where h^* and x^* are vertical and horizontal coordinates, respectively, of the surface of the ice sheet with respect to an origin at the center of the ice sheet and at set level; and H^* and X^* are the maximum values, respectively, of h^* and x^*. Figure 3 shows that Eq. 16 gives a first-order fit to the Antarctic ice sheet for $n = 1.6$. Shumsky (*13*) argues that the unglaciated elevation of Antarctica averaged about 0.5 km above sea level. Weertman (*14*) shows that the ice sheet will isostatically depress the bed to about one-third of the ice thickness, so that $h = 1.5$ ($h^* - 0.5$ km), where ($h^* - 0.5$ km) is the ice thickness above the unglaciated bed. Assuming that the density inversion occurs 1 km below the firn surface, as in Fig. 1, the buoyancy stress will act through ice of thickness $d = [1.5\ (h^* - 0.5\ \text{km})] - 1$ km. The surface slope of the ice sheet is:

$$\tan\theta = \frac{dh^*}{dx} = -\frac{n+3}{2n+4}\left(\frac{H^*}{X^*}\right)\frac{\left(\frac{x^*}{X^*}\right)^{(n+3)/(n+1)}}{\left(\frac{h^*}{H^*}\right)^{(2n+4)/(n+1)}} \quad (17)$$

We can now calculate L_w for various values of x^* and X^* by solving Eqs. 7 through 17 with $n = 1.6$, $C = 29.1$, and $B(f) = 1.38 \times 10^7$ bar^{-n} sec^{-1} obtained from the data of Higashi, Koinuma, and Mae (*7*), $\beta = 10^{-4}$ °C cm^{-1} obtained from theoretical temperature gradients in polar ice sheets (*15*), $d = 1.5\ h^* - 1.75$ km, $\rho = 0.92$ g cm^{-3}, $g = 980$ cm sec^{-2}, $\alpha = 15.3 \times 10^{-5}$ °C^{-1}, $\kappa = 1.18 \times 10^{-2}$ cm^2 sec^{-1}, $\eta_w = 1.8 \times 10^{-2}$ poise, $H_M = 80$ cal g^{-1}, and $J = 4.19 \times 10^7$ dynes cm^{-1} cal^{-1}. The results are shown in Fig. 4. According to Weertman's theory, a surge will not begin until $L_c \leqslant L_w$ at all values of x^*, and Fig. 4 shows that for $L_c \approx 2$ cm this condition is not approached until $X^* \approx 2900$ km. An ice sheet of this radius would extend to the continental shelf of Antarctica.

The principal assumptions we have made concern (i) the density inversion as a general feature of the Antarctic ice sheet, (ii) the estimation of basal sliding velocities from the Byrd Station core hole and the U.S. Geological Survey strain network data, (iii) the applicability of convection theory derived for a viscous fluid to a viscoplastic solid, and (iv) the equilibrium profile of the Antarctic ice sheet. If the assumptions made are valid, the Antarctic ice sheet will surge when the ice sheet approaches the continental shelf of Antarctica. Presumably the surge will begin when the critical thickness of the basal water layer extends to the edge of the ice sheet along one or more fronts. These fronts will then surge, causing a slumped region to proceed inland from each front until the entire ice sheet has collapsed to the postsurge equilibrium thickness.

Weertman's surge theory, modified to include the frictional heat of thermal convection, should apply to both polar ice sheets and Alpine glaciers. The basal water layer thickness is a function of the surface slope and the frictional heat generated by ice flow. Although no frictional heat from convection flow is possible for surging Alpine glaciers, their surface slopes are orders of magnitude greater than surface slopes of the Antarctic ice sheet (*16*).

Strong evidence suggests that the Antarctic ice sheet may have been more extensive in the past (*17*). However, it is not known whether the present equilibrium profile of the ice sheet was maintained during these past advances. If not, these advances may themselves have been surges, which suggests that our estimate of the controlling obstacle size is too large (*18*). Also, at present the Antarctic ice sheet probably does not behave dynamically as a unit, and possibly various sectors could surge independently. However, the larger the ice sheet becomes, the more it should behave as a unit, and, if it extended to the continental shelf, presumably it could be dynamically treated as such.

