*Proceedings of the National Academy of Sciences*
Vol. 65, No. 3, pp. 745–752, March 1970

# ACTH Receptors in the Adrenal: Specific Binding of ACTH-$^{125}$I and Its Relation to Adenyl Cyclase

**Robert J. Lefkowitz, Jesse Roth, William Pricer, and Ira Pastan**

DIABETES AND THYROID BIOCHEMISTRY SECTIONS, CLINICAL ENDOCRINOLOGY BRANCH, NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

*Communicated by James A. Shannon, December 22, 1969*

**Abstract.** Pure monoiodo ACTH-$^{125}$I was prepared that was biologically active and free of unlabeled ACTH. Extracts of adrenal cortex that contained ACTH-sensitive adenyl cyclase, bound ACTH-$^{125}$I; extracts that lacked the ACTH-sensitive cyclase did not bind ACTH-$^{125}$I. Unlabeled ACTH inhibited the binding of ACTH-$^{125}$I. Five ACTH derivatives which varied widely in biological activity were tested. All inhibited the binding of ACTH-$^{125}$I in direct proportion to their biological activity. Albumin, insulin, and four unrelated iodinated hormones were inert. The addition of excess hormone or acetic acid produced rapid dissociation of bound ACTH-$^{125}$I. This study demonstrates directly the binding of ACTH to its biologically significant site.

---

Adenosine 3′:5′-cyclic phosphate (cyclic AMP) in the adrenal appears to be the intracellular mediator of ACTH-stimulated steroidogenesis.[1] As with many other hormone-sensitive tissues, the sequence of events appears to be: (1) binding of hormone (ACTH) to the target (adrenal) cell, (2) activation of the enzyme, adenyl cyclase, and (3) a rise in the intracellular concentration of cyclic AMP.[2] Studies suggest that the binding sites for ACTH and other polypeptide hormones are located on the outer surface of cells, that ACTH-sensitive adenyl cyclase and the binding site are intimately associated and that the hormone-site interaction represents a rapid equilibration between hormone in the medium and a fixed number of sites on the cell.[3–7] Previous studies of hormone binding have either measured the binding of labeled hormone by target tissue without relating the binding to a specific biological action,[8–15] or have not measured uptake directly but rather inferred it from measurements of some known hormone action.[3, 4, 16–19]

In the present study we have measured the binding of biologically active ACTH-$^{125}$I to an adrenal extract and have related the binding to a specific biologically significant property, stimulation of adenyl cyclase.

To use labeled hormone for the study of binding of hormone to biologically significant sites, the labeled hormone must be biologically active, either stimulating the target tissue or specifically inhibiting binding by occupying the site. To be certain that the labeled hormone molecule itself is biologically active, the biological activity of the preparation must clearly exceed that which can be

ascribed to contamination with unlabeled hormone. The tissue or tissue extract should demonstrate a specific hormone-sensitive response to insure the presence of an intact binding site and hopefully to permit quantitative correlation between binding of hormone and its biological effect.

**Methods. Adrenal tissue:** An adrenal tumor that produces steroids in response to ACTH was obtained from Dr. Gordon Sato[20] and transplanted serially in $LAF_1$ mice. Adrenal particles that contained ACTH-sensitive adenyl cyclase were prepared from homogenates of the tumor by differential centrifugation.[6a] The 4,340 $\times$ $g$ and 10,800 $\times$ $g$ particles were lyophilized and stored at $-20°$.

