells

Ethanol molarity	—	1.0	10^{-1}	10^{-3}	10^{-5}	10^{-7}
Glycerol Release[a]	2.8[b] ± 0.5	2.0 ± 0.4	2.3 ± 0.2	2.3 ± 0.3	2.2 ± 0.7	2.0 ± 0.5

[a] Release expressed in nanomoles of glycerol/mg lipid/90 min; [b] Mean of 3 observations ± S.E.M.