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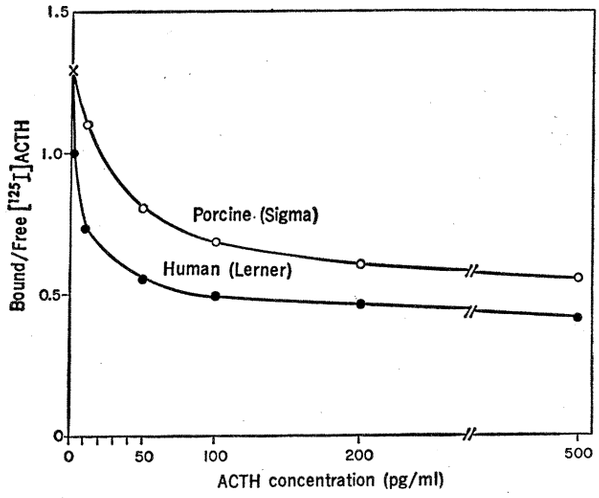
Radioreceptor Assay of Adrenocorticotropic Hormone: New Approach to Assay of Polypeptide Hormones in Plasma

Abstract. *Biologically active iodine-125–labeled adrenocorticotropic hormone (ACTH) binds specifically to ACTH receptors extracted from adrenals. Unlabeled ACTH at 1 picogram per milliliter significantly displaces labeled ACTH from these receptors. This system, which appears to be applicable to all polypeptide hormones, provides a rapid and sensitive method for measurements of biologically active ACTH in dilute whole plasma.*

Since its original application to the measurement of plasma insulin (*1*), radioimmunoassay has been used for the assay of many polypeptide hormones. In addition to the specificity and great sensitivity obtainable by the use of carefully selected antiserums to hormones, this method readily allows precise measurement of hormone concentrations on a large number of specimens. Disadvantages have been that the basis for specificity is immunological



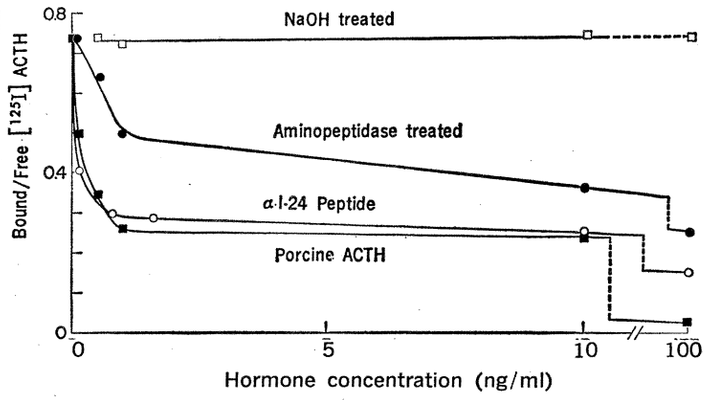


Fig. 1 (left). Standard curve for the radioreceptor assay. The ratio of bound to free porcine [^{125}I]ACTH is plotted as a function of hormone concentration. Human ACTH, bioassayed (*14*) at 140 unit/mg, was routinely used as the standard. Porcine ACTH, bioassayed (*7*) at 64 unit/mg, is shown for comparison. With storage at −20°C, the [^{125}I]ACTH showed a progressive decrease in binding to ACTH receptors with time. When freshly prepared, about 70 percent of the labeled hormone was bound in the presence of "excess" receptors. Presumably the remainder was biologically inert. Accordingly, in each assay the maximum amount of tracer bindable was determined and an appropriate correction was applied in calculating the ratio of bound (B) to free (F) ACTH; if 35 percent of the tracer is bound, and 70 percent is bindable, then B/F is equal to 35 percent/35 percent or 1. In this experiment, each tube in the assay contained 100 μl of plasma from a hypopituitary patient. When 200 μl of plasma was used, all points on the curve were slightly lower; when 50 μl of plasma was used, all points on the curve were slightly higher and were indistinguishable from those obtained when all plasma was omitted. Fig. 2 (right). Reactivity of ACTH and ACTH derivatives. The ratio of bound to free [^{125}I]ACTH is plotted as a function of hormone concentration. In plotting the data for the α1-24 peptide, a fully active synthetic ACTH consisting of the first 24 amino acids of ACTH, an appropriate correction was applied to account for its lower molecular weight. The aminopeptidase-treated ACTH (*7*) on bioassay was much less active than the porcine ACTH. The NaOH-treated ACTH (*7*) had trivial biological biological activity (less than 0.006 percent that of native porcine ACTH). Biological activity of these substances was measured as activation of adenyl cyclase in adrenal tumor particles (*10*).

rather than biological and that long incubation periods (2 to 7 days) are usually required to achieve maximum sensitivity (*2*). Analogous competitive protein binding assays have been developed in which other materials, such as thyroxine binding globulin, are used in place of specific antibodies; but again the structural features of the ligand that determine the degree of binding are not identical with those needed for biological activity (*3*).