**Adrenal extract:** Phosphatidylethanolamine (Folch Fr. V-Pierce Chem. Co.), 16 mg, was suspended in 4 ml of Tris-HCl (1.0 $M$, pH 7.8), sonicated at full intensity for 4 half-minute periods in a Branson model LS75 Sonifier, and clarified by centrifugation at 30,000 $\times$ $g$ for 10 min. The clarified emulsion was mixed with 6 ml of 0.01 $M$ Tris-HCl, pH 7.8, that contained 0.5 $M$ sucrose, $1.67 \times 10^{-3}$ $M$ $MgCl_2$, 0.04 $M$ NaF, and $1.67 \times 10^{-3}$ $M$ dithiothreitol (Calbiochem). After the 4340 $\times$ $g$ particles were added, equivalent to 1.0 to 1.5 gm of adrenal tissue, the mixture was passed through a French pressure cell three times at 12,000 pounds per square inch and centrifuged at 105,000 $\times$ $g$ for 60 min in a Spinco model L ultracentrifuge. The clear supernatant was dialyzed for 90 min against 100 vol of 0.01 $M$ Tris-HCl, pH 7.8, that contained 0.001 $M$ dithiothreitol and 0.001 $M$ $MgCl_2$. This material after dialysis, which contained essentially all of the ACTH-sensitive adenyl cyclase of the original particles, was used as the "adrenal extract" for binding studies. Although it contained small fragments of membrane on electron microscopy, the adrenal extract was sedimented in sucrose gradients and was included in agarose gels (Biogel A 15M) like a globular protein in the molecular weight range of 6 to 8 $\times$ $10^6$.[21]

**ACTH bioassay:** ACTH biological activity was measured as stimulation of adenyl cyclase activity in 10,800 $\times$ $g$ adrenal particles, expressed as picomoles of $^{32}P$-labeled cyclic AMP produced from labeled ATP in 15 min.[6a,b] A few hormone preparations were also assayed by measurement of corticosterone production in hypophysectomized rats.[22]

**ACTH:** A single lot of chromatographically purified porcine ACTH was used for all studies, except when noted. Supplied as a lyophilized powder (Sigma), it was stored at $-20°$ either as the powder, or at 1 to 5 mg/ml in 0.05 $M$ acetic acid. Its potency, listed by the manufacturer as 150 units/mg, was established as 64 units/mg by comparing it in the adenyl cyclase assay with a commercial oxycel ACTH (Parke Davis); the latter had been assayed at 16 units/mg *in vivo* against a USP standard.

**Leucine aminopeptidase-treated ACTH:**[23] Five mg of ACTH and 75 units of enzyme (DFP treated, Worthington) were dissolved in 1.0 ml of buffer, 0.005 $M$ $(NH_4)_2CO_3$, 0.0025 $M$ $MgCl_2$, pH 8.5. After 10 hr at 37°, the solution was passed over a 1 $\times$ 50 cm column of G-50 fine Sephadex (Pharmacia) in 0.1 $M$ ammonium acetate, pH 4.5. Under these conditions with this lot of G-50 fine Sephadex, ACTH alone was recovered in a single narrow zone 0.4 the distance between the void volume (dextran blue) and iodide. In this experiment, most of the material was recovered in a peak which was indistinguishable from that of ACTH. The fractions corresponding to the peak were pooled, lyophilized, redissolved in 1 ml of 0.05 $M$ acetic acid, and stored at $-20°$ until use. Biological activity was 10 units/mg.

**Periodate borohydride-treated ACTH:**[24] Ten mg ACTH were dissolved in 3 ml of water and treated serially with $NaIO_4$ and $KBH_4$. The product was desalted by filtration on G-15 Sephadex in 0.1 $M$ ammonium acetate, pH 4.5. The material in the void volume was lyophilized, redissolved in 2 ml of 0.05 $M$ acetic acid, and stored at $-20°$. Its biological activity was 2 units/mg.

**NaOH-treated ACTH:**[25] Three mg ACTH were dissolved in 10 ml of 0.1 $N$ NaOH, boiled in a water bath for 10 min, cooled, and neutralized with HCl. It was desalted on Sephadex G-15, reconstituted, and stored as above. Its biological activity was 0.004 units/mg.

**Trypsin-treated ACTH:**[26] Ten mg ACTH and 0.1 mg trypsin (TPCK treated, Worthington) were dissolved in 1.0 ml of 0.1 *M* ammonium acetate, pH 7.5. After 2 hr at 37°, 1 mg soybean trypsin inhibitor (Worthington) was added, and the solution was filtered on Sephadex G-50 fine (see above). All of the $A_{280}$ was recovered in a broad zone at about 0.8 the distance between the void volume and iodide. The fractions corresponding to the $A_{280}$ were pooled, lyophilized, reconstituted, and stored as above. The material had a biological activity of 0.002 units/mg.

