Isolated Adrenal Cells: Assay of ACTH in Rat Serum[1] (35326)

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Determination of ACTH concentration in serum has been limited by the sensitivity of the currently employed bioassay methods (1). The development of a trypsin technic for the dispersion of rat adrenal tissue (2) and the establishment of optimal conditions for incubation of the isolated cells (Sayers, Swallow, and Giordano, unpublished data) has provided an accurate and specific biological assay with a prerequisite sensitivity for the analysis of small volumes of rat serum.

Methods and Materials. Either Teflon ware or silicone coated glassware was used throughout. Blood samples were obtained at 8 to 9 a.m. from the abdominal aorta of intact rats under ether anesthesia (IRBE), from hypophysectomized rats under ether anesthesia (HRBE), and from intact, decapitated (application of a large bowel clamp to the neck) rats (IRD). Blood was drawn into vacutainer, siliconized, tubes, allowed to clot, and centrifuged at 300 rpm, 4° for 10 min. If the serum was not used immediately it was frozen and stored at −20° in 1.0-ml volumes.

Prior to assay, 1.0 ml of serum and 50 mg of Quso G32, microfine precipitated silica granules (Philadelphia Quartz Company) are mixed at room temperature for 5 min and centrifuged at 3000 rpm, 4°, for 10 min. The supernatant is poured off and 1.0 ml of 0.1 *N* HCl in 0.9% NaCl is added to the Quso adsorbate. The contents of the tube are mixed and centrifuged. The eluate is tested for ACTH activity in a 64-sample assay.

Suspensions of cells of the rat adrenal cortex were prepared by the method of Swallow and Sayers (2) with modifications (Sayers, Swallow, and Giordano, unpublished data). Briefly, the modified method is as follows. Adrenal quarters are placed in Krebs–Ringer buffer (KRB) which contains glucose (0.2 g/100 ml) and trypsin (0.25 g/100 ml); maintained at 37° and agitated with a siliconized glass paddle driven at 500 rpm. Freed cells in suspension are periodically withdrawn, pooled (total volume about 100 ml) and collected as a pellet by centrifugation. The pellet is suspended in 60 ml of KRB which contains glucose (0.2 g/100 ml), bovine serum albumin (BSA) (0.5 g/100 ml), calcium (7.65 m*M*), and lima bean trypsin inhibitor (0.1 g/100 ml).

ACTH standard (USP, 1.5 Intravenous Units/vial) is dissolved in a vehicle which consists of 0.5% BSA brought to pH 3.5 with HCl. Appropriate dilutions made with vehicle are delivered in a volume of 0.1-ml to 0.9-ml aliquots of cell suspension. Serum and Quso eluate were added in volumes of 10 to 200 μl. Vehicle only, in a volume of 0.1 ml, is added to duplicate samples of cell suspension to serve as blanks for the system. The mixture (cell suspension plus vehicle, ACTH, serum, or Quso eluate) is incubated for 60 min at 37° in a Dubnoff incubator (66 oscillations/min), atmosphere 95% O_2, 5% CO_2. Response is expressed as net production of corticosterone (B): B in sample to which ACTH or unknown is added minus B in vehicle blanks.

Steroid analysis was performed on methylene chloride extracts of each incubate by a modification of the fluorescent method of Silber *et al.* (3).

Results. B production and quantity of ACTH added to the cell suspension are related by a straight line for small quantities (0.05 to 1.0 μU) of hormone (Fig. 1). This is

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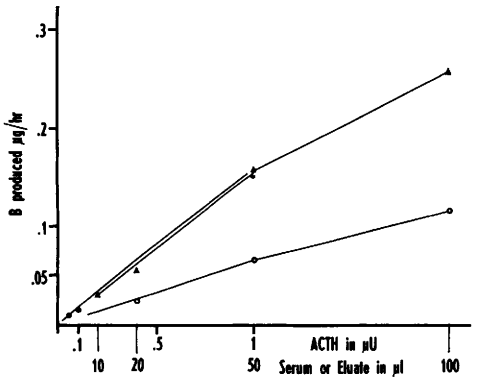

FIG. 1. A plot of net corticosterone (B) production (µg/hr) by isolated adrenal cells: ACTH Standard (µU) (●); volume of serum (µl) (○); or volume of Quso eluate (µl) (▲).

the applicable range for the assay of ACTH in small volumes of serum (Fig. 1).

A slight stimulatory effect is observed when serum from hypophysectomized rats (HRBE) is added directly to the system and definite inhibition is seen with the addition of serum from IRBE or IRD (Table I). Both of these nonspecific responses were eliminated with prior adsorption of serum with Quso and subsequent elution of the Quso adsorbate. Serum of hypophysectomized rats and possibly serum of intact rats contains a cofactor not available in the KRB medium and this cofactor is either not adsorbed by Quso or adsorbed and not eluted.

A comparison was made between the responses of the assay system to the direct addition of serum and the addition of Quso eluates of the same serum. The results for two such comparisons, serum from intact rats bled under ether anesthesia (IRBE) and serum from intact rats decapitated (IRD) are presented in Fig. 1 and in Tables I and II. Note the significantly greater response to eluate than to the serum itself.

