LTW(m) tumors gained significantly less weight (115 ± 5 g, $P < .01$). Among partners without tumors, there was again no difference in weight gains between controls (166 ± 5 g) and rats in parabiosis with a breast tumor–bearer (175 ± 10 g). Rats in parabiosis with a LTW(m) tumor–bearer gained less weight (141 ± 7 g, $P < .05$). (These data represent weight gained during the entire period of parabiosis. Tumors were present only for the final 46 days of the experiment. Postmortem study of these rats revealed no obvious metastases in either member of any pair.)

The results indicate that the transplantable LTW(m) tumor exhibits characteristics suitable for an animal model of tumor-induced anorexia. It causes decreased food intake in Wistar Furth rats when the tumor first becomes palpable. The effect is reversible on removal of the tumor. The tumor-implanted rats appear healthy and behave normally. They are free of some of the common biochemical derangements associated with cancer, notably hypercalcemia and altered liver function. The anorexia produced by the LTW(m) tumor could result from the release of some as yet unidentified substance by the tumor (*8*). The results of the parabiosis experiment support this concept. Animals in parabiosis with LTW(m)-bearing rats gained less weight than controls in parabiosis with tumorless rats or with rats whose tumors were nonanorexigenic, suggesting that an anorexigenic substance produced by the LTW(m) tumor is circulated into the partner, reducing its appetite and weight gain. Previous data from the study of certain strains of obese mice in parabiosis support the concept of circulatory transfer of an anorexigenic substance, despite the low rate of cross-circulation (*9*).

Many substances, including biogenic amines, steroid hormones, lipids, amino acids, peptides, lactate, and oligonucleotides seem to affect appetite (*1*, *8*). One of these substances could be responsible for tumor-induced anorexia. In particular, tumor-induced anorexia could be caused by a circulating peptide that exerts a depressant effect on appetite. The hypothesis is supported by investigations demonstrating that many peptides affect appetite (*10*). Clinical experience also is consonant with the concept of an anorexigenic peptide, as the tumors often associated with anorexia commonly produce ectopic peptide hormones (*11*). Since another Leydig cell tumor of rats, the Rice D6, produces a parathyroid hormone–like substance and hypercalcemia (*12*), it may be that the LTW(m) tumor is a secretor of peptide hormones. Parathyroid hormone is unlikely to be produced by the LTW(m) tumor, however, because animals with this tumor do not evidence hypercalcemia, hypophosphatemia, or elevated alkaline phosphatase.

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4. Inbred male Wistar Furth rats 8 to 14 weeks of age (Charles River) were used in all the experiments. Cryopreserved tumors were in at least their third serial passage since thawing when used. Donor tissue was taken from rats given tumors 30 to 45 days earlier. Fragments of donor tissue 1 mm^3 in size were implanted subcutaneously with a trocar. The animals were maintained in separate cages on a 12-hour light-dark cycle at a temperature of 22°C. They were given unrestricted access to Purina Rat Chow and water and were weighed three times weekly. Unpaired *t*-tests were used in statistical comparisons involving two groups. In statistical comparisons involving three groups, a one-way analysis of variance and the Newman-Keuls procedure for a posteriori contrasts were used.
5. Blood was obtained by cardiac puncture following ether anesthesia. Chemistries were determined with a Technicon SMA-12 autoanalyzer. Hematocrit, calcium, phosphate, urate, total protein, albumin, bilirubin, and blood urea nitrogen were within normal limits [B. M. Mitroka and H. M. Rawnsley, *Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals* (Masson, New York, 1977), pp. 122–124]. There were no significant differences in serum glutamic-oxaloacetic transaminase or lactic dehydrogenase between the two groups.
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Pentapeptide (Proctolin) Associated with an Identified Neuron

Abstract. *Individual neurons can be recognized and identified anatomically, physiologically, and biochemically in the insect central nervous system. Biochemical analyses of extracts prepared from one such identified neuron show it to be associated with a bioactive pentapeptide called proctolin. This peptide may be a neurotransmitter, and a preparation is established in which its physiological action can be studied at the cellular level.*