**Iodo ACTH:** Two types of iodinated ACTH were prepared. "ACTH-$^{127}$I" was prepared in milligram quantities at very low specific radioactivity for measurements of biological activity of iodinated ACTH. "ACTH-$^{125}$I" was prepared in microgram quantities at very high specific radioactivity for studies of binding of ACTH to adrenal extract. For both iodinations the buffer was sodium phosphate 0.01 *M* pH 7.4, and the oxidizing agent was chloramine T.[27] The degree of iodination was estimated after each addition of chloramine T by exposing an aliquot of the iodination solution to 25 mg of Dowex-1X10 (200–400 mesh $Cl^-$ form, Biorad) in 1.0 ml of 0.1 *N* acetic acid; $I^-$ adsorbs to the resin whereas ACTH-I is unadsorbed. At the completion of the iodination, an aliquot was analyzed more precisely by chromatoelectrophoresis; ACTH adsorbs at the origin, damaged hormone migrates with the plasma proteins and $I^-$ migrates about twice that distance.[28] Immediately after iodination, ACTH-I was separated from uniodinated ACTH by chromatography on carboxymethylcellulose (microgranular, Reeve Angel Co.) and stored at −20° (Fig. 1).

**"ACTH-$^{125}$I"** Typically, 100 μg of ACTH and 1000–2000 μCi of Na$^{125}$I (Union Carbide) in 500 μl of buffer were mixed with chloramine T, 3 μg in 30 μl of water; in molar equivalents, ACTH/I/chloramine T approximated 25/1/15. With 2 to 4 further

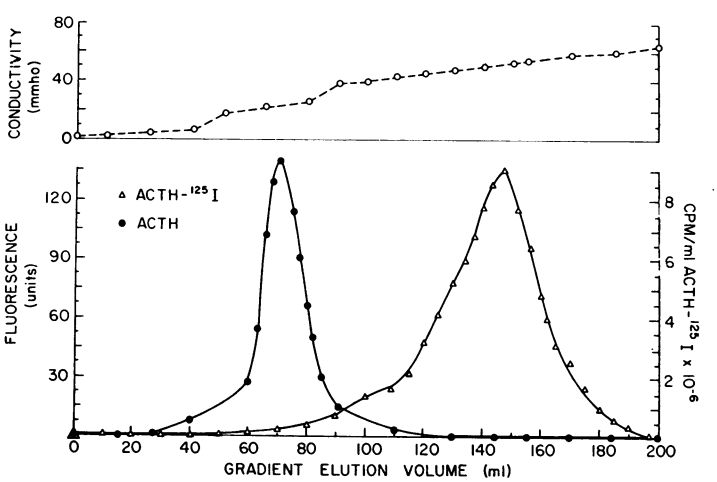



FIG. 1.—Separation of ACTH-I from unlabeled ACTH. 100 μg of ACTH that had been labeled with 1350 μCi of $^{125}$I ([ACTH]/[I] = 25/1) was applied to a 1 × 12 cm column of carboxymethylcellulose that had been equilibrated in ammonium acetate, 0.01 *M*, pH 4.5. After 500 ml of starting buffer had passed over the column, a gradient to 0.6 *M* ammonium acetate, pH 5.2 was applied. Unlabeled ACTH was measured by its $A_{280}$ or by fluorescence at 280 to 350 mμ expressed in arbitrary units/ml. ACTH-$^{125}$I was expressed as cpm/ml. Up to 15 mg of ACTH have been fractionated satisfactorily under these conditions. The wide separation was achieved by the use of the microcrystalline form of carboxymethylcellulose, the large volume of starting buffer, and the shallow gradient, created by use of a Varigrad apparatus in which successive compartments contained ammonium acetate as follows: 35 ml 0.01 *M*, pH 4.5; 35 ml 0.1 *M*, pH 4.8; 50 ml 0.2 *M*, pH 4.8; 75 ml 0.3 *M*, pH 5.0; 150 ml 0.6 *M*, pH 5.2.

additions of chloramine T, 90% of the $^{125}I$ was bound to ACTH. On chromatoelectrophoresis at the completion of iodination approximately 90% of the radioactivity was ACTH-I, with less than 2% in the region of damaged hormone.

