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.