Bioactive peptides are found in neurons and may be neurotransmitters (*1*). Little is known of the functions of peptide-containing neurons in the brain because they are difficult to locate, identify and study with single-cell physiological techniques. Our understanding of neuronal function has profited immensely from the study of single identified neurons in simplified systems (*2*). The power of this approach resides in the recognition of individual neurons, which are accessible for repeated study in different individuals of the same species. Physiological information can therefore be accumulated over time, and biochemical studies can be performed on precisely homologous individual neurons collected and pooled from many animals. Although most individually characterized neurons are found in invertebrates, most neuropeptides have been characterized in vertebrates. An exception is a highly bioactive pentapeptide called proctolin (Arg-Tyr-Leu-Pro-Thr) (*3*). It is found in the central nervous system of a number of insect species, but has not yet been associated with specific neurons. We now show evidence that proctolin is associated with an individually identified neuron, and we establish a preparation in which sites and modes of action of a neuropeptide can be studied at the cellular level. The evidence depends on a chromatographic characterization of extracts made from single, individually identified neurons.

Each segmental abdominal ganglion of

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Table 1. Summary of results of thin-layer chromatography of proctolin and LW cell extract. Cellulose plates were run to 15 cm (origin to solvent front) in the solvent systems indicated (*n*But, *n*-butyl alcohol; HAc, acetic acid; HForm, formic acid; MEK, methyl ethyl ketone). Proctolin was detected on the plate by the presence of a single ninhydrin spot. A parallel lane on which LW extract was chromatographed was divided into 15 1-cm fractions, scraped, eluted, and bioassayed. Proctolin and proctolin-like bioactivity always appear in the same 1-cm fraction. In solvent system IV, bioactivity was detected in fraction 7 as well as in fraction 5 (the ninhydrin spot is in fraction 5). Bioactivity in fraction 7 cannot therefore be proctolin, but appears to have a similar bioactivity.

Solvent system			Proctolin	LW extract
I	*n*But:HAc:H_2O	4:1:1	6	6
II	*n*But:HAc:H_2O	4:1:2	9	9
III	*n*But:HForm:H_2O	12:3:5	13	13
IV	MEK:HAc:H_2O	15:5:3	5	5 and 7

the central nervous system of orthopterous insects (cockroaches, locusts, grasshoppers, crickets, and mantids) contains 300 to 500 monopolar neurons. The neuronal cell bodies form a cortex around the central core of neuropil and axon tracts. Some of the cell bodies are particularly conspicuous because they are white and opaque when living ganglia are illuminated against a dark background (Fig. 1A). We have studied one bilateral pair of white neurons that lie ventrolaterally in the abdominal ganglia of cockroaches and crickets. The position and appearance of this pair of neurons are consistent in different individuals of the American cockroach, *Periplaneta americana*, and the European cricket, *Gryllus bimaculatas*. They are repeated serially as a single pair in adjacent abdominal ganglia (*4*). We call these neurons the lateral white (LW) neurons. The LW neuron soma can be distinguished from other cells by its opaqueness and by other criteria; for example, it has a high affinity for the vital dye neutral red (Fig. 1B), which in invertebrates specifically stains monoamine-containing neurons (*5*). Furthermore, the soma contains a singular vacuole-like inclusion that appears to be a specialization of the endoplasmic reticulum. Similar structures are found in some vertebrate neurosecretory neurons (*6*). Intracellular recording and dye-filling of the LW neurons reveal consistent features of anatomy and physiology sufficient to characterize them as identified neurons (*7*).

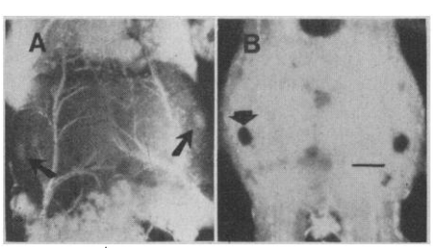

Fig. 1. (A) Lateral white cells as they appear in a living abdominal ganglion of a cockroach. Ventral view, anterior at top. (B) Neutral red staining of the living abdominal ganglion of the cricket. The LW cells (arrow) stain preferentially, suggesting the presence of a biogenic amine. Scale bar, 100 μm.

Proctolin is bioactive on a variety of invertebrate preparations (*8*). We have developed a sensitive and convenient bioassay for proctolin, using a locust leg extensor muscle preparation that generates a heart-like rhythm of myogenic contractions (*9*). The frequency of contractions is increased by as little as $10^{-10}M$ proctolin. Furthermore, the assay can detect proctolin-like bioactivity in small volume (0.5 to 1.0 μl) samples of crude and purified LW cell extracts (Fig. 2A).