In ACTH, the *N*-terminal amino acid, which is serine, is necessary for biological activity. Tyrosine in positions 2 and 23 each account for 15% of the hormone's $A_{280}$; tryptophane in position 9 accounts for essentially the rest of the $A_{280}$.[29] Leucine aminopeptidase removes serine slowly, but once serine is removed, tyrosine no. 2 is released quite promptly.[23] When unlabeled ACTH with an aliquot of ACTH-$^{125}I$ was treated with aminopeptidase and filtered (see above), the location of the ACTH was unchanged but its tyrosine/tryptophane[29] was reduced to 58%, its $A_{280}$ to 85%, its biological activity to 15%, and its $^{125}I$-radioactivity to 15% of the original mixture. If ACTH and ACTH-I react about the same with the enzyme, then these data indicate that the leucine aminopeptidase-treated hormone was 85% de-Ser, de-Tyr and 15% was intact and that ACTH-I was labeled virtually only in Tyr-2.

**"ACTH-$^{125}I$"** Typically, 15 mg of ACTH, 1 mg of $Na^{127}I$, and 1–5 $\mu$Ci of $Na^{125}I$ in 2.0 ml of buffer were mixed with chloramine T, 0.3 mg in 0.003 ml of water; ACTH/I/chloramine T was approximately 1/2/0.5. With 2 to 4 further additions of chloramine T, about 60% of the I was bound to ACTH. After purification on carboxymethylcellulose, ACTH-$^{127}I$ had, by measurement of $A_{295}$, $A_{305}$, $A_{325}$ at pH 4.5, 8.4, 9.0, 10.0, and 12.5, tyrosine/monoiodotyrosine/diiodotyrosine of 1/1/1.[30] These data confirmed that in our experiments the two tyrosines differed widely in reactivity for I and indicated that the ACTH-$^{127}I$ was a mixture of at least three different species of ACTH-I. The biological activity of this material was 32 units/mg both by adenyl cyclase activation (Fig. 2) and by *in vivo* bioassay.

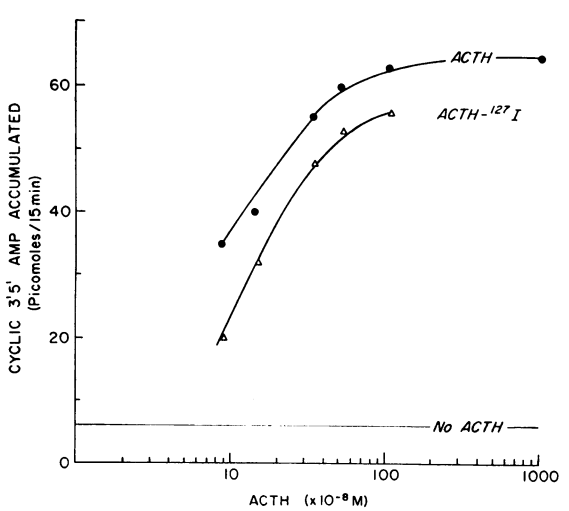



FIG. 2.—Biological activity of *ACTH-I*. ACTH-$^{127}I$, which was free of unlabeled ACTH and in which [tyrosine]/[monoiodotyrosine]/[diiodotyrosine] was 1/1/1, was compared with the unlabeled ACTH from which it was derived for its capacity to stimulate adenyl cyclase activity in adrenal particles, expressed as picomoles of $^{32}P$-labeled ATP converted to cyclic AMP during 15 min of incubation. As reported elsewhere,[6a] the production of cyclic AMP is linear for almost the entire 15-min period, the destruction of cyclic AMP is insignificant, and the identification and recoveries of product are reproducible. Each point on the graph represents two determinations in each of two experiments.

