The Synthesis of β -Corticotrophin-(1-24)-tetracosapeptide (Synacthen) of High Specific Radioactivity

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The need for an ACTH* molecule labelled sufficiently to be detectable at concentrations found in animal circulation caused us to examine ways whereby this specific radioactivity (25–50 Ci/mmol) could be achieved. The method chosen was catalytic tritium-halogen exchange with a suitably protected intermediate. Further, to obtain labelling at a specific site it was decided to utilize the susceptible tyrosine-23 residue and to avoid affecting the equally susceptible amino acid residues, namely methionine, histidine and tryptophan, in the *N*-terminal portion of the molecule. This was achieved by the iodination of protected β -ACTH-(11-24)-tetradecapeptide to give the corresponding [23-di-iodotyrosine]-tetradecapeptide, which was then coupled with protected β -ACTH-(1-10)-decapeptide. Catalytic exchange of iodine for tritium in the protected tetracosapeptide proceeded smoothly, and deprotection with aqueous trifluoroacetic acid was followed by purification on a column of CM-cellulose. [3,5- $^3\text{H}_2$]Tyrosine-23-labelled β -ACTH-(1-24)-tetracosapeptide was characterized by t.l.c. and column chromatography and amino acid analysis and shown to behave identically with unlabelled synthetic material prepared in the usual way (Schwyzer & Kappeler, 1961). Intermediates that were isolated were characterized by elemental and amino acid analysis. The overall yield over the tritiation, deprotection and purification stages was 40%.

The specific radioactivity of the product was 46 Ci/mmol. Bioassay in an isolated adrenal-cell system (Lowry & McMartin, 1972) showed the labelled peptide to possess the full steroidogenic activity of unlabelled material.

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Lowry, P. J. & McMartin, C. (1972) *J. Endocrinol.* in the press

Schwyzer, R. & Kappeler, H. (1963) *Helv. Chim. Acta* **46**, 1550

* Abbreviation: ACTH, adrenocorticotrophin; the amino acid sequence of β -ACTH-(1-24)-tetracosapeptide is Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro.

Observations on the Fate of Adrenocorticotrophin in Isolated Rat Adrenal-Cell Suspensions and the Separation of an Enriched Adrenocorticotrophin-Sensitive Steroidogenic Cell Fraction

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The preparation of adrenal-cell suspensions by trypsin dispersal and the fluorometric assay of 21-hydroxy steroids elaborated in response to ACTH* were based on the method of Sayers *et al.* (1971) as modified by Lowry & McMartin (1972).

After a 3–6 min lag submaximal ACTH concentrations induced an increased rate of steroid production that remained approximately constant for 2 h. However, when β -ACTH-(1-24)-tetracosapeptide was incubated with cells for 1 h and the extracellular fluid (prepared by gentle centrifugation) was assayed with fresh cells only 5% of the original ACTH activity was detectable. This finding suggests that ACTH is either inactivated in solution (e.g. by metabolism) or extensively adsorbed on cells. Evidence against the latter possibility has been obtained by using [$^3\text{H}_2$]tyrosine-23-labelled β -ACTH-(1-24)-tetracosapeptide (46 Ci/mmol; Brundish & Wade, 1972). After a 30 min incubation with cells nearly all the radioactivity was found in the supernatant.

An explanation for the finding that steroid output continues after the ACTH has been inactivated might be provided by the report by Kitabchi *et al.* (1971) that isolated adrenal cells contain decreased activities of the phosphodiesterase responsible for hydrolysing cyclic AMP (adenosine 3':5'-cyclic monophosphate). However, density-gradient fractionation has suggested that the cell-type that responds to ACTH constitutes only a fraction of the total cell population, so that enzyme measurements on crude cell suspensions could be misleading. Cells (1 ml) were layered on a linear 2–10% (w/v) albumin gradient (5 cm) and centrifuged for 5 min at 20g. Large cells (10–20 μm) at the bottom of the gradient were responsible for the steroidogenic activity induced by ACTH. Preliminary electron microscopy suggests that the large rapidly sedimenting cells come mainly from the zona fasciculata and to a smaller extent from the zona intermedia.

The enriched cell fractions should provide a more sensitive system for the investigation *in vitro* of specific biochemical changes induced by ACTH.

Brundish, D. E. & Wade, R. (1972) *Biochem. J.* **129**, 23 P
Kitabchi, A. E., Wilson, D. B. & Sharma, R. K. (1971) *Biochem. Biophys. Res. Commun.* **44**, 898

* Abbreviation: ACTH, adrenocorticotrophin.

