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.

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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-

Table 1. Preparation of bovine coherin from an acetone powder of posterior pituitary tissue.

Steps	Protein yield (mg/g)	Inhibition dose (μg/kg)	Coherence	Enrichment factor
Powder extraction	179	16	—	—
DEAE-cellulose	22	2	+	8
Sephadex G-50	9.8	1.5	++	11
Sephadex G-25	7.3	1.0	++	16
Sephadex G-10	0.35	0.3	+++	53
Electrophoresis*	0.014	0.08	++++	200

* Continuous electrophoresis, *p*H 6.0, 3.5, and 2.8.

tractions (expressed in percent) initiated at the cephalad end of a 12-cm segment of jejunum, which are propagated in uninterrupted sequence through the entire segment. Substances that inhibit intestinal contraction are compared for coherence after doses are adjusted to equal inhibitory levels (5 minutes of inhibition). The mean value for coherence in the normal fasted dog with chronic jejunal fistula is 26 percent. One to 2 hours after intravenous injection of the new peptide coherence increases to 62 percent (hence the name coherin). This increase of 36 percent ($P < .001$) is compared to an increase of 4 percent after an injection of saline (control), a decrease of 4 percent for oxytocin, and an incease of 8 percent for [8-arginine]-vasopressin. Thus coherin appears to promote a rhythmic pattern of propagative motor activity in the gut. Other hormonal substances including adrenocorticotropic hormone, cortisone, and thyroid-stimulating hormone cause negligible changes in coherence.

Inhibition of intestinal contraction is a useful response for locating biologically active components in the fractionation of pituitary tissue, and gains specificity when used in conjunction with determinations of coherence. This measurement (of inhibition period) lends itself to convenient quantitation and gives typical logarithmic dose response curves.

The procedure for isolation of coherin, derived after extensive exploration, is summarized in Table 1. Acetone-dried bovine posterior pituitary powder (10 g) (Miles Laboratories) (*5*) was homogenized (ice bath; 3 minutes; Sorvall Omni-Mixer) in 50 ml of ammonium acetate, 0.2*M*, *p*H 5.6. After centrifugation of the homogenate for 40 minutes at 18,000*g* and 5°C, the supernatant was filtered through 3 g of Celite (Johns Manville, Analytical) and transferred to a column (50 by 610 mm) of diethylaminoethyl (DEAE)-cellulose (Eastman 7392) that had been equilibrated with the 0.2*M* ammonium acetate; the components of the supernatant were eluted with ammonium acetate, 0.05*M*, *p*H 5.6 (Fig. 1A).

Eluates were assayed for vasopressor (*6*) and oxytocic (*7*) activity and for inhibition of intestinal contraction. Active inhibitory fractions were also assayed for their ability to produce coherence. To assess the degree of homogeneity, fractions were subjected to electrophoresis (Whatman 3MM filter paper) at *p*H 2.0, 3.5, 6.0, and 8.5. Molecular weights of active fractions were estimated on thin layers of Sephadex G-50, superfine (*8*). Fractions containing coherin activity were pooled, lyophilized, dissolved in 5 ml of 0.05*M* ammonium acetate, *p*H 5.6, and transferred to a column (50 by 990 mm) of Sephadex G-50, superfine. The coherin active peak was then passed through Sephadex G-25 and G-10 in analogous stages (Fig. 1, B to D).

The DEAE-cellulose stage of preparation allows virtually complete separation of hemoglobin from neurophysin-bound oxytocin, and vasopressin (*9*) and from the free unbound peptides. Upon passing through the Sephadex G-50 column, neurophysin-bound oxytocin and vasopressin are eluted in peak I (Fig. 1B) with the rapidly moving components (*10*). Peak II (Fig. 1B) which represents mainly free unbound peptides low in oxytocin and vasopressin contains the coherin activity. Sephadex G-25 removes remaining traces of neurophysin-bound vasopressin and oxytocin in the fast-moving fraction (*11*); the unbound peptides including coherin are eluted in peak II (Fig. 1C). As judged by behavior on paper electroporesis, the product obtained after repeated passes through columns of Sephadex G-10 (Fig. 1D) required further purification. Continuous flow electrophoresis (Beckman-Spinco Apparatus, model CP) (*12*) proved most suitable (Fig. 1E), for it made possible the convenient removal of traces of vasopressin and oxytocin by virtue of their relatively high cationic mobilities in acidic solution (*13*).

