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Corticotropin/lipotropin common precursor-like material in normal rat extrapituitary tissues*

(pro-opiomelanocortin/procorticotropin/pro-lipotropin/ectopic hormone production/nonendocrine tissue hormone production)

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ABSTRACT **A M_r 26,000 corticotropin (ACTH)-like material is present in glacial acetic acid extracts of all normal rat extrapituitary tissues. In the present study, β-melanotropin (β-MSH) immunoactivity was detected in glacial acetic acid extracts of normal rat extrapituitary tissues. β-MSH immunoactivity was also present in all extracts (mean ± SEM, fmol/mg of protein): brain, 71.0 ± 16.3; stomach, 11.5 ± 1.6; kidney, 8.9 ± 0.8; colon, 8.2 ± 1.1; small intestine, 6.5 ± 1.1; liver, 4.3 ± 0.5; and heart, 3.2 ± 0.5. Except in brain extracts, β-MSH and ACTH immunoactivities of tissue extracts were strongly correlated to each other (r = 0.79; n = 42). When tissue extracts (except brain) were passed through a Sephadex G-75 (superfine) column, ACTH and β-MSH immunoactivities were eluted in a single peak corresponding to M_r 26,000. In contrast, for brain extracts, the M_rs of major peaks of ACTH and β-MSH immunoactivities were 4,500 and 8,000, respectively; a smaller peak of M_r 26,000 ACTH/β-MSH-like material was also eluted. Specific anti-ACTH immunocolumns, which did not bind purified synthetic β-MSH, adsorbed both ACTH and β-MSH immunoactivities of all tissue extracts except those of brain. One-third of the β-MSH immunoactivity in brain extracts adsorbed to the anti-ACTH immunocolumn, but two-thirds of β-MSH immunoactivity passed through the column. We conclude that ACTH and β-MSH immunoactivities are present in all normal rat extrapituitary tissues and exist in most tissues on the same molecule. This M_r 26,000 molecule is closely related to the pituitary ACTH/β-lipotropin common precursor.**

Until the 1960s, it was believed that corticotropin (ACTH) was produced exclusively by the pituitary gland. Since that time, however, a great deal of evidence has accumulated to indicate that ACTH is also produced by a wide range of extrapituitary tissues (1–6).

In 1977 and 1979, Odell *et al.* (7, 8) reported that extracts of all carcinomas studied, regardless of histological type, contained an ACTH-like material. These ACTH-like materials, which were biologically inert or weakly bioactive, were reported to be considerably larger molecules than standard pituitary ACTH (6–8). In control studies, immunoactive ACTH was occasionally detected at the limits of assay sensitivity in a small number of extracts of normal tissues (7, 8). In follow-up studies using a much more sensitive ACTH immunoassay, an ACTH-like material which was immunoactive, but not bioactive, was found in a wide range of normal rat extrapituitary tissues (9). On the basis of gel chromatography, the M_r of the major fraction of these tissue ACTH-like materials was 26,000. Trypsin treatment digested this M_r 26,000 ACTH to a M_r 4,500 immunoactive and bioactive ACTH.

Eipper and Mains (10) found a M_r 26,000–27,000 common precursor containing the sequences of both ACTH and β-lipotropin by utilizing the ACTH-secreting At-T-20 mouse pituitary tumor cell line and primary pituitary cell cultures. β-Lipotropin contains the entire amino acid sequence of β-melanocyte-stimulating hormone (β-MSH; melanotropin). Previous studies from our laboratory demonstrated that β-MSH *per se* was not present in extracts of normal human pituitaries, nor was it detectable in blood (11). The material reacting in immunoassays developed against synthetic human β-MSH was probably β-lipotropin (11).

In the present study, we measured β-MSH and ACTH immunoactivities of glacial acetic acid extracts of a wide variety of normal rat extrapituitary tissues and established that both immunoactivities reside on the same molecule. These studies offer further evidence that normal nonendocrine rat tissues contain the ACTH/β-lipotropin common precursor molecule.

METHODS

Tissue Extraction. Glacial acetic acid extracts of tissue from adult Sprague–Dawley rats were prepared by methods to be described elsewhere. Recoveries averaged 86% and 82% for β-MSH and ACTH (SD, ± 7%), respectively, as determined in at least five independent studies of each tissue. Contamination of tissue extracts or glassware with purified or standard β-MSH and ACTH was prevented.

