amplified the action of imipramine in this test; Prange *et al.* (*2*) had shown that T3 augmented the antidepressant action of imipramine. Further, Breese *et al.* (*5*) showed that T3 alone was active in the dopa plus pargyline test. Subsequently, Prange and Wilson (*9*) found that T3 alone given briefly in moderately large doses was comparable to imipramine in antidepressant activity. This paradigm, taken with our findings of activity of TRH in hypophysectomized mice, suggests that TRH may also possess antidepressant value but by a mechanism independent of thyroid hormone action.

Note added in proof: Since this report was submitted for publication, Prange and Wilson have established in clinical studies with TRH that there is an immediate (within 2 hours) antidepressant effect in patients with unipolar depression (*10*).

N. P. PLOTNIKOFF
Experimental Therapy Division, Abbott Laboratories, North Chicago, Illinois

A. J. PRANGE, JR., G. R. BREESE
Departments of Psychiatry and *Pharmacology,*
University of North Carolina School of Medicine, Chapel Hill 27514

M. S. ANDERSON
Experimental Therapy Division, Abbott Laboratories

I. C. WILSON
Departments of Psychiatry and *Pharmacology, University of North Carolina School of Medicine*

References and Notes

1. A. J. Prange, Jr., and M. A. Lipton, *Nature* **196**, 588 (1962); ———, G. N. Love, *ibid.* **197**, 1212 (1963); A. J. Prange, Jr., *Amer. J. Psychol.* **119**, 994 (1963).
2. A. J. Prange, Jr., I. C. Wilson, A. M. Rabon, M. A. Lipton, *Excerpta Med. Int. Cong. Ser.*, No. 180 (1968), p. 332; *Amer. J. Physiol.* **126**, 457 (1969); I. C. Wilson, A. J. Prange, Jr., T. K. McClane, A. M. Rabon, M. A. Lipton, *N. Engl. J. Med.* **282**, 1063 (1970).
3. A. J. Prange, Jr., I. C. Wilson, A. Knox, T. K. McClane, M. A. Lipton, *Amer. J. Psychol.* **127**, 191 (1970).
4. M. S. Anderson *et al.*, *N. Engl. J. Med.* **285**, 1279 (1971).
5. G. R. Breese, T. D. Traylor, A. J. Prange, Jr., *Psychopharmacologia*, in press.
6. G. M. Everett, *Excerpta Med. Int. Congr. Ser.*, No. 122 (1966), p. 164.
7. C. Y. Bowers, H. G. Friesen, P. Hwang, H. J. Guyda, K. Folkers, *Biochem. Biophys. Res. Commun.* **45**, 4, 1033 (1971); L. S. Jacobs, P. J. Snyder, J. F. Wilber, R. D. Utiger, W. H. Daughaday, *J. Clin. Endocrinol.* **33**, 996 (1971).
8. N. P. Plotnikoff, A. J. Kastin, M. S. Anderson, A. V. Schally, *Life Sci.* **10**, 1279 (1971).
9. A. J. Prange, Jr., and I. C. Wilson, in preparation.
10. ———, *Psychopharmacologia* **26**, 82 (1972).
11. Supported in part by NIMH grant MH-15631, NIMH career scientist award MH-22536 to A.J.P., and by National Institute of Child Health and Human Development grant 5-K4-HD-24, 585-03 to G.R.B.

19 April 1972; revised 21 August 1972

Arthropod Molting Hormone: Radioimmune Assay

Abstract. *A radioimmune assay for the arthropod molting hormone, ecdysterone, has been developed. The sensitivity of the assay is 200 picograms or 25 times the maximum sensitivity of the bioassay. Closely related steroids also bind the antibody, but with lower affinities.*

Ecdysterone (β-ecdysone, crustecdysone) induces molting in many arthropodan species (*1*). One technical problem that has hindered research related to ecdysterone is the lack of a precise physicochemical technique for quantifying tissue and circulating titers of this hormone. In the standard bioassay for crustecdysone either *Calliphora* (*2*) or *Musca* (*3*) is used as the assay organism, but like most bioassays it is relatively insensitive.

A number of laboratories have investigated a gas-liquid chromatographic method (GLC) for quantifying either α- or β-ecdysone (*4*). While these procedures are promising, the problems inherent in purifying biological samples for GLC as well as interfering side reactions in the derivatization of ecdysones tend to make this alternative less attractive at present.

Another technique for measuring steroid hormones, which are often present in vanishingly small quantities, is the radioimmune assay (*5*). By conjugating the hormone to a large carrier protein [such as bovine serum albumin (BSA)], the steroid is rendered haptenic and can elicit an immunogenic response. Since the specificity of the antibody response is dependent on the homogeneity of the haptene configuration, a derivative is first formed at one position, which is then reacted with the carrier protein. Derivatization of ecdysterone at the 6-keto function was chosen for a number of reasons. Theoretically the limit of detection by this method is dictated primarily by the specific activity of the labeled standard available.