Extracts of the LW neuron (*P. americana*) were prepared by dissecting the neuron's cell body from both sides of the living abdominal ganglion under isotonic physiological saline (*10*). The dissected and isolated cells were transferred to 50 μl of 2*N* acetic acid at room temperature. Control cells were removed from regions adjacent to the LW soma and transferred to a second extraction tube. For each extract sample, this procedure was repeated on abdominal ganglia in different individuals, and as many as 50 LW neurons and an equivalent number of control cells were accumulated. Samples were centrifuged, and the supernatant was removed and dried. The dried samples were then dissolved in physiological buffer and bioassayed before and after incubation with proteolytic enzymes. Other samples were taken up into one of a variety of solvents and solvent systems for column, thin-layer, and reverse-phase high-performance liquid chromatography (HPLC) (*11*). Fractions of chromatographed samples were then tested for proctolin-like bioactivity. In each experiment, standards of authentic proctolin or [^{3}H]proctolin were chromatographed under the same conditions (*12*). The presence of proctolin in the eluted fractions was determined either by liquid scintillation counting or bioassay.

The results show that proctolin is specifically associated with extracts of the LW cell and is not found in control cells. Bioactivity of the crude LW cell extract suggests that each cell may contain 0.05 to 0.1 pmole of proctolin (*13*). Individual abdominal ganglia contain 0.3 to 0.8 pmole of proctolin. The bioassay is able to detect proctolin-like bioactivity in a 1-μl portion of dilute extract containing 1 percent of the extract of a single LW cell. Proctolin-like bioactivity is abolished by incubation with leucine aminopeptidase, but is not decreased when crude LW cell extract is incubated with trypsin (*14*). Authentic proctolin is resistant to trypsin, but is destroyed by leucine aminopeptidase. This shows that the bioactive component of the LW cell is a peptide, possibly proctolin. Confir-

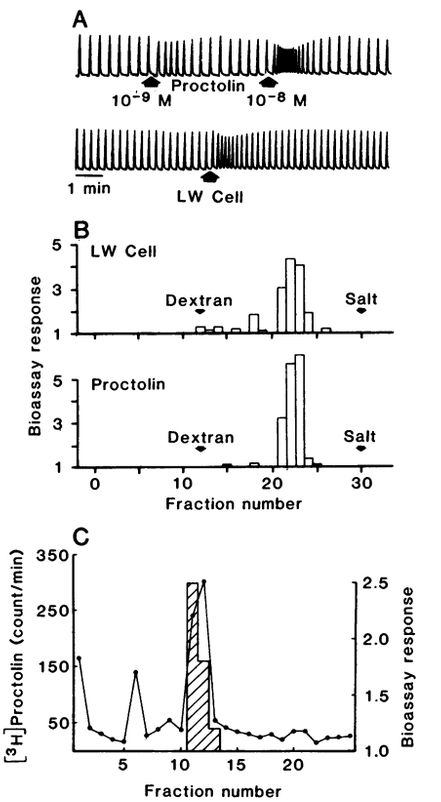

Fig. 2. (A) Proctolin bioassay. Rhythmic contractions of the locust leg muscle are recorded isotonically with a photoelectric movement detector. Arrows indicate the time of application of 1-μl portions of 10^{-9} and $10^{-8}M$ proctolin (upper trace) and a 1-μl portion of LW cell extract containing 1/50th of one cell. The responses are qualitatively similar. (B) Column chromatography (Bio-Gel P2) of LW cell extract and authentic proctolin. The correspondence of elution suggests that the active component of the cell extract has a molecular size similar to that of proctolin (648.8 daltons). (C) Reverse-phase HPLC (C_{18}) of LW cell extract and [^{3}H]proctolin. The peak response for [^{3}H]proctolin and the peak biological activity of the LW cell extract (shaded area) correspond almost exactly. Minor peaks in the chromatographed [^{3}H]proctolin are tritiated impurities.

mation is provided by the results of chromatographic experiments. In these, proctolin-like bioactivity, without exception, elutes with authentic or [^{3}H]proctolin. Molecular sieving (Fig. 2B) shows that the bioactive component has a molecular size indistinguishable from that of proctolin (648.8 daltons). Proctolin-like bioactivity and authentic proctolin could not be separated in thin-layer chromatography with four different solvent systems (Table 1). Moreover, in reverse-phase (C_{18}) HPLC, extract bioactivity, [^{3}H]proctolin, and authentic proctolin were not separated (Fig. 2C).

