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The Uptake of Glycerol Ethers by the Lactating Goat Mammary Gland

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The mammary uptake of blood triglycerides, in the form of chylomicrons and low-density lipoproteins, accounts for 50–60% of milk-fat triglyceride in the goat (Linzell, 1968), but it is not known if the fat is taken up as intact triglyceride, partial glycerides or free fatty acids. Data relevant to this problem include the known mammary uptake of plasma free fatty acids (Annison, Linzell, Fazakerley & Nichols, 1967), the release of lipoprotein lipase by the mammary gland (Barry, Bartley, Linzell & Robinson, 1963) and the change in the $^{14}\text{C}/^3\text{H}$ ratio that occurs during the transfer of doubly labelled chylomicron triglyceride into milk fat (West, Annison & Linzell, 1967). The reutilization for mammary fat synthesis of the products of triglyceride hydrolysis vitiated quantitative interpretation of the latter data, and largely prevents the direct study of the uptake of partial glycerides. The use of glycerol ethers, however, which function as analogues of mono- and diglycerides in their acceptance of activated fatty acids (Gallo, Vahouny & Treadwell, 1968), provides unequivocal data on uptake since the ether linkage is not susceptible to lipase action in mammary tissue.

Stabilized emulsions of trilinoleoyl glycerol ether, 2-linoleoyl 1-oleoyl glycerol ether, 1-oleoyl glycerol ether, 2-oleoyl glycerol ether or ^{14}C -labelled glycerol monoethers (particle size 0.5–3.0 μm) were infused into the arteries of lactating goats over 90–120 min. The goats were milked at 1 h intervals for 5 h, then anaesthetized, and samples of mammary tissue were removed for analysis.

The glycerol triether was not taken up, and only traces of the glycerol diether and the glycerol 1-monoether could be detected in mammary tissue. In contrast, 10–20% of infused 2[^{14}C]-linoleoyl

glycerol ether was extracted by the udder, and about 20% of the ether taken up appeared in milk as 'triglyceride'. The specificity of the mammary gland for glycerol 2-monoethers was checked further by infusing a mixture of 2-oleoyl glycerol ether and 1-oleoyl glycerol ether: only the glycerol 2-monoether appeared in mammary tissue and milk.

The results support the view that the 2-mono-glyceride pathway of triglyceride synthesis is operative in goat mammary tissue (Dimick, McCarthy & Patton, 1966), in addition to the glycerol 3-phosphate pathway (Pynadath & Kumar, 1964), and are in line with the failure of 1-monoglycerides to act as precursors of triglyceride (Pynadath & Kumar, 1964). Further, the findings suggest that the hydrolysis of blood triglyceride to 2-monoglyceride and free fatty acids in the capillaries of the mammary gland is a prerequisite of uptake.

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Toxicity of Methylxanthines and Local Anaesthetics towards Muscle

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Although the methylxanthines are often added to tissue preparations to act as inhibitors of phosphodiesterase and hence of the breakdown of 3':5'-cyclic AMP, they are at the concentrations generally used (1–5 mM) very toxic to muscle. The tissue goes into rigor, suffers deterioration of condition and exhibits a diminished rate of incorporation of amino acids into protein. The capacity to induce contraction and rigor has been known for some years (Axelsson & Thesleff, 1958), though the mechanism is not fully understood. That the drugs may release Ca^{2+} from the sarcoplasmic reticulum has been considered (Bianchi, 1961; Weber & Hertz, 1968), and if so we may expect to see other effects on metabolism. In this we are not disappointed. Either compound at 5 mM enhances respiration of isolated diaphragm muscle by about 40%, and by about 20% when added at 1 mM. Again the mechanism of this stimulation is not clear. There

is little stimulation of lactate or glycerol output such as is seen on addition of adrenaline or when respiration is enhanced by addition of octanoate.

Caffeine-induced contractures can be prevented by various local anaesthetics, again by mechanisms thought to involve removal of Ca^{2+} from the scene of activity (Feinstein, 1963, 1964; Hauser & Dawson, 1968), and it is noteworthy that their addition (at 1 mM) to isolated diaphragm suppresses the enhancement of respiration induced by the methylxanthines and restores the appearance of the tissue to normal. Surprisingly, however, the rate of amino acid incorporation into protein is not restored to normal, but depressed further. Those compounds most successful in suppressing enhancement of respiration by methylxanthines (butacaine, dibucaine and tetracaine) were most potent in inhibiting protein synthesis in the tissue. Conversely procaine, cocaine and lignocaine had little influence on either parameter. The mechanism of this inhibition is again not clear, but is not explicable solely in terms of inhibition of amino acid uptake by the tissue, though this also occurs. Bloxam (1967a,b) noted the toxicity of various anaesthetics to liver, including interference with amino acid transport; these results in effect extend such observations to muscle.

The initial motivation of these experiments was the question whether the action of insulin on protein metabolism in muscle is influenced by the intracellular concentration of 3':5'-cyclic AMP. None of the treatments influenced the relative extent of stimulation of incorporation by insulin. Coupled with lack of direct evidence for any influence of the hormone on the 3':5'-cyclic AMP content of muscle, it appears unlikely that the effect of insulin on protein synthesis in this tissue involves the cyclic nucleotide.

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The Formation of *N*-Hydroxymethylcarbazole as a Metabolite of *N*-Methylcarbazole

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The biological demethylation of methylamines has been postulated as occurring via one of two intermediate pathways. One route suggested is that the

N-oxide is first formed, which rearranges to yield the demethylated product plus formaldehyde. The other suggestion is that the methyl group is first oxidized to yield a carbinol.

However, only with compounds having a carbonyl group adjacent to the nitrogen atom have the proposed hydroxymethyl derivatives been detected (for fuller discussion see McMahon, 1966).

We now report the formation of *N*-hydroxymethylcarbazole as a metabolite of *N*-methylcarbazole both *in vitro* and *in vivo*.

After incubation of *N*-methylcarbazole with a fortified hepatic microsomal preparation obtained from rabbit, one major and three minor metabolites were detected by chromatographic techniques. The principal compound had chromatographic properties and responses to spray reagents identical with those of authentic *N*-hydroxymethylcarbazole. Elution from chromatography plates showed that these compounds had identical u.v.-absorption spectra.

Treatment of urine, obtained from a rabbit after administration of *N*-methylcarbazole, with β -glucuronidase also produced an ether-soluble metabolite identical with the major product of the oxidation *in vitro*.

The minor metabolites observed in the microsomal oxidation of *N*-methylcarbazole were recognized as carbazole, 3-hydroxycarbazole and possibly 3-hydroxy-*N*-methylcarbazole, the first by comparison with authentic material or by comparison with the phenolic metabolite obtained from carbazole (Johns & Wright, 1964).

The demethylation of *N*-methylcarbazole was compared in various species by using the Nash assay for formaldehyde. The results showed a wide species variation in the order: mouse < rat < hamster < rabbit < cavy.

The detection of a hydroxymethyl compound as a metabolite from a methylamine supports the concept that this is an early step in dealkylation. In this case, like those observed by earlier workers, the methyl group was attached to a relatively non-basic nitrogen atom in the substrate. It may be that the basicity of a compound decides whether complete dealkylation or the formation of a hydroxymethyl derivative is the ultimate route of metabolism.

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