**Results.** When ACTH-$^{125}I$ was mixed with adrenal extract and filtered on G-75 Sephadex, more than half the radioactivity was recovered in the void volume with the extract (Fig. 3). Unlabeled ACTH inhibited the binding of ACTH-$^{125}I$. With this hormone at $6.6 \times 10^{-5}$ *M* (300 $\mu$g/ml) or more, inhibition was complete; at lower concentrations, ACTH was less effective so that at $2.2 \times 10^{-7}$ *M* (1 $\mu$g/ml) the binding of ACTH-$^{125}I$ was unaffected (Fig. 3, 4*a*). In the presence of adrenal extract and excess ACTH, the ACTH-$^{125}I$ migrated as a single peak indistinguishable from that of ACTH-$^{125}I$ alone (Fig. 3).

When fractions from the void volume that contained the bound ACTH-$^{125}$I were mixed with 2 *N* acetic acid and filtered again in acid, all of the ACTH-$^{125}$I was recovered in the ACTH zone. Most of this ACTH-$^{125}$I, after neutralization, bound again to a fresh aliquot of adrenal extract. In other experiments, the addition of 1 mg of ACTH was followed by rapid dissociation of bound ACTH-$^{125}$I.

Synthetic ACTH 1-24 peptide (Ciba), which has biological activity approximately that of native ACTH, completely inhibited binding of ACTH-$^{125}$I to the extract (Fig. 4*a*). Aminopeptidase-treated ACTH and periodate-treated ACTH, which had moderately reduced biological activity, inhibited binding of ACTH-$^{125}$I but to a lesser extent than unmodified ACTH (Fig. 4*a*). Trypsin-treated ACTH and NaOH-treated ACTH, which retained trivial biological activity, and insulin, which has no stimulating activity in the adrenal, did not affect binding of labeled ACTH (Fig. 4*a*). In all cases, the capacity of the derivatives to inhibit binding of ACTH-$^{125}$I to the adrenal extract, closely paralleled their capacity to stimulate adenyl cyclase in adrenal particles (Fig. 4, Table 1).

Dilution of the extract caused proportionate changes in binding and enzyme activity. Occasional adrenal extracts, which for unexplained causes failed to bind ACTH-$^{125}$I, had also lost the hormone-stimulated enzyme activity. Freezing and storage for 24 hours regularly reduced the binding capability of adrenal extracts; enzyme activity was also reduced. Repeat freezing and storage for an additional 24 hours caused

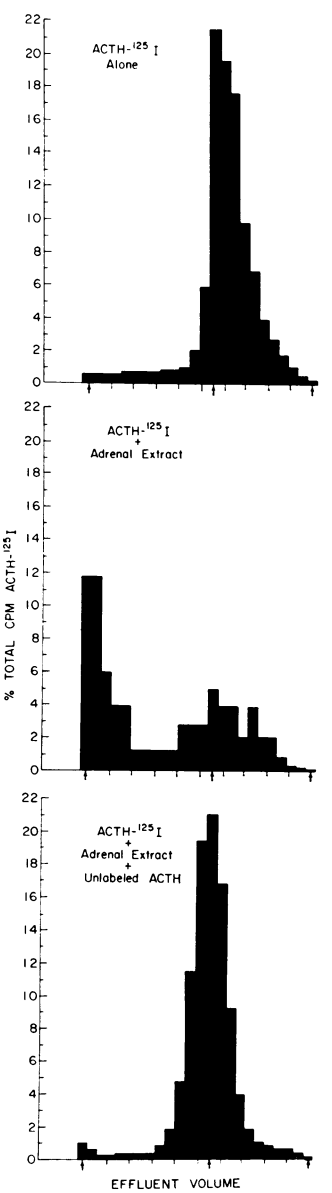



FIG. 3.—Binding of ACTH-$^{125}$I to adrenal extract. ACTH-$^{125}$I ($2 \times 10^{-12}$ *M*) with or without ACTH, with or without adrenal extract, was filtered on a $1 \times 50$ cm column of G-75 Sephadex at 4°. The buffer throughout was 0.01 *M* Tris-HCl, pH 7.8 that contained 0.001 *M* $MgCl_2$, 0.001 *M* dithiothreitol, a human serum albumin, 1 mg/ml. Fractions were 1 ml. The arrows denote the location of the peaks of unretarded material (dextran blue), unlabeled ACTH ($A_{280}$), and salt ($^{131}$Iodide).