Macromolecules that bind estrogen, testosterone, progesterone, aldosterone, and cortisol have been identified in cytoplasmic and nuclear constituents of respective target tissues (*4*). The estrogen binding substance from the cytosol of rabbit uterus has been used for the measurement of estrogen in plasma extracts (*5*). In contrast to the soluble cytoplasmic and nuclear binding sites for these steroid hormones, polypeptide hormones, including adrenocorticotropic hormone (ACTH), appear to act by binding to specific receptors which are on the outer surface of cells. In most cases, the receptor hormone complex, in some undetermined way, activates adenyl cyclase, an enyme bound to the plasma membrane that markedly accelerates the conversion of adenosine triphosphate to cyclic adenosine monophosphate; the latter appears to be the intracellular mediator of ACTH action (*6*).

Monoiodo [^{125}I]ACTH of very high specific radioactivity that retains at least half of the biological activity of native ACTH and adrenal extracts that contain both the ACTH receptors and the ACTH-sensitive adenyl cyclase have been prepared (*7*). With these reagents we have developed a rapid, sensitive assay for ACTH based on competition of ^{125}I-labeled and unlabeled ACTH for binding sites on specific ACTH receptors (*8*). Reactivity in this system appears to be based on the biological properties of ACTH. Of the ACTH derivatives tested, only those which retain biological activity occupy the receptors (*7*). The assay permits easy measurements of ACTH in unextracted plasma in a few hours. This method which, in principle, is widely applicable to other hormones, has the advantages of immunoassay and also affords greater speed and a specificity that is biological rather than immunological.

Monoiodo [^{125}I]ACTH was prepared at very high specific radioactivity by labeling ACTH (porcine, Sigma, 64 unit/mg) lightly with ^{125}I$^-$ (molar ratio of ACTH to iodide approximately 20 to 1) and separating the iodinated ACTH from uniodinated ACTH by chromatography on carboxymethyl cellulose (*7*).

To obtain adrenal receptors we transplanted ACTH-sensitive adrenal tumors serially in mice (*9*). The tumors were homogenized, and the particulate fractions, which contain all of the ACTH-sensitive adenyl cyclase, were separated by differential centrifugation (*10*). When stored at −20°C, the lyophilized particles remained stable for at least 6 months. The particles which had sedimented at 10,800*g* or 4,300*g* were disrupted in a French pressure cell in the presence of phosphatidylethanolamine and fluoride (*7*). The mixture was centrifuged at 100,000*g*. The supernatant, after dialysis, was used as the ACTH receptor extract in the assay (*7, 8*). Extracts were stored in portions at −20°C or below and were stable for at least 10 to 14 days if they were not thawed and refrozen.

The binding of [^{125}I]ACTH by adrenal receptors is highly specific (*7*). Adrenal extracts that contained the ACTH-sensitive cyclase bound the hormone; other tissue extracts were inert. Biologically active [^{125}I]ACTH was bound; other labeled polypeptide hormones were unreactive. The degree of competition by an ACTH derivative for receptors appears to be proportional to its biological activity.

Further studies have indicated that the binding of ACTH to receptors is rapid, reaching equilibrium in a few hours even at 1°C. Other experiments showed that there are two orders of receptors, one with an apparent association constant K of about 10^{12}, the other with K of about 10^{7} (*10*); and, although the high affinity sites represent only about 0.1 percent of the total receptors, they confer high sensitivity to the system.

For the assay of hormone in plasma a series of tubes was prepared so that each contained, in a total volume of 1 ml, adrenal extract (*7*) (200 to 250 μl), [^{125}I]ACTH (1.0 to 1.5 pg, 700 to 1000 count/min), and either 50 to 200 μl of patient's plasma or 50 to 200 μl of plasma from patients with hypopituitarism to which had been added unlabeled ACTH (0 to 10 ng of human ACTH). All procedures were performed at 1°C. Diluent was 0.01M tris-HCl buffer, pH 7.8, which contained 0.001M $MgCl_2$, 0.001M dithiothreitol, 0.1 percent human serum albumin. Tubes (12 by 75 mm) were flint glass and were siliconized prior to use. After 1 hour at 1°C, about 10 mg of Quso (microfine silica, Philadelphia Quartz Co.) was added, which adsorbed the free but not the receptor-bound [^{125}I]ACTH (*11*). After centrifugation for 5 minutes the radioactivity in the supernatants and the Quso was counted separately.

