the lower layers of the epidermis contained 0.6 nmole/mg., whereas the two upper layers contained 1.8 and 3.9 nmoles/mg. of skin.

These changes in lipid composition may be attributed to temperature differences and the supply of oxygen or glucose. The increase of triglycerides in the upper layers may be due to an increase in phosphatidate phosphohydrolase activity caused by starvation of glucose. The activity of this enzyme was found to be rate-limiting in liver homogenates from fed rats and to be increased in starved rats (Vavrečka, Mitchell & Hübscher, 1968). The increase of fatty acids in the upper layers of skin may be due to increased phospholipase activity.

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Comparison of Steroid Sulphate and Free Steroids as Substrates of a Crystalline Oxidoreductase

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3 β -Hydroxypregn-5-en-20-one sulphate is now reported to be a substrate for crystalline preparations of 20 β -hydroxy steroid-NAD oxidoreductase (EC 1.1.1.53) from strains of *Streptomyces* (Hübener, Sahrholz, Schmidt-Thomé, Nesemann & Junk, 1959). Kinetic constants for this sulphate [K_m (substrate) 20 μ M with 145 μ M-NADH; K_m (NADH) 4.5 μ M with 100 μ M-substrate; V_{max} 4.0 μ moles/min. $\times$ mg. of enzyme at pH 5.2 at 25°] were similar to those for the corresponding acetate [K_m (substrate) 18 μ M; K_m (NADH) 4.9 μ M; V_{max} 2.7 μ moles/min $\times$ mg. of enzyme] and 4-en-3-one, progesterone [K_m (substrate) 22 μ M; K_m (NADH) 4.6 μ M; V_{max} 20 μ moles/min. $\times$ mg. of enzyme]. 3 β -Hydroxypregn-5-en-20-one itself behaved anomalously, undergoing only partial reduction, and it appeared to inhibit competitively (K_i 34 μ M) the reduction of pregn-4-ene-3,20-dione. This anomalous behaviour was not characteristic of 3 β -hydroxy-5-ene compounds in general. For example, 17 α ,21-dihydroxypregn-4-ene-3,20-dione [K_m (substrate) 7 μ M; V_{max} 8.9 μ moles/min. $\times$ mg. of enzyme] and 3 β ,17 α ,21-trihydroxypregn-5-en-20-one [K_m (substrate) 9 μ M; V_{max} 3.7 μ moles/min. $\times$ mg. of enzyme] behaved normally.

To investigate whether the sulphate and related free steroid substrates reacted at the same centre, the total initial rates of reaction were determined for various concentrations of each of the three possible binary mixtures of 3 β -hydroxypregn-5-en-20-one sulphate, acetate and pregn-4-ene-3,20-dione. In every case the possibility that the substrates reacted independently was excluded. However, the rates of reaction of the mixtures were lower than would result from simple mutual competitive inhibition (Pocklington & Jeffery, 1969).

The similar substrate behaviour of the ionic sulphate and hydrophobic acetate of 3 β -hydroxypregn-5-en-20-one suggests that the region of C-3 is not involved in enzyme-substrate interactions. Steroids and steroid sulphates occur widely, and some general implications of the findings are discussed.

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Stimulation by Glucose of Steroidogenesis from Acetate by the Superovulated Rat Ovary *in vitro*

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Glucose stimulates the incorporation *in vitro* of [14 C]acetate into sterols and steroids by slices of superovulated rat ovary; this effect appears to involve the provision of neither carbon nor energy and occurs in the absence of measurable pentose-cycle activity (Flint & Denton, 1969). Since malate, pyruvate, lactate and isocitrate also stimulate steroidogenesis from acetate the possibility has been considered that these effects are the result of an increased availability of substrates for NADP-malate dehydrogenase and NADP-isocitrate dehydrogenase. These enzymes may play a more important role than glucose 6-phosphate dehydrogenase in the production of NADPH for steroidogenesis in this tissue.

The total activities of NADP-malate dehydrogenase and NADP-isocitrate dehydrogenase are both about four times that of glucose 6-phosphate dehydrogenase (which is present at a total activity of 10.8 units/g. wet wt.), and their K_m values for NADP $^+$ are 0.24 and 0.48 respectively of that of glucose 6-phosphate dehydrogenase. All three enzymes are cytoplasmic.

