proteins might be synthesized by the liver under the control of oestradiol.

Liver slices from immature fish incorporated L-[¹⁴C]leucine into material precipitable with trichloroacetic acid, the incorporation being inhibited by puromycin. Addition of carrier egg protein to an extract of slices and precipitation with antiserum gave no radioactivity in the precipitate. Liver slices from immature fish that had received an injection of 1 mg of oestradiol benzoate/kg 5 days previously gave a rate of incorporation of L-[¹⁴C]leucine into the trichloroacetic acid precipitate greater than that of the controls and also gave incorporation of L-[¹⁴C]leucine into the egg protein immunoprecipitate, the rate being about 40% of that for the trichloroacetic acid precipitate.

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Steroid Metabolism by Foetal Sheep Adrenals

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The development and control of steroid-metabolizing enzyme systems in the foetus has received little attention. It is of interest therefore that destruction of the foetal sheep pituitary or infusion of synthetic adrenocorticotrophic hormone into the foetus *in utero* (Liggins, 1968, 1969) delays or advances parturition respectively. It has been hypothesized consequently that the developing pituitary-adrenal axis is responsible for the timing of parturition.

This preliminary study is concerned with the transformation *in vitro* of pregnenolone and progesterone by foetal sheep adrenals. Steroid metabolism has been investigated in adrenals from fetuses of different gestational age, from a foetus whose birth was prematurely induced by the intraperitoneal infusion of Synacthen *in utero* (790 µg over 76 h) and from the adult animal. Isotopically labelled pregnenolone and progesterone were incubated for 2 h at 39.5°C in Krebs-Ringer bicarbonate-glucose medium with ovine adrenal tissue from (a) adult non-pregnant ewe, (b) foetus at 122 days gestation, (c) foetus at 143 days gestation and (d) foetus at 116 days gestation after Synacthen infusion *in utero*. Steroids were extracted, isolated after extensive chromatography and derivative formation, and measured by procedures previously described (Pierrepoint, Anderson, Griffiths & Turnbull, 1969).

Foetal and adult adrenal tissues extensively metabolized pregnenolone. In the foetal adrenal at

122 days progesterone was the main product from pregnenolone, in contrast with the Synacthen-stimulated and term foetal adrenals, and also the adult gland, where there was a large conversion of this substrate into corticosteroids. Cortisol and corticosterone were the major products from pregnenolone in the Synacthen-stimulated foetal adrenal and in the adult, whereas a relative inactivity of the 11β-hydroxylation enzyme system was demonstrated in the term adrenal, 11-deoxycorticosterone and 11-deoxycortisol being the chief metabolites.

The effect of infused Synacthen on progesterone metabolism was not only to produce a greater transformation of the steroid but also to increase the production of cortisol and corticosterone compared with the unstimulated gland of similar gestational age. In the foetal tissue at 122 and 143 days 11-deoxycorticosterone was the major metabolite of progesterone, the yields of corticosterone and cortisol being relatively small. Progesterone was converted mainly into corticosterone by adult adrenal tissue.

No 3β-hydroxy steroid sulphokinase-transferase activity was demonstrated in either foetal or adult sheep adrenal tissue.

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The Distribution of ¹³¹I-Labelled Adrenocorticotrophin in the Normal Guinea Pig

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The organ distribution of injected adrenocorticotrophin has been investigated by using labelled and unlabelled preparations (Richards & Sayers, 1951; Sonenberg, Keston & Money, 1951). These were often of lower purity than those currently available and the doses were outside the physiological range. The present experiments were carried out with synthetic α¹⁻²⁴-adrenocorticotrophic hormone labelled at high specific radioactivity with ¹³¹I and the doses injected were about 20–30-fold less than those previously reported.

Normal unanaesthetized guinea pigs were injected intravenously with α¹⁻²⁴-adrenocorticotrophic hormone (CIBA, Basle, Switzerland)

labelled with [^{131}I]iodine by the method of Landon, Livanou & Greenwood (1967) at specific radioactivities of $500\ \mu\text{Ci}/\mu\text{g}$. The injected solution also contained ^{125}I -labelled plasma albumin or [^{125}I]iodide. The animals were then killed at various intervals and the radioactivity due to both isotopes in a portion of each tissue was measured in an automatic spectrometer.