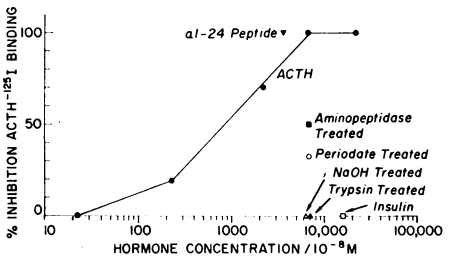
*Upper panel*–ACTH-$^{125}$I was mixed with 1 ml of buffer and applied to the column.

*Middle panel*–ACTH-$^{125}$I was mixed with 1 ml of adrenal extract. After 5 min at 1°, the mixture was applied to the column.

*Lower panel*–ACTH, at a final concentration of $2 \times 10^{-4}$ *M*, was mixed with 1 ml of adrenal extract. After 5 min at 1°, ACTH-$^{125}$I was added. After 5 min more, the mixture was applied to the column.

complete loss of both functions. Sonication or boiling of adrenal particles destroys ACTH-stimulated enzyme activity.[31] Extracts of sonicated or boiled particles did not bind ACTH-$^{125}$I.

Extracts in which normal rat kidney was substituted for adrenal, or in which all tissue was omitted did not bind ACTH-$^{125}$I. The gel filtration pattern of ACTH-$^{125}$I was completely unaltered in the presence of human serum albumin



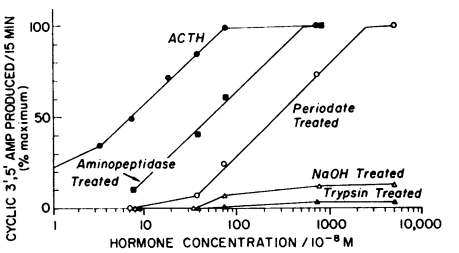




FIG. 4(*a*).—Inhibition of ACTH-$^{125}$I binding by ACTH and derivatives. Hormones, at the concentrations indicated, were mixed with 1.0 ml of adrenal extract. After 5 minutes at 1°, ACTH-$^{125}$I was added at a final concentration of $2 \times 10^{-12}$ *M*. After an additional 5 min the mixture was applied to a column of G-75 Sephadex (see legend to Fig. 3). To determine the extent of inhibition of binding by each unlabeled hormone, 1.0 ml of the same extract was mixed with ACTH-$^{125}$I without unlabeled hormone and after 5 min filtered on an identical column;

$$\% \text{ inhibition} = \left(1 - \frac{\% \text{ ACTH-}^{125}\text{I bound with unlabeled hormone}}{\% \text{ ACTH-}^{125}\text{I bound without unlabeled hormone}}\right) \times 100$$

Each point is the mean of two experiments.

(*b*).—Simulation of adenyl cyclase by ACTH and ACTH derivatives. Four ACTH derivatives were compared with the ACTH from which they were derived for their capacity to stimulate the adenyl cyclase of adrenal particles (see Fig. 2 legend and *Methods*). Each point on the ACTH curve represents two determinations in each of three assays, and for each derivative, two determinations on one assay. Since in this assay system, cyclic AMP production is proportional to the concentration of enzyme protein, it was convenient to combine the data of three experiments. Biological activity is expressed as % maximum, where 100% represents cyclic AMP production with ACTH in excess and 0% represents cyclic AMP production in the absence of ACTH.

TABLE 1. *Inhibition of ACTH-$^{125}$I-binding and adenyl cyclase activation by ACTH and derivatives.*

| Hormone | Inhibition of ACTH-$^{125}$I-binding: ACTH derivative/ native ACTH | Adenyl cyclase activation: ACTH derivative/ native ACTH |
|---|---|---|
| $\alpha_{1-24}$ Peptide | 1.70 | 1.1* |
| Aminopeptidase-treated ACTH | 0.14 | 0.16 |
| Periodate-treated ACTH | 0.05 | 0.03 |
| NaOH-treated ACTH | 0 | 0.00006 |
| Trypsin-treated ACTH | 0 | 0.00003 |
| Insulin | 0 | 0 |

* This value for the 1–24 peptide represents the data of others obtained by ascorbic acid depletion *in vivo*.[34]

The biological activity of ACTH derivatives as determined by inhibition of ACTH-$^{125}$I binding (column 1) and activation of adenyl cyclase (column 2) is compared to that of equimolar concentrations of native ACTH.