The presence of pyruvate carboxylase (total activity 7.8 units/g. wet wt.) and NAD-malate dehydrogenase (total activity 307 units/g. wet wt.) in the superovulated rat ovary suggests that the pyruvate-malate cycle, previously described in liver (Lowenstein, 1961; Shrago & Lardy, 1966; Walter, Paetkau & Lardy, 1966) and in adipose tissue (Kornacker & Ball, 1965; Ballard & Hanson, 1967), may operate in this tissue. This cycle provides a means by which mitochondrial or cytoplasmic NADH may be converted into cytoplasmic NADPH for steroidogenesis.

The effect of glucose on steroidogenesis from acetate could be the result of glycolytic pyruvate priming the pyruvate-malate cycle. The effects of lactate and pyruvate can also be explained in these terms. The intracellular concentration malate is increased in the presence of glucose from 16 to 364 μ M. The effect of isocitrate may be due to its metabolism by NADP-isocitrate dehydrogenase.

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Absorption and Tissue Uptake of α -Tocopherol in the Rat

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Tracer doses of DL-[5-Me-³H] α -tocopherol were administered orally to groups of six rats housed individually in metabolic cages with precautions to prevent coprophagy. The net absorption (determined from faecal radioactivity) and tissue contents of radioactivity were measured after 7 days.

The effects of (1) the vitamin status of the animal, (2) the vehicle (Tween 80, alcohol or arachis oil) and (3) decreased serum lipoprotein concentrations on the absorption and tissue distribution of radioactivity were studied.

Rats deficient in vitamin E had the same net absorption as supplemented animals, but significantly more radioactivity in liver ($P < 0.02$) and kidneys ($P < 0.005$).

Net absorption of the vitamin was the same whether given in Tween 80, alcohol or arachis oil. The amounts of radioactivity in the liver but not in kidneys of groups given the vitamin in alcohol or arachis oil were significantly different ($p < 0.05$). When Tween administration was compared with

arachis oil the resulting tissue contents of radioactivity with Tween were very much greater ($P < 0.001$).

Prior lowering of serum lipoprotein concentrations in rats by prolonged dietary orotic acid did not affect net absorption, but radioactivity in adrenals ($P = 0.05$), adipose tissue ($P < 0.01$) and liver ($P < 0.001$) were significantly greater and in heart significantly lower ($P < 0.025$) than in control animals.

It is concluded that, although net absorption remains unchanged, the vitamin E status of the animal, the nature of the vehicle in which α -tocopherol is administered and the serum lipoproteins concentrations affect the tissue distribution of the ingested vitamin.

The Distribution of Vitamin D₃ Metabolites in Human Serum and Tissues

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In an earlier communication Mawer & Stanbury (1968) described the persistence for several months of more-polar metabolites in human serum after the injection of isotopically labelled vitamin D₃ (cholecalciferol).

Methods have now been developed that allow the separation of the 'polar metabolite fraction' into its several components (Mawer & Backhouse, 1969).

In the present communication the changes with time within the polar metabolite fraction of human serum will be described. Initially the principal component was peak ivb, which is eluted with diethyl ether and co-chromatographs in our silicic acid system with authentic 25-hydroxycholecalciferol (Blunt & DeLuca, 1969). This peak possesses biological activity in the rat line test approximately equal to that of cholecalciferol. Three other peaks of radioactivity were observed, none of which possessed biological activity and only one, peak vi, eluted by methanol-ether (1:1, v/v), increased in concentration with time and continued to increase after peak ivb had started to decrease.

The concentration of peak ivb and its rate of appearance in serum may be related to the previous vitamin D status of the subject. When the serum vitamin D activity, as assayed in the rat line test, was low, peak ivb was produced more rapidly and represented a higher proportion of the injected dose.

Results will also be presented describing the metabolite pattern in the tissues of a leg amputated 4 days after the subject had received intravenous [4-¹⁴C]cholecalciferol. The percentage of the injected dose present in each tissue when extra-