The LW cell is the first to be associated with proctolin. In view of their accessibility for intracellular electrophysiological studies, the LW neuron and other neurons in the insect central nervous system may provide a simple model in which to investigate the precise sites and modes of action of a putative peptide neurotransmitter. Furthermore, the highly selective staining with neutral red suggests the presence of a biogenic amine (*5*). The LW neuron may, therefore, also provide a preparation in which functional and anatomical implications of amine and peptide co-localization (*15*) can be studied in a well-characterized cellular system.

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10. The isotonic physiological saline contained 140 m*M* NaCl, 5 m*M* KCl, 5 m*M* $CaCl_2$, 4 m*M* $NaHCO_3$, 1 m*M* $MgCl_2$, 5 m*M* trehalose, 5 m*M* trimethylaminoethane sulfonic acid, 100 m*M* sucrose, at *p*H 7.2.
11. Bio-Gel columns were eluted with 10 percent formic acid; thin-layer chromatography plates (cellulose) were divided into 1-cm fractions, scraped, and eluted with 2*N* acetic acid (solvent systems are given in the text). For reverse-phase HPLC a μ-Bondapack C_{18} column (Waters Associates) and a single M45 pump (Waters) were used. The liquid phase was 12 percent acetonitrile in 50 m*M* ammonium acetate, *p*H 4.5, pumped at 2500 pounds per square inch and 1.3 ml/min. Thirty-second fractions were collected.
12. Authentic proctolin was purchased from Sigma. [^{3}H]Proctolin was prepared by catalytic conversion (New England Nuclear) of an I_2-Tyr^2 analog of proctolin synthesized in Prof. T. Kaiser's laboratory in the Department of Chemistry, University of Chicago, by B. DeGrado.
13. This was estimated by comparing the response in the bioassay to known concentrations of authentic proctolin delivered to the same preparation in the same volume. It can be regarded as a low estimate because no account is made of the efficiency of the extraction procedure.
14. Samples were incubated for 2 hours at 37°C with leucine aminopeptidase (0.3 mg/ml). Trypsin was used at 1 mg/ml, and incubations up to 6 hours did not reduce the proctolin-like bioactivity. Enzymes were heat-inactivated before samples were tested in the bioassay.
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Thyroidectomy Increases Rat Hepatic Ferritin Iron

Abstract. *Studies of the hepatic polysome profiles of hypothyroid rats demonstrated contamination of the free monosome peak with ferritin. This led to the serendipitous observation of a fivefold increase in ferritin iron in livers and kidneys of hypothyroid rats.*

Evidence suggests that thyroid hormone (T_3) exerts control over general and specific messenger RNA (mRNA) transcription in the nuclei of responsive cells, an effect mediated by the interaction of T_3 with nuclear receptor sites (*1*). Also, some data suggest that T_3, directly or indirectly, affects the translation of mRNA (*2–6*). To investigate this possibility we isolated and analyzed by sucrose gradient sedimentation free and membrane-bound polysomes from thyroidectomized and euthyroid rats. Our studies appeared to indicate the presence of a large monosome peak in the free ribosomal fraction; however, additional studies described herein indicated that this was an artifact due to contamination with ferritin.

Free and membrane-bound hepatic polysomes were isolated from the livers of Sprague-Dawley rats by the method of Ramsey and Steele (*7*). Fractions of liver homogenate containing either membrane-bound or free polysomes were centrifuged through discontinuous sucrose gradients 1.3*M* and 2.0*M* designed to retard monoribosomes, ribosomal subunits, and other small macromolecules while allowing the polysomes to form pellets (*8*). We then sedimented purified polysomes in linear sucrose density gradients; the polysomes were subsequently fractionated through a continuous ultraviolet (254 nm) absorbance monitor to generate polysome profiles. Representative profiles from the livers of ten thyroidectomized and ten euthyroid rats are shown in Fig. 1, A and D, respectively. Whereas membrane-bound polysomes (not shown) were essentially the same with respect to mean and median polysome size, the free polysomes from thyroidectomized rats showed a large absorbance peak in the 75*S* portion of the gradient; this peak was almost absent in the free polysomes from the euthyroid rats. Daily administration of intraperitoneal T_3 to thyroidectomized rats (20 μg of T_3 per 100 g for 6 days or 300 ng of T_3 per 100 g for 28 days) largely eradicated this abnormal peak (Fig. 1, B and C). Furthermore, whereas a 5-day fast did not produce a large 75*S* peak,

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