β-MSH Radioimmunoassay. We determined the β-MSH immunoactivity of tissue extracts by radioimmunoassay with a rabbit anti-human β-MSH antibody that had been used in studies in our laboratory for quantifying human β-MSH in unextracted plasma (11) and showed complete crossreaction with β-lipotropin. Synthetic human β-MSH-(1–22) was kindly provided by the National Pituitary Agency. Assay sensitivity was 5 pg per tube. The within-assay coefficient of variation was ±8%; the between-assay coefficient was 15%.

ACTH Radioimmunoassay. ACTH immunoactivity of tissue extracts was assayed by a radioimmunoassay to be described elsewhere.

Sephadex G-75 Column Chromatography. Aliquots (3–4 ml) of tissue extracts were applied to a Sephadex G-75 (superfine) column (1.6 × 90 cm) and eluted with 0.05 M sodium phosphate, pH 8.0/0.5% bovine serum albumin/0.5% mercaptoethanol at 4°C. Fractions (1.8–3.6 ml) were collected and assayed directly for β-MSH and ACTH immunoactivities.

Anti-ACTH Immunocolumn. Anti-ACTH immunocolumns and control columns (normal rabbit gamma globulin columns)

Abbreviations: ACTH, corticotropin; β-MSH, β-melanotropin; Con A, concanavalin A.

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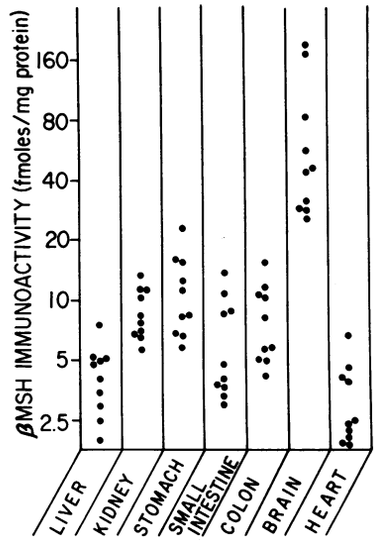

FIG. 1. Concentrations of β-MSH in normal rat tissues as measured by radioimmunoassay.

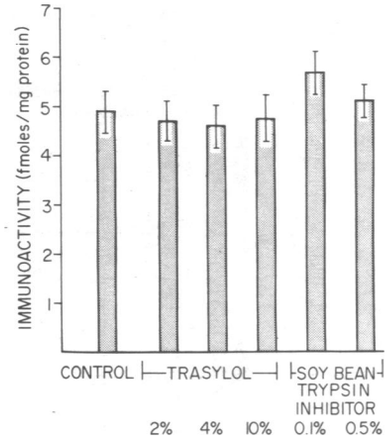

FIG. 3. Effect of enzyme inhibitors on β-MSH immunoactivity of glacial acetic acid extracts of rat liver (0.80 mg of protein) measured in 1 ml of 0.05 M sodium phosphate, pH 8.0/0.5% mercaptoethanol/0.25% bovine serum albumin with or without enzyme inhibitors at the indicated concentrations. Bars represent mean ± SD for triplicate incubations.

were prepared. Aliquots (3 ml) of tissue extracts in ACTH radioimmunoassay buffer were applied to both anti-ACTH immunocolumns and control columns (0.6 × 7 cm), and eluates (4 ml) were directly assayed by β-MSH and ACTH radioimmunoassays.

Concanavalin A Column Chromatography. Concanavalin A (Con A) bound to Sepharose 4B (Pharmacia) was used to prepare columns in 0.1 M sodium phosphate/0.15 M NaCl, pH 8.0. The tissue extracts were applied to the column and eluted first with buffer and then with 0.2 M methyl α-D-glucopyranoside.

RESULTS

β-MSH Immunoactivity in Extracts of Normal Rat Tissues. Fig. 1 shows the levels of β-MSH immunoactivity in extracts of 10 sets of normal rat extrapituitary tissues. All extracts contained β-MSH-like materials in detectable amounts (mean ± SEM, fmol/mg of protein): brain, 71.0 ± 16.3; stomach, 11.5 ± 1.6; kidney, 8.9 ± 0.8; colon, 8.2 ± 1.1; small intestine, 6.5 ± 1.1; liver, 4.3 ± 0.5; and heart, 3.2 ± 0.5. Representative dose–response curves of tissue extracts are shown on logarithm–logit transformation in Fig. 2. The slopes of the response lines were −0.993, −1.043, −1.107, −1.010, and −0.964 for kidney, stomach, small intestine, brain, and colon, respectively, with all values in the 95% confidence range of those of β-MSH standard (mean ± SD, −0.998 ± 0.114; n = 10).

