The Effect of Progesterone and Oestradiol on Rat Uterine Monoamine Oxidase Activity

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It appears that rat uterine monoamine oxidase (EC 1.4.3.4) activity may be under hormonal control (G. G. S. Collins, J. Pryse-Davies, M. Sandler & J. Southgate, unpublished work). Groups of 30 rats were injected on 4 consecutive days with oestradiol and progesterone (3.3mg/kg subcutaneously); a third group of untreated animals was used as controls. The enzyme activities of whole uterine homogenates were measured with [¹⁴C]tryptamine, [¹⁴C]dopamine (3,4-dihydroxy[¹⁴C]phenethylamine), [¹⁴C]tyramine and [¹⁴C]benzylamine as substrates (Robinson, Lovenberg, Keiser & Sjoerdsma, 1968); the relative rates of oxidation were 1.00, 2.21, 4.05 and 26.32 by homogenates from untreated rats. Compared with these values, the activity of uteri from progesterone treated animals was higher but differed according to the substrate employed. In contrast, oestradiol pretreatment decreased monoamine oxidase activity. Michaelis constants were not invariably altered by hormonal pretreatment, the effect again being substrate-dependent. The pH optimum with kynuramine (Kraml, 1965), tyramine and dopamine as substrates was between 7.5 and 8.0, whereas with benzylamine a second peak of activity occurred at pH 6.5.

The inhibitory effect of oestradiol could be mimicked *in vitro* and appeared to be competitive with the substrate. In contrast with rat liver (Hawkins, 1952), addition of FAD (0.01–1mM) caused an increase in uterine monoamine oxidase activity to up to 180% of control values.

Uterine monoamine oxidase from the three groups of rats was solubilized by using a modification of the method described by Youdim & Sandler (1967). Electrophoresis on 5% polyacrylamide gel (Youdim, Collins & Sandler, 1968) separated the enzyme into three bands of activity, one migrating towards the anode (MAO₁) and two towards the cathode (MAO₂ and MAO₃). The activity of each band was measured with kynuramine as substrate; of the three bands the activities of MAO₁ and MAO₂ were similar in each group, whereas that of MAO₃ prepared from oestradiol-treated rats was 17% and that from progesterone-treated rats 184% of the same band from untreated animals. In addition, this steroid-sensitive band was more heat-stable than either MAO₁ and MAO₂. The bands of activity appear to represent a polymeric series having molecular weights of about 175 800, 371 500 and 676 100.

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Mixed Lipolytic Activities of an Enzyme Preparation from the Pancreas

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Lipolytic enzymes from various tissue sources have been shown to aggregate with lipid to form high-molecular-weight complexes (Sarda, Maylie, Roger & Desnuelle, 1964; Gelotte, 1964; Downey & Andrews, 1965; Schnatz & Cortner, 1967; Okuda & Fujii, 1967, 1968). These lipoprotein complexes can be delipidized to leave enzyme subunits of much lower molecular weight, which can be reaggregated to higher-molecular-weight forms by incubation and/or sonication with lipid.

In the present study a high-molecular-weight lipolytic preparation, demonstrating the above characteristics, was obtained from acetone-dried pig pancreas powder (Miller, 1969). Chromatography of the preparation on Sephadex G-200 showed that the lipolytic activity was associated with a protein fraction excluded from the gel and eluted at the void volume of the column, and therefore having a molecular weight greater than 800 000. It also formed an insoluble complex with polyvinylpyrrolidone, thus indicating its lipoprotein nature (Cornwell & Kruger, 1961). This high-molecular-weight fraction with lipolytic activity was capable of hydrolysing both the cholesteryl esters in a plasma substrate and triglyceride emulsion in the free or albumin-activated state.

This high-molecular-weight preparation could be converted by acetone or ether extraction into a delipidized product, which on chromatography gave a second protein peak of much smaller molecular weight (15 000–20 000). This molecule also showed the same substrate specificity as the high-molecular-weight parent molecule.

Incubation of this low-molecular-weight preparation with a triglyceride emulsion at 37°C resulted in the production of a fraction that was excluded from Sephadex G-200 and whose substrate specificity and showed it to have lipolytic activity characteristic of cholesterol esterase and triglyceridase.

Since the parent high-molecular-weight preparation, the low-molecular-weight preparation and the reconstituted high-molecular-weight preparation all demonstrated the lipolytic activities associated with cholesterol esterase and triglyceridase, it is postu-

lated that these activities are mediated by a single enzyme.

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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-mono-glycerides to act as precursors of triglyceride (Pynadath & Kumar, 1964). Further, the findings suggest that the hydrolysis of blood triglyceride to 2-mono-glyceride 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