Ecdysterone (40 mg) was converted to the oxime acetic acid ether in a 4 percent solution of aminooxyacetic acid (*6*) in pyridine, overnight, at 40°C. The identity of the oxime acetic acid ether derivative and its methyl ester was established by infrared and ultraviolet spectrum analyses and thin-layer chromatography in three different solvent systems (*7*). The oxime derivative was coupled to BSA by way of the isobutylchloroformate mixed anhydride intermediate. The protein-ecdysone conjugate was then dialyzed against running distilled water overnight and lyophilized (*8*).

The conjugate (4 mg) was suspended in equal volumes of Freund's complete adjuvant and saline and injected subcutaneously and intramuscularly into each of three New Zealand white rabbits. A 1-mg booster injection was administered in similar fashion 6 weeks after the initial injection. Blood was collected 9 days after the booster injection and analyzed for antibodies to crustecdysone. Control serum was collected from uninjected animals.

The antibody response was assayed according to a method modified from

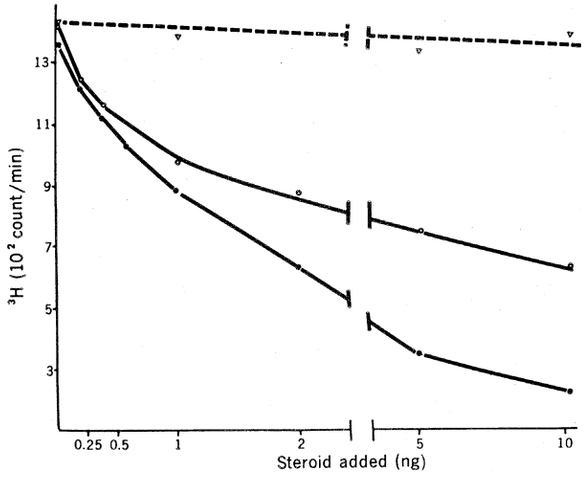

Fig. 1. Inhibition of [^{3}H]ecdysterone binding by rabbit antiserum in the presence of increasing amounts of various unlabeled steroids; (●), ecdysterone; (○), α-ecdysone; (△), 3β-hydroxy-5α-cholestan-6-one. All reaction tubes contained 1 percent antiserum and [^{3}H]ecdysterone (4000 count/min). Total volume was made up to 0.5 ml with borate buffer (*p*H 8.4). To facilitate precipitation of antibody-bound haptene, total serum concentration was brought up to a constant 10 percent in each reaction tube by the addition of control serum. Nonspecific binding of label by a 10 percent solution of control serum amounted to 150 count/min and has been subtracted from each of the above values.

Farr (*9*). Antiserum was incubated with ^{3}H-labeled and unlabeled ecdysterone in a borate buffer (*p*H 8.4) for 3 hours (*10*). The haptene-antibody conjugate was then precipitated by the addition of an equal volume of saturated $(NH_4)_2SO_4$, centrifuged, and washed with two volumes of 50 percent saturated $(NH_4)_2SO_4$. The pellet was dissolved in H_2O, and the radioactivity was counted in Aquasol (New England Nuclear) on a Beckman LS-233 scintillation counter (counting efficiency, 46.7 percent).

Initial experiments showed that while control serums bound only a minimal amount of the labeled ecdysterone (2 percent), a 10 percent solution of antiserum would bind virtually 100 percent. Titrations showed a linear relation between the amount of labeled ecdysterone bound and the percentage of antiserum in the incubation mixture. As might be expected, above the 2 percent antiserum dilution (where 50 percent of the labeled ecdysterone is bound) the system shows nonlinear characteristics. In a similar manner, treating a 2 percent antiserum mixture with increasing amounts of [^{3}H]ecdysterone resulted in a linear binding of haptene at concentrations below 3.0 ng/ml. At values above 3.0 ng/ml, a tendency toward saturation was observed.

The radioimmune assay for ecdysterone depends on the decrease in the amount of labeled haptene bound by a fixed amount of antiserum in the presence of increasing concentrations of unlabeled ecdysterone. Under optimum conditions (Fig. 1) as little as 200 pg of ecdysterone decreases by 14 percent the observed amount of label bound in the precipitate while 2 ng of competitive ecdysterone decreases the amount of label bound by more than 50 percent. Since this assay depends on the recognition of the molecular configuration of the haptene by the antibody, it would not be surprising if related steroids were also bound by the system and competed for binding with the labeled ecdysterone, albeit with different affinities. As is indicated, α-ecdysone does compete for the haptene binding sites but not so effectively as unlabeled ecdysterone. Furthermore, inokosterone has also been shown to bind to the antibody. Neither 3β-hydroxy-5α-cholestan-6-one nor cholesterol (not shown) will compete for binding in this system. The resolution of competing ecdysone analogs should be possible as a consequence of their different binding affinities to the antibody.