To examine the possibility that the proteolytic activity contained in tissue acetic acid extracts may affect the β-MSH radioimmunoassay, tissue β-MSH immunoactivity was measured in the presence of enzyme inhibitors. Addition of enzyme inhibitors [Trasylol (aprotinin) at up to 2.83 trypsin inhibitor units per tube and soybean trypsin inhibitor at up to 0.5%] in the incubation medium of the β-MSH radioimmunoassay did not modify β-MSH immunoactivity. As an example, Fig. 3 shows the data obtained with liver extracts. In addition, elution profiles of [^{125}I]iodo-MSH through Sephadex G-50 column after 48-hr incubation with or without tissue extracts were indistinguishable.

β-MSH and ACTH immunoactivities in the extracts expressed in molar concentrations correlated strongly to each other for all tissues other than brain (r = 0.789; n = 42) (Fig. 4). Brain β-MSH and ACTH immunoactivities showed a weaker correlation (r = 0.502; n = 9) that was not statistically significant.

Sephadex G-75 Column Chromatography. Fig. 5 shows elution profiles, from Sephadex G-75 (superfine) columns, of both

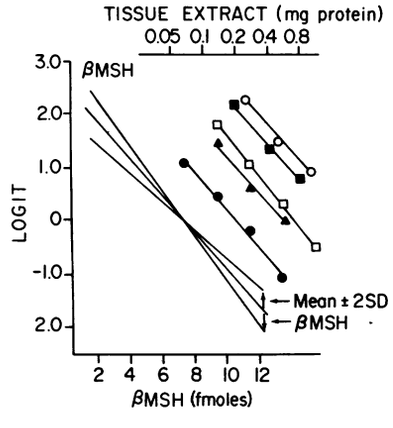

FIG. 2. Logarithm–logit dose–response lines for β-MSH-like materials extracted from normal rat tissues. ■, Kidney; ▲, stomach; ○, colon; ●, brain; □, small intestine.

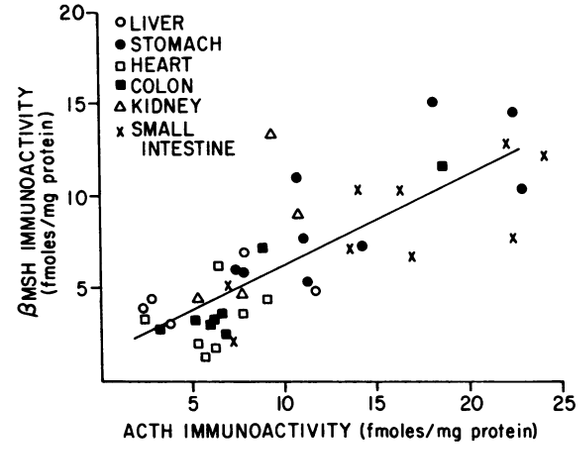

FIG. 4. Relationship between ACTH and β-MSH immunoactivities extracted from normal rat tissues. Except for brain extracts, there was significant correlation between the two values (r = 0.789; n = 42).