Coherin differs markedly from authentic samples of synthetic oxytocin (Parke, Davis) and [arg^8]vasopressin (Sandoz) with respect to electrophoresis, chromatography, and biological activity. The oxytocic and rat pressor activity of coherin is less than 5 international units per milligram. Electrophoretic migration rates relative to bromophenol blue (10 volt/cm, 0.2 to 1.0 ma/cm) are: *p*H 2, −0.78 (cathodal); *p*H 6.0, + 0.05 (anodal); *p*H 10.9, + 1.16 (anodal). The isoelectric point is *p*H 6.0. Coherin is relatively thermostable, losing only 10 percent of its activity when heated in a boiling water bath at *p*H 3 for 1 hour.

The molecular weight is approximately 4000. Amino acid analysis gives the following residues per mole: lysine, 0.59; histidine, 0; arginine, 0; aspartic acid, 3.9; threonine, 0.11; serine, 0.22; glutamic acid, 4.0; proline, 5.1; glycine, 4.7;

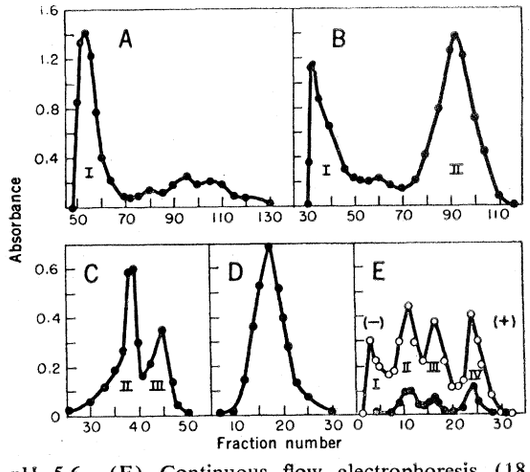

Fig. 1. Elution curves in the preparation of coherin. ●, Absorbance at 280 nm; ○, absorbance at 230 nm. (A) DEAE-cellulose (column, 50 by 610 mm); activity is in peak I. (B) Sephadex G-50, superfine (column, 50 by 990 mm). Activity is in peak II; (the ratio of the elution volume to the void volume, V_e/V_o, is 2.9). (C) Sephadex G-25, superfine (column, 20 by 1460 mm) activity is in peak II; $V_e/V_o = 1.9$. (D) Sephadex G-10 (column, 10 by 1050 mm), third rerun ($V_e/V_o = 1.6$). Eluent for A was 0.05*M* ammonium acetate, *p*H 5.6; the eluent for B to D was ammonium acetate, 0.05*M*, *p*H 5.6. (E) Continuous flow electrophoresis (18 volt/cm; 0.83 ma/cm); the electrolyte was 0.2*M* acetic acid, *p*H 2.75. The sample was applied at the top, +75 mm from the vertical midline at the rate of 0.4 mg/hour. The flow rate of the eluent was 0.5 ml/min; the coherin activity is in peak II.

alanine, 0.90; cystine (½), 4.9; valine, 0; methionine, 0; isoleucine, 3.3; leucine, 3.1; tyrosine, 2.9; phenylalanine, 0.88. A sample of partially purified coherin subjected to electrophoresis at *p*H 8.7 on polyacrylamide gel (20 percent) gave a single weak band ($R_F = 0.485$) when stained with Coomassie blue.

Although complete homogeneity must still be demonstrated, our product gives a single spot on paper electrophoresis (*p*H 1.9, 6.0, 8.5, and 11.0) and represents a purification of about 200-fold, on the basis of intestinal inhibiting activity (Table 1).

Our results suggest that we have isolated from bovine posterior pituitary powder a new factor with unique biological activity and with chemical and physical properties distinct from those of the known hormones of the posterior pituitary gland. Although the identity of coherin as a hormone remains to be established, the potency of the preparation and the nature of its activity suggests such a role. If indeed it proves to be a hormone involved in the regulation of peristaltic function, our understanding of the mechanisms of gastrointestinal motor activity will be greatly enhanced.

