BIOASSAY OF CORTICOTROPHIN USING THE SHEEP'S TRANSPLANTED ADRENAL GLAND

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Measurement of minute amounts of adrenocorticotropic hormone (ACTH) in body fluids at present depends upon biological assay. We here report a very sensitive method of ACTH assay using the transplanted gland in sheep whose ACTH secretions have been suppressed with 16α-methyl-9α-fluoro-Δ¹ cortisol (dexamethasone).

The animals used were three Merino ewes. The left adrenal had been transplanted to a vascular anastomosis with the left carotid and jugular vessels and enclosed in a skin loop 4–8 months previously, using the technique of McDonald, Goding & Wright (1958). The right adrenal was removed 2–4 weeks later. Experiments were conducted on each animal in turn at monthly intervals, when blood was collected from the adreno-jugular vein before and after carotid-arterial injections of test fluid. A fluorescent technique (De Moor, Raskin & Steeno, 1960) was used to measure cortisol—the main glucocorticoid secreted by the sheep (Bush & Ferguson, 1953)—in 2 ml. samples of adrenal effluent plasma. The rate of cortisol secretion was calculated from concentration and adrenal plasma flow per minute.

Complete suppression of adrenocortical secretion was achieved by 10–12 mg. dexamethasone given as three intramuscular injections over 18 hr. preceding the experiments. Preliminary work indicated that under these conditions the maximum response in cortisol secretion occurred 6–11 min. after beginning a 2 min. perfusion of a standard dose of ACTH.

On the day of assay the adreno-jugular vein loop was catheterized and pure adrenal venous blood collected using a system of inflatable cuffs (McDonald & Reich, 1959). Heparin was given intravenously at intervals to prevent clotting. The adrenal venous effluent was first collected for 5 min. (control sample). Two millilitres of the test fluid (plasma for assay or acidified saline–gelatin solution containing ACTH standard) was then injected evenly into the carotid artery loop over 2 min. At exactly 6 min. from the start of the injection blood was collected for 5 min. (response sample). Both 5 min. collections were measured, centrifuged, and the cortisol content of each plasma sample determined. The difference in cortisol content between the samples was used as a measure of response to the perfused test fluid.

In Fig. 1 the responses to at least three doses of the ACTH standard are plotted for five separate experiments. In each case there was a linear relationship between response and the log dose of ACTH injected. Sensitivity and slope varied between different sheep and on different days in the same sheep, but for the day of assay linearity was found over a dose range of 0·005–0·05 m-u. ACTH. Acidified saline–gelatin solution alone and plasma drawn after noon from normal human subjects.
gave no response. Preliminary results indicate measurable concentrations of ACTH in plasma drawn at 8 a.m. from normal subjects (0·2–0·3 m-u./100 ml. plasma) and elevated levels following surgery (0·37 m-u./100 ml. plasma in one subject 30 min. after herniorrhaphy). Very high levels of ACTH were encountered in two subjects who had received 4 g./day of Metopirone for 2 days (both over 1 m-u. ACTH/100 ml. plasma). Plasma from excited sheep assayed at 0·5–1·05 m-u./100 ml. Previous estimates of ACTH concentration in normal human plasma vary from below 1 m-u./100 ml. (Lipscomb & Nelson, 1962) to over 100 m-u./100 ml. (Bornstein & Trewella, 1950). Recently Vance, Reddy, Nelson & Thorn (1962), using the assay procedure of Lipscomb & Nelson, found ACTH levels of 0·4–1·0 m-u./100 ml. in plasma drawn at 8–9 a.m. from normal human subjects but could not detect a rise after Metopirone administration.

The log dose–response curve calculated from mean responses for all assay animals has a slope of 28 and standard deviation of 0·71. The precision of the method would be increased by using two assay animals in a cross-over design. In our experiments an interval of three weeks between assays in the same sheep has been adhered to, but shorter intervals could possibly be used. The effects of repeated perfusion of human plasma on the transplanted adrenal gland remain to be determined.

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Fig. 1. Relation between ACTH dosage and cortisol secretion on 5 different days. The vertical axis represents the difference in cortisol secretion (µg.) between response and control samples. Results from three different sheep.

REFERENCES