The assay lends itself to fast quantitative analysis of ecdysterone and related steroids (*11*). Moreover, the lower limit of 200-pg sensitivity can be improved by the use of ecdysterone with a higher specific activity. The biological conversion of highly labeled α-ecdysone has already resulted in ecdysterone with a specific activity of approximately 50 c/mmole (*12*). The use of such biosynthesized labeled ecdysterone should extend the sensitivity of this assay to approximately 25 pg.

DAVID W. BORST
JOHN D. O'CONNOR
Department of Biology,
University of California at Los Angeles,
Los Angeles 90024

References and Notes

1. A. Krishnakumaran and H. A. Schneiderman, *Biol. Bull.* **139**, 520 (1970).
2. E. Becker and E. Plagge, *Biol. Zentralbl.* **59**, 326 (1939); D. Adelung and P. Karlson, *J. Insect Physiol.* **15**, 1301 (1969); J. A. Thomson, F. R. Imray, D. H. S. Horn, *Aust. J. Exp. Biol. Med. Sci.* **48**, 321 (1970).
3. J. N. Kaplanis, L. A. Tabor, M. J. Thompson, W. E. Robbins, T. J. Shortino, *Steroids* **8**, 625 (1966).
4. M. Katz and Y. Lensky, *Experientia* **26**, 1043 (1970); E. D. Morgan and A. P. Woodbridge, *Chem. Commun.* **1971**, 475 (1971); N. Ikekawa, F. Hattori, J. Rubio-Lightbourn, H. Miyazaki, M. Ishibashi, C. Mori, *J. Chromatogr. Sci.* **10**, 233 (1972).
5. F. G. Peron and B. V. Caldwell, *Immunological Methods in Steroid Determinations* (Appleton-Century-Crofts, New York, 1970).
6. Eastman Chemical Co., Rochester, New York.
7. The three solvent systems were (i) chloroform and ethanol (60 : 40); (ii) chloroform and methanol (50 : 50); and (iii) chloroform, methanol, and water (60 : 30 : 5).
8. B. F. Erlanger, in *Methods in Immunology and Immunochemistry*, C. A. Williams and M. W. Chase, Eds. (Academic Press, New York, 1967), vol. 1, p. 144.
9. R. S. Farr, in *ibid.*, vol. 3, p. 66.
10. ^{3}H-Labeled ecdysterone at 6 c/mmole was purchased from New England Nuclear.
11. Results in our laboratory have indicated that the addition of known amounts of ecdysterone to the hemolymph of intermolt crayfish (*Procambarus* sp.) results in the expected inhibition of radioligand formation.
12. D. S. King, personal communication.
13. Supported by PHS grant NS 08990 and by NOAA office of sea grant USDC 2-35208. D.W.B. is supported by an NSF predoctoral fellowship. We acknowledge the gift of ecdysterone from D. H. S. Horn (Commonwealth Scientific and Industrial Research Organization, Australia), the gift of α-ecdysone from both D. King (Zöecon Corp., Palo Alto, Calif.) and L. I. Gilbert (Northwestern University, Evanston, Ill.), and the gift of 3β-hydroxy-5α-cholestan-6-one from H. Emmerich (Cologne). In addition, critical discussion with Dr. G. E. Abraham (University of California Medical Center, Los Angeles) proved invaluable.

18 August 1972

Coherin: A New Peptide of the Bovine Neurohypophysis with Activity on Gastrointestinal Motility

Abstract. *A factor with potent activity in the regulation of mammalian gastrointestinal motor function has been isolated from the bovine posterior pituitary gland by a process allowing minimal dissociation of neurophysin-bound complexes and the separation of free unbound peptides. This substance alters the frequency, amplitude, rhythm, and duration of peristaltic contraction.*

Evidence is presented for the presence in the bovine neurohypophysis of a new factor which may be involved in the normal physiological regulation of intestinal motility in mammals. We now describe the isolation and some of the properties of this factor.

Examination of a large number of neurohypophyseal fractions has revealed one fraction with a unique property: the capacity to induce prolonged, rhythmic, integrated contractions of the jejunum in vivo beginning about 1 hour after intravenous injection of 1 μg/kg and lasting for periods in excess of 5 hours. This substance also inhibits jejunal contraction within 5 seconds after it is injected, and the inhibition lasts for periods up to 20 minutes. It induces changes in the electro-enterogram of the Biebl loop from a random to an organized and coherent pattern of electrical cycles (the basal electrical rhythm) in contiguous segments of jejunum (*1*).

To quantify peristaltic activity we have used awake trained dogs with Thiery-Vella (*2*) or Roux-en-Y (*3*) fistulas of the small intestine or with the Biebl loop (*4*). Contractions were monitored by pressure transducers (Statham P23D6) attached to four separate balloons inserted in tandem into the jejunal lumen at intervals of 3, 6, and 3 cm, respectively.

In the fasted unanesthetized dog with Roux-en-Y fistula the main criteria for activity of pituitary fractions on the intestinal tract were: (i) inhibition of jejunal contraction after intravenous injection of a dose of 1 μg per kilogram of body weight, and (ii) coherence of intestinal contraction for a period of at least 2 hours after the intravenous injection. Coherence is defined as the con-