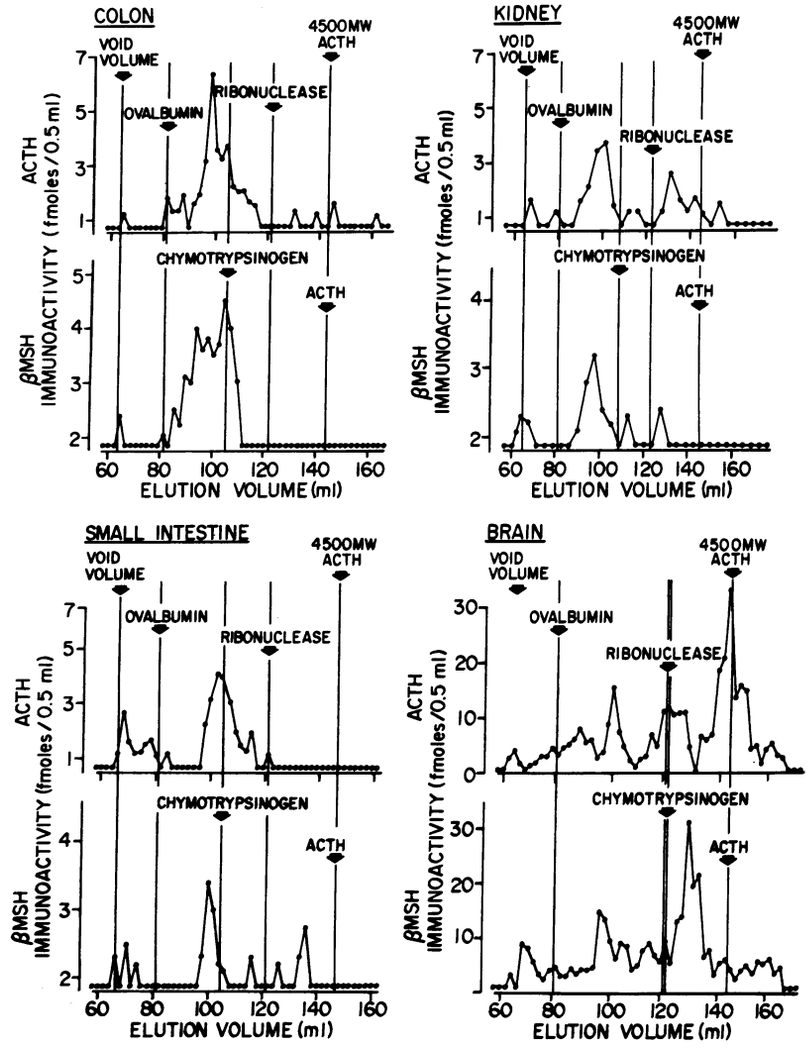

FIG. 5. Sephadex G-75 gel filtration elution profiles of β-MSH and ACTH immunoactivities in rat tissue extracts.

β-MSH and ACTH immunoactivities in several normal rat tissue extracts. With the exception of brain extract, most β-MSH and ACTH immunoactivities were eluted in a single peak corresponding to M_r 26,000. In contrast, the M_rs of the major peaks of β-MSH and ACTH immunoactivities of brain extracts were 8,000 and 4,500, respectively, corresponding to the M_rs of lipotropin and bioactive ACTH. Brain extracts contained only a small peak of β-MSH and ACTH immunoactivities corresponding to a M_r of 26,000.

Anti-ACTH Immunocolumn Studies. Sixty-two percent to 86% (mean ± SEM, 79% ± 4%) of the ACTH immunoactivity of normal rat tissue (excluding brain) extract was removed after passage through anti-ACTH immunocolumns (Table 1). Similar anti-ACTH columns removed 85.2 ± 3.5% (mean ± SEM) of standard ACTH. Between 16.7% and 77.8% (mean, 57 ± 11%) of the β-MSH immunoactivity of normal rat tissue (excluding brain) extract was also removed after passage through anti-ACTH immunocolumns. Such immunocolumns did not adsorb β-MSH standard. About one-third of the β-MSH immunoactivity in brain extracts was adsorbed to anti-ACTH immunocolumns; two-thirds of β-MSH immunoactivity passed through the columns. The mean percentages of β-MSH and ACTH activities bound to the anti-ACTH columns were not significantly different ($P \geq 0.2$).

Con A Column Chromatography. Extracts of small intestine, colon, and kidney showed a small peak of ACTH immunoactivity eluting in the void volume (Fig. 6). More than 80% of the ACTH immunoactivity bound to Con A and was eluted after application of 0.2 M methyl α-D-glucopyranoside. One hundred percent of standard ACTH eluted in the void volume, and >90% of ^{125}I-labeled thyrotropin bound to Con A and eluted after the addition of methyl α-D-glucopyranoside. These two control peptides/proteins showed characteristic elution patterns when applied to the Con A column alone or mixed with tissue extract.

DISCUSSION

In the past 6 years, studies in this laboratory have revealed that extracts of a wide variety of histological types of carcinomas taken from patients without clinical evidence of ectopic hormone production contained large quantities of immunologically active ACTH (7, 8), MSH (12), and human choriogonadotropin (CG) (7, 13). Control studies of normal human tissues indicated that human choriogonadotropin was detectable in all tissues studied (7, 14–16). Later it was demonstrated that extracts of nonendocrine normal human tissues also contained a somatotropin-like material (17).