^{131}I -labelled adrenocorticotrophic hormone disappeared rapidly from the plasma and was concentrated mainly in the kidney. By contrast neither [^{125}I]iodide nor ^{125}I -labelled albumin was found to accumulate at this site. There was no evidence for preferential adrenal uptake of either radioactive isotope. Extraction of tissues for adrenocorticotrophic hormone by the method of Richards & Sayers (1951) showed that a significant proportion of the renal ^{131}I radioactivity had the physicochemical characteristics of the hormone.

In similar experiments the intra-adrenal distribution of radioactivity was determined after serial sectioning of the frozen gland (Grunbaum, Geary & Glick, 1956). ^{131}I -labelled adrenocorticotrophic hormone was concentrated in the glomerulofascicular border and in the reticular zone. On the other hand ^{125}I -labelled albumin was found mainly in the reticular zone, and [^{125}I]iodide was uniformly distributed throughout the gland.

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Membrane-Bound Penicillinase of *Bacillus licheniformis* 749/C

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In *Bacillus licheniformis* 749/C there is apparently only one structural gene for penicillinase although the enzyme is found in the growth medium and in the membrane (Dubnau & Pollock, 1965). The extracellular form is soluble and the amino acid sequence is known (Ambler & Meadway, 1969). It has proved difficult to solubilize the cell-bound form by using agents that disrupt only non-covalent bonds (Lampen, 1967), although trypsin releases

the enzyme in a form that is similar to the exo form except for the absence of the terminal lysine residue (Ambler & Meadway, 1969). As the two forms are so closely related, comparative studies were undertaken to reveal properties of importance in determining the cell-bound state.

The intracellular enzyme is distributed approximately equally between the two subcellular membranous fractions (a) the plasma membrane and (b) a vesicle fraction released from cells on preparing protoplasts. They are also differentiated by their role in the secretion of penicillinase (Sargent, Ghosh & Lampen, 1968, 1969).

Both intracellular forms of penicillinase were solubilized in 0.1% sodium deoxycholate and 0.05M-pyrophosphate buffer, pH 9.0. Previous studies suggested the enzyme would remain particulate after removal of deoxycholate, and therefore the molecular weight in the presence and absence of deoxycholate was determined by gel filtration on Bio-Gel A-5M, with columns calibrated with protein standards, which were run with the sample or in a consecutive experiment.

Both cellular forms of penicillinase are eluted in the presence of deoxycholate with a molecular weight of 45000. In the absence of deoxycholate plasma-membrane penicillinase is eluted at a molecular weight of 600000 whereas vesicle-fraction penicillinase is eluted at a molecular weight of 24000 (i.e. identical with that of the exo form), and deoxycholate is separated from the proteins. Both changes in molecular weight are readily reversible. In contrast the elution volume of exoenzyme and four other proteins is unchanged in the presence or absence of deoxycholate.

Ammonium sulphate, pH 8.0 (25%, w/v), added to deoxycholate solubilizes of plasma membrane converts the plasma-membrane form into the exo form by a time- and temperature-dependent reaction. Vesicle-fraction penicillinase is converted into the exo form at pH 9.0 without salt in the absence of deoxycholate after partial purification. As these conditions are unlikely to involve changes in covalent bonding, the two cell-bound forms of penicillinase probably represent conformational variants of the exo form. The vesicle-fraction penicillinase we suggest is a hydrophobic but monomeric variant of the exo form, which in the presence of deoxycholate binds up to 50 molecules of deoxycholate/molecule to reach a molecular weight of 45000. The plasma-membrane form we consider to be an aggregate of 25 molecules that depolymerizes to monomers in the presence of deoxycholate but that then bind deoxycholate on their hydrophobic faces in the manner suggested for the vesicle-fraction enzyme.

The results certainly indicate marked organizational differences between the soluble exo form and

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