Lowry, P. J. & McMartin, C. (1972) *J. Endocrinol.* in the press

Sayers, G., Swallow, R. L. & Giordano, N. D. (1971) *Endocrinology* **88**, 1063

Structure-Activity Relations for the Nicotinamide-Adenine Dinucleotide-Linked 20 β -Hydroxy Steroid Oxidoreductase Activity of Cortisone Reductase with Special Reference to Ring B

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Kinetic studies of the reduction by cortisone reductase of the 20-oxo group in a small series of steroids indicated that ring A may be situated towards the extremity of the substrate-binding site of the enzyme (White & Jeffery, 1972). Studies now described relate particularly to the B ring. The ratio apparent K_m /apparent V is abbreviated Π , lower values indicating more efficient substrate utilization (units, min $\cdot$ mg of enzyme $\cdot$ l $^{-1}$).

The relationship of rings A and B to the rest of the molecule differs in 9 β ,10 α -pregn-4-ene-3,20-dione and pregn-4-ene-3,20-dione. The former was a much worse substrate (Π about 68) than the latter (Π about 0.18), indicating (i) that around rings A and B the binding site is considerably bigger than a normal steroid (or very flexible) and (ii) that the B ring probably normally assists substrate binding. 19-Norpregn-4-ene-3,20-dione (Π about 1.6) was a poorer substrate than pregn-4-ene-3,20-dione, indicating that the 10 β -methyl group may normally assist favourable binding, presumably through hydrophobic interactions. The 6-oxo derivative of pregn-4-ene-3,20-dione (Π about 0.65) was a poorer substrate than the parent compound, but 3 β -hydroxypregn-5-en-20-one and its 7-oxo derivative were utilized with similar efficiency (Π about 0.4). The effect of the 6-oxo group was evidently not caused solely by its bulk, because 3 β -hydroxy-6-methylpregn-5-en-20-one (Π about 0.4) behaved similarly to 3 β -hydroxypregn-5-en-20-one. Both 6-hydroxypregn-4-ene-3,20-diones were poorer substrates than pregn-4-ene-3,20-dione, but the 6 β -hydroxy compound (Π about 2) was poorer than the 6 α -hydroxy compound (Π about 0.72), suggesting favourable hydrophobic interactions between the protein and the β -side of this part of ring B.

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White, I. H. & Jeffery, J. (1972) *Eur. J. Biochem.* **25**, 409

Structure-Activity Relations for the Nicotinamide-Adenine Dinucleotide-Linked 3-Hydroxy Steroid Oxidoreductase Activity of Cortisone Reductase with Special Reference to the Ring A/B Junction and Ring D

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Kinetic studies of the reduction by cortisone reductase of the 3-oxo group of various androstane and oestrane steroids were made by using the spectrophotometric technique of Gibb & Jeffery (1971). The structural differences between the compounds covered features important for various biological activities (e.g. androgenic activity), and the best substrate (5 α -androstane-3,16-dione) was utilized roughly 1000 times more efficiently than the worst (5 β -androstane-3,17-dione). 5 α -Oestrane-3,17-dione (A/B *trans*, relatively flat molecule) 5 α ,10 α -oestrane-3,17-dione (A/B *cis*, ring A on β -side of rings B, C and D) and 5 β -androstane-3,17-dione (A/B *cis*, ring A on α -side of rings B, C and D) were substrates, showing that the steric relationship between the reacting group and the major part of the molecule could be altered considerably without preventing utilization. The change in a polar group in going from 5 α -androstane-3,16-dione (K_m about 90 μ M, V about 188 μ mol $\cdot$ min $^{-1}$) to 5 α -androstane-3,17-dione (K_m about 130 μ M, V about 2.7 μ mol $\cdot$ min $^{-1}$), or in a non-polar group in going from 17 β -hydroxy-5 β -oestrane-3-one (K_m about 844 μ M, V about 147 μ mol $\cdot$ min $^{-1}$) to 17 β -hydroxy-5 β -androstan-3-one (K_m about 224 μ M, V about 0.98 μ mol $\cdot$ min $^{-1}$) showed the marked influence of certain small structural changes. Various such comparisons are made. The view is put forward that the steroid-binding site is bigger than a steroid, or very flexible, and that substrates are not limited to binding with a particular oriented juxtaposition extending over much of the molecule.

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Gibb, W. & Jeffery, J. (1971) *Eur. J. Biochem.* **23**, 336