Action of Adrenocorticotrophin on a Preparation of Isolated Cells from Rat Adrenals

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Several methods have been described for the preparation of adrenal cell suspensions (Kloppenborg, Island, Liddle, Michelakis & Nicholson, 1968; Swallow & Sayers, 1969; Halkerston & Feinstein, 1968). We now report results obtained by a modification of the method proposed by Kloppenborg *et al.* (1968).

Decapsulated glands were quartered and digested with collagenase (Sigma type I; 5 mg/ml) in Krebs-Ringer bicarbonate buffer, pH 7.4 containing glucose (0.2%) and albumin (3%, w/v). The preparation was filtered through nylon gauze (mesh 61 μ m) and washed twice by centrifugation (5 min at 200g) and resuspension. The equivalent of one adrenal yielded 600 000–1 000 000 cells. After incubation corticosterone content of cells plus medium was assayed by a specific and sensitive protein-binding technique adapted to assay corticosterone over the range 0–10 ng. With mouse serum (1%) as the binding protein source and correction for incomplete removal of unbound corticosterone by the adsorbing agent (Florisol), a linear relationship was obtained between unbound/bound steroid and total steroid (Schulster, Tait, Tait & Mrotek, 1970).

Before incubation each sample (1 ml, equivalent to the cells derived from one gland) contained 27.7 ± 3.2 ng of corticosterone (mean $\pm$ S.E.M. of seven experiments); in the absence of adrenocorticotrophin the value obtained after incubation for 1 h was 36.3 ± 7.0 ng of corticosterone (mean $\pm$ S.E.M. for six experiments). With increasing amounts of adrenocorticotrophin (Third International Standard: Bangham, Mussett & Stacke-Dunne, 1962), 1 h incubations resulted in a sigmoid log (dosage)–response curve. The minimum effective dose, ED₅₀ and the minimum dose for maximum response (25–60 ng/gland per min) were 10^{-6} , 10^{-4} and 10^{-3} i.u./ml respectively. The log (response) was proportional to log (dose of adrenocorticotrophin) in the range 10^{-6} – 10^{-3} i.u./ml (index of precision 0.13).

Previous experiments *in vivo* have shown that adrenocorticotrophin achieves its maximum steroidogenic response within 10 min (Garren, Ney & Davis, 1965), whereas a longer time-period (30 min) has been reported for incubations *in vitro* of cell suspensions prepared by using trypsin (Kitabchi, Thompson & West, 1970). Our preparations, however, showed a rapid steroidogenic response to adrenocorticotrophin (2×10^{-2} i.u./ml);

corticosterone production rate became constant certainly within 10 min after adrenocorticotrophin addition.

The rapidity, sensitivity and magnitude (up to 300-fold stimulation over basal production) of the response elicited by adrenocorticotrophin make the adrenal-cell suspension a useful system for studying the biochemistry of the steroidogenic response.

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Ribonucleic Acid Polymerase from Rat Prostate Nuclei and Nucleoli

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Much insight has been gained recently into the metabolism and binding of testosterone and dihydrotestosterone within rat prostate cells. It would appear that one result of these activities could be to bring about both qualitative and quantitative changes in RNA synthesis. Consequently studies were initiated on the solubilized RNA polymerase(s) of rat prostate gland. RNA polymerase enzyme was extracted by a modified version of the method developed originally by Roeder & Rutter (1969) for extraction of RNA polymerase activity from sea-urchin oocyte nuclei and rat liver nuclei. The method involves solubilization of the enzyme activity from purified nuclei by ultrasonication at high ionic strength, decrease of the ionic strength and removal of chromatin by centrifugation, followed by ammonium sulphate fractionation.

Enzyme activity isolated by this method from rat prostate nuclei showed it to be dependent on added DNA, required all four ribonucleoside triphosphates and was greater in the presence of Mn²⁺ and denatured DNA than with Mg²⁺ and native DNA. On 15–30% glycerol gradients the