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.

- Blank, H., Rosenberg, E. W. & Sarkany, I. (1961). *J. Invest. Derm.* **36**, 303.
 Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
 Halprin, K. M., Ohkawara, A. & Adachi, K. (1967). *J. Invest. Derm.* **49**, 559.
 Vavrečka, M., Mitchell, M. P. & Hübscher, G. (1968). *Biochem. J.* **110**, 52P.
 Yardley, H. J. & Godfrey, G. (1964). *J. Invest. Derm.* **43**, 51.

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

By T. POCKLINGTON and J. JEFFERY. (*Department of Obstetrics and Gynaecology and Department of Chemical Pathology, University of Aberdeen*)

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.

We thank the Medical Research Council for a research studentship to T.P., and C. F. Boehringer und Soehne G.m.b.H. (Dr H. U. Bergmeyer) for gifts of enzyme and cofactor.

- Hübener, H. J., Sahrholz, F. H., Schmidt-Thomé, J., Nesemann, G. & Junk, R. (1959). *Biochim. biophys. Acta*, **35**, 270.
 Pocklington, T. & Jeffery, J. (1969). *Biochem. J.* **112**, 331.

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

By A. P. F. FLINT and R. M. DENTON. (*Department of Biochemistry, University of Bristol, BS8 1TD*)

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.