In the absence of unlabeled ACTH, the [^{125}I]ACTH was bound to the adrenal receptors (Fig. 1). Unlabeled ACTH at 1 pg/ml reproducibly caused a significant fall in the ratio of bound to free ACTH (Fig. 1). The ability of an ACTH derivative to displace [^{125}I]ACTH was directly related to its biological activity (*7*) (Fig. 2).

Twenty-one plasmas from 19 subjects have been assayed. We did not detect ACTH in three patients with deficiencies of pituitary ACTH (two panhypopituitarism, one isolated deficiency) and two patients in whom excess corticosteroids suppressed ACTH production (one hyperfunctioning adrenal adenoma and one patient taking 60 mg of prednisone daily) (Fig. 3). Of seven normal adults, ACTH was undetected in three, while in the other four values ranged up to 88 pg/ml. In patients who had hyperadrenocorticism due to excessive production of ACTH from the pituitary (six patients) or from an extrapituitary tumor (two patients), plasma ACTH ranged from about 200 pg to 10,000 pg/ml (Fig. 3).

The plasma ACTH was independently measured by radioimmunoassay (*11*) or bioassay in vivo (*12*) in the following cases. In one patient who had ectopic ACTH syndrome due to a carcinoid tumor (*13*), ACTH was 260 pg/ml by radioimmunoassay, 230 to 360 pg/ml by conventional bioassay, and 250 pg/ml by radioreceptor assay. During a remission of the disease, ACTH was undetectable by both radioimmunoassay and by our assay. In one patient with a pituitary tumor, ACTH was 7500 pg/ml by radioreceptor assay and 7000 pg/ml by radioimmunoassay.

The successful application of ACTH receptors to assay of ACTH in plasma represents the first time that target tissue receptors have been used for the measurement of a polypeptide hormone. A substantial advantage of the radioreceptor assay is its specificity for biologically active ACTH; reactivity in the system is proportional to biological activity. Another virtue is that the desired sensitivity is intrinsic to the receptor, since in vivo it is responding to fluctuations in hormone concentrations that are within the physiologic range. In this system 1 pg of ACTH per milliliter is uniformly detectable, so that the sensitivity of the assay is 5 pg/ml when the plasma is diluted 1 to 5. This system provides not only a direct method for studying the interaction of hormones with their target tissues, but also a general approach to the assay of biologically active substances in body fluids.

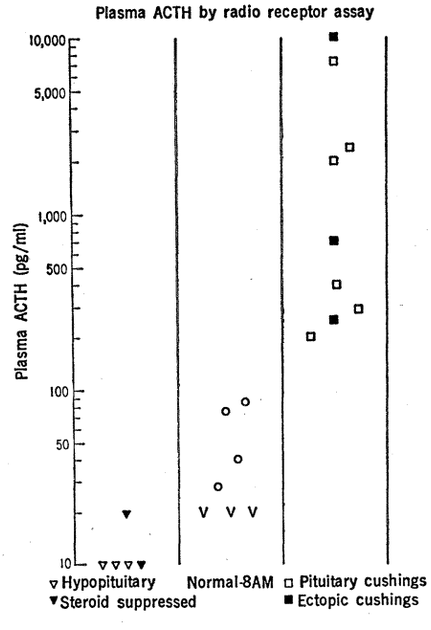


Fig. 3. Plasma ACTH by radioreceptor assay. Blood was drawn in the morning into heparin and immediatey centrifuged, and the plasma was stored at −20°C until assayed. Plasma ACTH, on a logarithmic scale, is plotted for each patient. The open and closed triangles indicate values below the limit of sensitivity of that particular assay. The lower limit of sensitivity for the four patients assayed in column 1 was 5 pg/ml. Two of the values for ectopic Cushing's syndrome were on samples of plasma from one patient, which were drawn 3 years apart. Two of the values for pituitary Cushing's syndrome were on samples of plasma from one patient drawn before and 2 weeks after total adrenalectomy.

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