Concerning the ectopic ACTH syndrome caused by cancer, it was found (7, 8, 12) that extracts of all histological types of carcinomas contained high M_r immunoactive ACTH and β-MSH. Concentrations of ACTH and β-MSH (lipotropin) in extracts of

Table 1. Recovery of ACTH and β-MSH immunoactivities after passage through anti-ACTH immunocolumn

Tissue	ACTH immunoactivity, fmol/0.5 ml			β-MSH immunoactivity, fmol/0.5 ml		
	Anti-ACTH immunocolumn	Control column	% bound to immunocolumn	Anti-ACTH immunocolumn	Control column	% bound to immunocolumn
Liver	1.1	4.2 ± 0.3*	73.8	2.0	2.9 ± 0.4	31.0
Kidney	1.4 ± 0.4	11.3 ± 2.4	87.5	2.0	6.0 ± 0.8	66.7
Stomach	1.9 ± 0.3	12.8 ± 1.3	85.2	2.0	7.3 ± 0.9	72.6
Small intestine	1.7 ± 0.8	9.7 ± 1.3	82.5	2.0	8.7 ± 3.6	77.0
Colon	3.0 ± 0.8	19.4 ± 1.1	84.5	2.0	9.0 ± 3.2	77.8
Heart	1.1	2.9 ± 1.3	62.1	2.0	2.4 ± 0.2	16.7
Brain	8.3 ± 4.6	59.3 ± 9.0	86.0	19.2 ± 2.3	30.4 ± 1.2	36.8

Aliquots (3 ml) of tissue extracts were applied to anti-ACTH immunocolumns and control (normal rabbit serum) columns (0.6 × 7 cm) equilibrated and eluted with the assay buffer. Eluates (4 ml) were subjected directly to ACTH and β-MSH radioimmunoassays. Recoveries (mean ± SEM) of standard ACTH after passage through an anti-ACTH immunocolumn and control column were 14.8 ± 3.5% and 96.5 ± 6.0%, respectively. Recoveries of β-MSH standard after passage through an anti-ACTH immunocolumn and control column were 96.5 ± 6.0% and 99.6 ± 4.1%, respectively.

*Mean ± SEM for triplicate columns.

carcinomas averaged 22,359 and 21,877 pg/g, respectively; extracts of normal human tissues contained <1,000 pg/g. However, because of previous findings that normal tissue contained human choriogonadotropin- and somatotropin-like material, it seemed possible that normal tissues might contain an ACTH-like material in amounts less than 1,000 pg. Because the ACTH immunoassay reacts with rat ACTH as well as human ACTH, we could study this question in an animal model whereas our previous studies were limited to difficult-to-obtain human tissues.

Our most recent studies (9) show that small amounts of ACTH immunoactivity are present in a wide range of normal extrapituitary tissues in rats. This ACTH-like material was shown to have the following characteristics: (*i*) a M_r of ≈26,000; (*ii*) no detectable ACTH biological activity as assessed in a very sensitive *in vitro* bioassay; and (*iii*) conversion to M_r 4,500 immunoactive and bioactive ACTH by trypsin exposure. The present studies show that the same ACTH-like substance also (*iv*) possesses β-MSH immunoactivity and (*v*) is a glycoprotein. All of these characteristics suggest that this normal-tissue ACTH material is similar to the normal pituitary ACTH precursors (ACTH/lipotropin common precursor), as previously described by Eipper and Mains (10).

Although some investigators (18–20) have suggested that experimental artifacts account for the apparent presence of such hormone-like materials in nonendocrine tissue extracts, we do not believe that our data can be so explained. The method of extraction used greatly affects the reliability of the data ob-