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28 June 1972; revised 21 August 1972

Eyes Have a Role in Photoperiodic Control of Sexual Activity of Coturnix

Abstract. *In blinded Japanese quail* (Coturnix coturnix japonica) *encephalic photoreception of the stimulus from long photoperiods is sufficient to induce and maintain normal gonadal function in females (egg laying) and in males (enlargement of the cloacal gland). However, the termination of sexual activity by short days is dependent on these birds having experienced long days at the time of blinding.*

The importance of day length in controlling reproductive activity in many species of birds is well established. More than 35 years ago Benoit (*1*) reported that growth and development of testes of the domestic duck could be stimulated by direct photostimulation to the brain. In one of his experiments, severance of the optic nerve in immature male ducks decreased the photoresponsiveness to one-fifth of the normal. He concluded that if encephalic photoreception has any physiological role in avian reproduction the nature of its function would be supplemental to the eyes.

Recent studies have indicated that the eyes, or vision, are not necessary for photoperiodic induction of gonadal growth in the chicken (*Gallus domesticus*) (*2*), the common coturnix or Japanese quail (*Coturnix coturnix japonica*) (*3, 4*) and house sparrow (*Passer domesticus*) (*5*). Furthermore, after maturity, blinded and intact birds are equally responsive to changes in environmental light, as indicated by the testes weight in common coturnix (*6*), and by time of oviposition in the domestic fowl (*7*).

Both retinal and encephalic photoreception may be functional in the control of reproduction, but their interaction has not been determined. We have found that the eyes are involved in the termination of reproduction in birds or at least in Japanese quail. We now present a hypothesis that assigns different roles of ocular and encephalic photoreceptors in photoperiodic control of avian reproduction.

Independent studies with the common coturnix or Japanese quail (*Coturnix coturnix japonica*) at the University of Tokyo and the University of California at Davis were sufficiently similar in design so that it is possible to consider the data complementary.

The experimental Japanese quail used in Tokyo were derived from the stock that had been maintained at the University of Nagoya. Birds were first reared as a group under continuous light (LL). They were kept on short days (light : dark, LD 8 : 16, 20 lux, incandescent light) from 3 weeks of age and then were transferred to individual cages. Ten birds were blinded at 5 weeks of age, when the ovary was still undeveloped. When the birds were 8 weeks of age, the light regimen was changed to constant light (LL) and continued for 40 days when it was changed to LD 8 : 16. The time from onset of LL to sexual maturity (first egg), and ovipositing time of each egg laid, were recorded. Except during the summer season, the room temperature was controlled at 18° to 22°C. At 8 weeks of age half of the blinded birds and intact controls of the same hatch were subcutaneously injected around the skull with 0.1 to 0.3 ml of India ink (Pelikan) in an attempt to insulate the brain from the impinging light. The ink had been passed through a Sephadex G-25 column to remove toxic materials. In a preliminary test, ink injection reduced the amount of light to the brain indefinitely to less than 1/100 of that in the untreated controls.

At the University of California at Davis (UCD), the experimental birds were from the UCD random-bred line of common coturnix. They were held under long daily photoperiods (LD 16 : 8, white fluorescent light, 200 lux) to 4 weeks of age then transferred to individual cages in two bioclimatic chambers in which temperature was controlled at 23°C (relative humidity 55 percent) and short photoperiods (LD 6 : 18) were provided. The birds were kept on short days until 15 weeks of age. Thereafter they were exposed to alternate periods of long (LD 16 : 8) and short (LD 6 : 18) days, each of which continued for several weeks. In addition to the periods of short days, further periods of complete darkness for 1 or 2 weeks were given to hasten gonadal regression. At 6 weeks of age, half of the birds in each chamber were pinealectomized, and at 7 weeks of age all of the birds in one chamber were blinded. The endocrine responses of the testes were assessed, indirectly, by the width of the cloacal gland and by the amount of its foamy secretion. A close relation between this gland and testicu-