(Pentex) up to 10 mg/ml. Active adrenal extracts did not bind or alter in any way the location on gel filtration of purified iodinated arginine vasopressin, insulin, follicle stimulating hormone, or placental lactogen.

**Discussion.** Based on the following considerations, we have concluded that we have demonstrated directly the binding of ACTH to its biologically significant receptor site. ACTH-$^{127}$I that was labeled with an average of 2 iodine atoms/molecule of hormone and free of any unlabeled ACTH had specific adenyl cyclase stimulating activity that was half that of pure ACTH with an identically shaped response curve over a broad range of hormone concentrations. We have assumed that ACTH-$^{125}$I prepared in a similar way, but with only 1 iodine atom/molecule of ACTH, had at least as much biological activity, and therefore was capable of binding to the biologically significant ACTH-binding sites. Activation of adenyl cyclase, the biological property observed, is generally considered to be a specific essential part of the chain of events through which this hormone stimulates steroidogenesis.[1] If the enzyme can be activated by ACTH, the ACTH-binding site must be contained within the system. The capability of a tissue extract to bind ACTH-$^{125}$I was generally related to its content of ACTH-sensitive adenyl cyclase. Similar preparations that lacked the ACTH-sensitive cyclase did not bind ACTH-$^{125}$I. Nonspecific binding of labeled proteins, often a problem in other systems and indeed a problem when we used 4,340 $\times$ $g$ particles, was essentially nonexistent with the adrenal extract; no other iodinated hormones were bound. Finally, the capability of each unlabeled hormone to inhibit binding of ACTH-$^{125}$I was always proportional to its biological activity. It should be noted that it is immaterial to the argument whether the ACTH derivatives that we prepared contained hormone that was uniformly reduced in activity or a mixture of fully active and fully inactivated species.

That all of the hormone preparations had binding capabilities that paralleled their biological activities was fortuitous but not a necessary condition for the system. The capability of a labeled hormone to bind or of an unlabeled hormone to inhibit binding is a measure of its affinity for the binding site. The capability of a hormone to activate the adenyl cyclase reflects both its affinity for the site and the efficiency with which the hormone-site complex activates enzyme. By contrast, the standard biological assays not only fail to distinguish these two functions but in addition include in their results many other variables. For example, *in vitro* measures of steroidogenesis would include rates of formation, distribution and fate of cyclic AMP, binding of cyclic AMP to receptors, as well as the pathway for steroid synthesis and release. *In vivo* assays further include alterations in distribution, degradation, renal losses and blood flow. Thus, measurements in our system allow much more specific and precise studies of structure-function relationships as well as very rapid screening of substances for potential biological activity, both stimulation and specific inhibition. In addition to providing the basis for precise specific bioassays, which combine the technical advantages of radioimmunoassay with a biological basis for specificity, this approach is widely applicable to the study of peptide hormone binding and to the isolation and characterization of hormone-specific binding sites.

The differential rate of iodination of the tyrosines, demonstrated by aminopep-

tidase digestion of the ACTH-$^{125}$I and spectral analysis of the ACTH-$^{127}$I, was surprising especially since the ionization of the phenolic groups in each of the tyrosines appears to be normal. Interpretation of physical studies that ACTH has a totally random structure,[32, 33] and the generalization that differential iodination of tyrosines in a protein indicates that one of the tyrosines is "buried" or inaccessible to solvent, clearly need further examination.

The authors thank R. Ney for the *in vivo* bioassays, G. Malan, A. Schneider, and H. Edelhoch for advice on the spectral analyses, and B. Sherman, G. Robertson, S. Rosen, B. Weintraub, and E. Steers for gifts of one or more reagents.

