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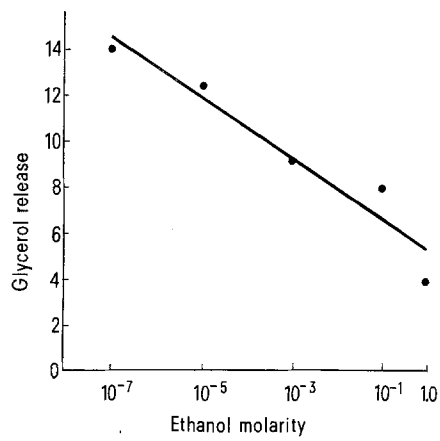
Ethanol Inhibition of Serum Stimulated Lipolysis in Isolated Fat Cells of the Rat

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.

Results. The data in the Table show that ethanol had no obvious effect on the basal release of glycerol from isolated fat cells. However, when lipolysis was provoked by rat serum, there was a linear correlation between the degree of inhibition of glycerol release and the concentration of ethanol in the incubation medium. The calculated regression line in the Figure clearly shows this.

Discussion. The lack of pharmacologic effect of ethanol (10^{-7} M–1.0 M) on the basal lipolysis of free fat cells obtained from fasted rats (Table) is in keeping with the observations of BIZZI and CARLSON[5]. The inhibition of hormone (i.e. serum) stimulated lipolysis, by ethanol, in isolated fat cells obtained from fasted rats over the concentration range of alcohol of 10^{-7} M–1.0 M, contrasts with the promotion of lipolysis, observed in fed rats, by ethanol at appromimately 5×10^{-2} M–5×10^{-1} M. However, it is known that adipose cells from fasted rats are more sensitive to lipolytic hormones than are cells from fed rats. This dual effect of ethanol, dependent upon the nutritional status of the fat cell, could be explained in the following terms: in lipocytes from fed rats there is present a quantity of dietry lipid from the circulation not as yet incorporated into the storage pool of neutral fat. If this is subsequently esterified with glyceride-glycerol formed from ethanol rather than glucose/alpha glycerophosphate (the alcohol would enter the fat cell at a faster rate than glucose owing to higher lipid solubility and smaller molecular mass) then glycerol formed via the α-glycerphosphate pathway is surplus to requirements and effuses from the fat cells. In the fasting state, lipogenesis is minimal, ethanol still penetrates the fat cells and becomes partitioned between the water soluble compartment and the lipid soluble compartment of the fat cell thus reducing available interface where the lipase(s) act(s), in effect blocking the enzyme from the substrate so inhibiting lipolysis[16].

Résumé. Il y a une connexion linéaire entre la concentration progressive de l'éthanol et l'inhibition de la lipolyse stimulée par le sérum dans les cellules libres du tissu adipeux de rats affamés. L'éthanol n'a pas affecté la décharge basale du glycerol.

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Comparison of Sterol Composition and Transformation in Chicken and Pigeon Intestines

During our studies using the spontaneously atherosclerosis-susceptible White Carneau pigeon, we noted that coprostanol and other 5β-stanols were absent from the feces of this species while chicken feces contained a significant amount of coprostanol[1,2]. This stimulated us to compare the nature of the sterols and their transformation products in the intestine and intestinal contents of these 2 species. Specifically, 2 aspects of sterol transformation in the intestine were examined: 1. formation of coprostanol and 2. the nature of plant sterols in the intestinal tissue and their relation to the plant sterols in the diet. The uptake of plant sterols by the avian intestines has previously been demonstrated[3,4].

Materials and methods. Adult White Carneau pigeons were obtained from the Palmetto Pigeon Plant (Sumter, S.C.) and were fed a mixed grain diet (Purina Pigeon Chow, Ralston Purina Co., St. Louis, Mo.). Roosters and hens obtained locally were fed chicken feed (Laudon Bros. Feed and Seed Co., Dover, Minn.). Cholesterol-4-^{14}C (specific activity, 60.9 mCi/mole) and reference sterols were obtained commercially. Intestinal tissue segments and their contents were obtained after the birds were killed. The intestinal tissues were rinsed with saline and 1 mM sodium taurodeoxycholate solution and weighed.

Sterols and steryl esters were extracted from the tissue samples asaportion of the total lipid extract, as described by FOLCH et al.[5] Free sterols and steryl esters were then separated from each other and other lipids by thin layer chromatography (TLC) on silica gel G with the solvent system, heptane-isopropyl ether-acetic acid (65:40:4, v/v/v)[6]. The sterols of the intestinal contents were extracted and purified as described previously for the fecal steroids[1,2].

After TLC, the trimethyl silyl ether derivatives of the sterols were identified by gas-liquid chromatography (GLC) in an F & M 402 high-efficiency gas chromatograph equipped with a flame ionization detector[7]. The sterols were chromatographed on glass columns packed with 3.8% W-98 on Diatoport (80 to 120 mesh)[1,2]. The sterols were quantitated by gas chromatography with 5α-cholestane as an internal standard[1,2,7].

Results and discussion. Sterols of the intestinal contents represent a mixture of sterols derived from the bile, intestinal wall, and diet and their bacterial products. On TLC, chicken intestinal contents contained a band corresponding to coprostanol, but no such band was found in pigeon intestinal contents. There was no band corresponding to coprostanone in either species. The presence of coprostanol derivatives in the chicken but not in the pigeon is in agreement with our previous studies on the fecal sterols of these 2 species[1,2].

On GLC, the band corresponding to coprostanol gave peaks corresponding to coprostanol and the 5β-stanols of

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