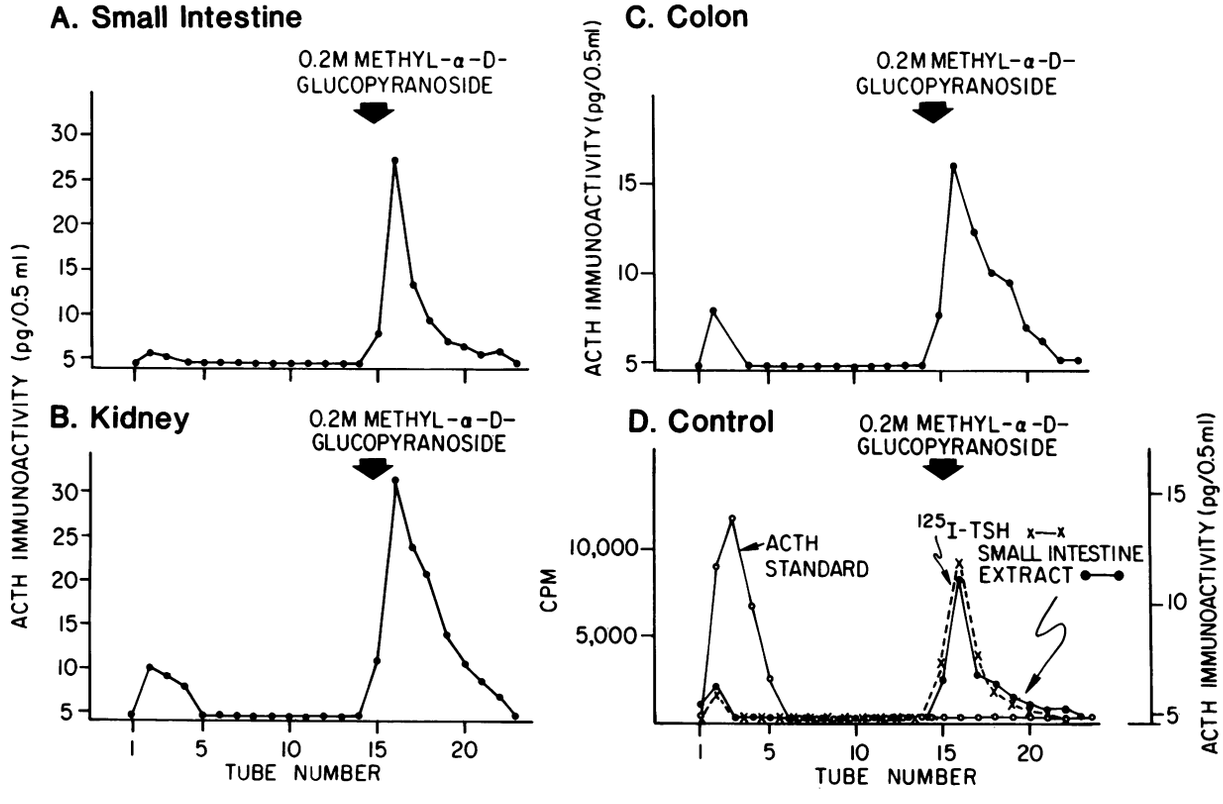

FIG. 6. Con A column chromatography of rat tissue extracts (*A*, *B*, and *C*) and of ACTH standard (a carbohydrate-free peptide) and ^{125}I-labeled thyrotropin (^{125}I-TSH), a glycoprotein, in the presence of small intestine extract (*D*).

tained (9). Furthermore, in our studies of both ACTH (9) and MSH (present data), there was careful control of factors affecting assay results (pH, osmolality, protein concentration, and enzymatic damage to [^{125}I]iodo-β-MSH).

Additionally, in earlier studies, Krieger *et al.* (21–26) and other investigators (27–39) demonstrated that ACTH and related proteins are extractable from the normal placenta and extrapituitary sites of the brain. Larsson (40–42), Orwoll *et al.* (43–45), and Tanaka *et al.* (46) have reported that ACTH immunoactivity is present in extracts of the gastrointestinal tract and pancreas of several species. Furthermore, the ACTH/β-lipotropin precursor is probably present in brain (14), placenta (26), gastrointestinal tract (34), and neoplasms (47, 48).

LeRoith *et al.* (49) demonstrated that extracts of annelid worms contain insulin. Livingston and Livingston (50), Cohen and Strampp (51), and Acevedo *et al.* (52) have demonstrated that a human choriogonadotropin-like material is produced by bacteria, and our studies suggest[§] (53) that it is extractable from fish. LeRoith *et al.* (53) have shown that bioactive ACTH and immunoactive β-endorphin were extractable from protozoa.

The studies showing the widespread presence of protein hormones in all nonendocrine mammalian tissues analyzed and the widespread presence of human choriogonadotropin (50–52, §), insulin (49), and ACTH (53) in lower vertebrates and nonvertebrates have led to the hypothesis that protein hormones are early evolutionary signal systems. It also appears possible that these "hormones" are involved in cell–cell communication at a tissue level in mammals. Furthermore, pertinent to so-called ectopic hormonal productions by cancers, our studies show that, because all tissues probably produce such hormones, "ectopic hormone production" is not ectopic.

[§] Yoshimoto, Y. & Odell, W. D., Sixth International Congress of Endocrinology, Seattle, WA, September, 1980.

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