[1] Haynes, R. C., Jr., *J. Biol. Chem.*, **233**, 1220 (1958).
[2] Sutherland, E. W., G. A. Robison, and R. W. Butcher, *Circulation*, **37**, 279 (1968).
[3] Pastan, I., J. Roth, and V. Macchia, these PROCEEDINGS, **56**, 1802 (1966).
[4] Taunton, O. D., J. Roth, and I. Pastan, *J. Clin. Invest.*, **46**, 1122 (1967).
[5] Schimmer, B. P., K. Ueda, and G. H. Sato, *Biochem. Biophys. Res. Commun.*, **32**, 806 (1968).
[6] (*a*) Taunton, O. D., J. Roth, and I. Pastan, *J. Biol. Chem.*, **244**, 247 (1969). (*b*) Krishna, G., B. Weiss, and B. Brodie, *J. Pharm. Exp. Therapeutics*, **163**, 379 (1968).
[7] Rodbell, M., *J. Biol. Chem.*, **242**, 5744 (1967).
[8] Goodfriend, T., and S. Y. Lin, *Clin. Research*, **17**, 243 (1969).
[9] Fong, C. T. O., L. Silver, D. R. Christman, and I. L. Schwarz, these PROCEEDINGS, **46**, 1273 (1960).
[10] Edelman, P. M., and I. L. Schwartz, *Amer. J. of Med.*, **40**, 695 (1966).
[11] Gorski, J., D. Toft, G. Shyamala, D. Smith, and A. Notides, *Rec. Prog. Horm. Res.*, **24**, 45 (1968).
[12] Sonenberg, M., A. S. Keston, and W. L. Money, *J. Clin. Endocrinol.*, **10**, 809 (1950).
[13] Edelman, I. S., and G. M. Fimognari, *Rec. Prog. Horm. Res.*, **24**, 1 (1968).
[14] Izzo, J. L., A. Roncone, M. J. Izzo, and W. F. Bale, *J. Biol. Chem.*, **239**, 3749 (1964).
[15] Lunenfeld, B., and A. Eshkol, *Vits. and Hormones*, **25**, 137 (1967).
[16] Stadie, W. C., N. Haugaard, J. B. Marsh, and A. G. Hills, *Amer. J. Med. Sci.*, **218**, 265 (1949).
[17] Birmingham, M. K., and E. Kurlents, *Endocrinology*, **62**, 47 (1958).
[18] Birnbaumer, L., and M. Rodbell, *J. Biol. Chem.*, **244**, 3477 (1969).
[19] Taunton, O. D., J. Roth, and I. Pastan, submitted for publication.
[20] Yasumura, Y., V. Buonassisi, and G. Sato, *Cancer Res.*, **26**, 529 (1966).
[21] Pastan, I., W. Pricer, and J. Blanchette-Mackie, in preparation.
[22] Lipscomb, H., and D. H. Nelson, *Fed. Proc.*, **18**, 95 (1959).
[23] White, W. F., *J. Amer. Chem. Soc.*, **77**, 4691 (1955).
[24] Dixon, H. B. F., *Biochem. J.*, **83**, 91 (1962).
[25] Reinhardt, W. O., I. I. Geschwind, J. O. Porath, and C. H. Li, *Proc. Soc. Exp. Biol. Med.*, **80**, 439 (1952).
[26] White, W. F., and W. A. Landmann, *J. Amer. Chem. Soc.*, **77**, 1711 (1955).
[27] Hunter, W. M., and F. C. Greenwood, *Nature*, **194**, 495 (1962).
[28] Berson, S. A., and R. S. Yalow, *J. Clin. Invest.*, **47**, 2725 (1968).
[29] Edelhoch, H., *Biochem.*, **6**, 1948 (1967).
[30] Edelhoch, H., *J. Biol. Chem.*, **237**, 2778 (1962).
[31] Pastan, I., and W. Pricer, unpublished observations.
[32] Bernstein, R. S., M. Wilchek, and H. Edelhoch, *J. Biol. Chem.*, **244**, 4398 (1969).
[33] Edelhoch, H., and R. E. Lippoldt, *J. Biol. Chem.*, **244**, 3876 (1969).
[34] Kappeler, H., and R. Schwyzer, *Helvet. Chim. Acta*, **44**, 1136 (1961).