Recovery of ACTH from serum has been established. Standard ACTH was added to serum from hypophysectomized rats. Standard ACTH added alone, serum plus ACTH added directly, and Quso eluate of serum plus ACTH added to aliquots of a suspension of isolated cells have been compared with respect to B production (Table III). It appears that more than 90% of ACTH added to serum from hypophysectomized rats is recovered by the Quso process. In the data presented a correction was made for B in the serum added directly to the cell suspension. The amount of B present in the serum (tested) was subtracted from the amount of steroid in the incubate. Extraction of Quso eluates gave no significant increase in fluorescence over reagent blanks.

TABLE I. Stimulatory and Inhibitory Factors in Rat Serum.

ACTH std			Net B production (µg)			
			Serum from IRBE[a]		Serum from HRBE[b]	
µU/ml of cell susp'n.	Net B (µg)	Vol tested (µl)	Direct addition	Quso eluate	Direct addition	Quso eluate
0.05	0.011	10	0.013	0.032	—	—
	0.008		0.012	0.028		
0.1	0.014	20	0.024	0.050	—	—
	0.016		0.026	0.054		
0.5	0.080	50	0.060	0.155	—	—
	0.082		0.071	0.155		
1.0	0.148	100	0.122	0.249	0.023	0.003
	0.156		0.112	0.268	0.020	0.004
5.0	0.441	200	—	—	0.026	0.005
	0.460				0.023	0.010

[a] IRBE = intact rats bled under ether anesthesia.

[b] HRBE = hypophysectomized rats bled under ether anesthesia.

TABLE II. ACTH in Serum of Intact Rats Decapitated (IRD).

ACTH std		Serum from IRD			
		Direct addition		Quso eluate	
μU/ml of cell susp'n.	Net B (μg)	Vol (μl)	Net B (μg)	Vol (μl)	Net B (μg)
0.05	0.018 0.020	50	0.003	50	0.011 0.011
0.1	0.038 0.040	100	0.009	100	0.025 0.023
0.5	0.201 0.228	—	—	—	—

Discussion. The isolated adrenal cell system fulfills the criteria of a good bioassay method. Accuracy at the low quantities of ACTH in serum is characterized by a coefficient of variation less than 10%. Sensitivity is characterized by a minimum effective dose (MED) of 0.05 to 0.1 μU (0.5 to 1.0 pg) of ACTH. Specificity is excellent and we have shown that relatively large quantities (10^7 pg) of insulin, glucagon, oxytocin, vasopressin, and angiotensin II do not induce increased steroidogenesis when added to isolated adrenal cells. MSH does stimulate, but the MED for this polypeptide is about 10^6 pg. It is most unlikely that these hormones would interfere with the assay of ACTH in serum.

The stimulatory factor in serum, we believe, is not a polypeptide hormone but rather a cofactor(s). The presence of such in serum and its manifestation of action in the assay system is not surprising in light of the fact that the incubation medium is Krebs–Ringer bicarbonate buffer with added glucose, albumin, and extra calcium.

The nature of the inhibitory factor is under investigation in our laboratory, but no definite statement can be made at this time. The factor does have potentially important implications from the standpoint of the physiology and pathology of the function of the adrenal cortex. From the standpoint of assay of ACTH it is indeed fortunate that Quso processing appears to eliminate the inhibitory factor as a complication in the assay of ACTH in serum.

Advantage has been taken of the chemical characteristics of ACTH; easily absorbed on

TABLE III. Recovery of ACTH Added to Serum of Hypophysectomized Rats.

ACTH std		HRBE serum + ACTH (50 μU/ml of serum)			
		Direct addition		Quso eluate	
μU/ml of cell susp'n.	Net B (μg)	Vol (μl)	Net B (μg)	Vol (μl)	Net B (μg)
0.05	0.010 0.008	—	—	—	—
0.1	0.020 0.020	—	—	—	—
0.5	0.095 0.095	50	0.208 0.198	50	0.246 0.229
1.0	0.175 0.168	100	0.280 0.270	100	0.390 0.401
5.0	0.415 0.425	—	—	—	—

glass at pH 6–8, and eluted therefrom at low pH (about pH 2). Berson and Yalow (4) have described the adsorption onto and elution from Quso of polypeptide hormones, particularly parathyroid hormone and ACTH. These authors employed the silicate to advantage for concentration of hormone in connection with radioimmunoassay.

The present data suggest that Quso processing coupled with the isolated adrenal cell system is an effective means for the determination of ACTH in serum. Serum from hypophysectomized rats exhibits no activity; more than 90% of ACTH added to serum from hypophysectomized rats is recovered; estimates of ACTH in rat serum fit with physiological concepts particularly the data herein presented that ACTH is at a considerably lower concentration in the serum of rats not subjected to a noxious stimulus (0.6 μU/ml) than that of rats subjected to ether anesthesia and hemorrhage (18 μU/ml). We expect to report data on groups of animals in various states of activity of the adenohypophyseal–adrenal cortex system with estimates of means and fiducial limits at a future date. The method is being adapted to the determination of ACTH in the serum of man.

Summary. Suspensions of isolated adrenal cells respond to ACTH with increased steroidogenesis. This isolated adrenal cell system has the prerequisite sensitivity, accuracy, and specificity for the determination of ACTH when coupled with Quso processing of serum. Quso mixed with serum adsorbs the ACTH therein; 0.1 *N* HCl quantitatively elutes the ACTH from the Quso. Substances in serum which interfere with the assay of ACTH in the isolated adrenal cell system are eliminated.

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