treated controls, respectively. Various human fetal cells, including skin and lung fibroblasts, showed lower binding of labeled EGF compared to HEPM cells and were less responsive to EGF. Adult liver and heart cells were able to bind labeled EGF to a similar extent as HEPM cells; however, EGF suppressed their growth and DNA synthesis, and changed their cell shape to a more fibroblastic appearance (not shown). It is noteworthy that a human oral epidermoid carcinoma cell line (CCL 17) (*13*) has an extremely high number of EGF receptors, approximately 60 percent of that found in A431 cells (3×10^6 per cell) (*14*) and that its basal ODC activity is higher than in other cell lines listed in Table 2.

Our data indicate that HEPM cells, compared to various human adult and fetal cells investigated in the present study, are the most sensitive to stimulation by EGF, whereas FGF and insulin at concentrations known to be stimulatory in other cell systems (*15*) do not stimulate HEPM cell growth to a significant extent. Thus, our findings, together with the proposed role of EGF in the control of growth of various target cells (*16*), suggest that HEPM cells are one of the target cells for EGF during development. These results are consistent with our previous studies showing that EGF affects terminal differentiation of the medial epithelial cells in the rodent palate as well as mesenchymal cells of the palatal shelves in organ culture (*3, 17*). We proposed (*18*) the presence of an embryonic form of EGF in the midgestation mouse embryo. It appears that this form of EGF, which may be similar to sarcoma growth factor (*19*), may be important in development of various embryonic and fetal tissues (*20*). Since HEPM cells are derived from the palatal shelves of a human embryo, these cells may be useful for studies on the physiological and biochemical role of hormones and growth factors in the regulation of embryonic and fetal development.

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Tumor-Induced Anorexia in the Wistar Rat

Abstract. *The transplantable Leydig cell tumor of Wistar rats, LTW(m), caused decreased food consumption and weight loss in the host within 2 weeks of implantation. The tumor was small, did not metastasize, and did not affect several parameters of biochemical function. When the tumors were removed, increases in food intake and body weight occurred within 72 hours and were sustained. Reimplantation of tumors caused anorexia to recur. Parabiotic pairs of rats with tumor in one partner also experienced weight loss. Those rats in parabiosis with tumor-bearing rats gained less weight than those in parabiosis with control rats. These observations suggest that the LTW(m) tumor causes anorexia and that this anorexia is mediated by a circulating substance.*

Tumor-induced anorexia is of major concern to physicians, as it often leads to the development of fatal cachexia (*1*). Its cause is unknown. Research has been hampered by the difficulty of human experimentation and the lack of well-characterized animal models. With humans there often is variability in the presentation of the syndrome, lack of tissue for study, and confounding of data by therapy. An animal model of tumor-induced anorexia should display several features. The tumors should be small and not metastasize. They must not cause structural or biochemical derangements that could produce nonspecific loss of appetite. Ideally, long-term survival of the animal should be possible, and loss of appetite should be reversible on removal of the neoplasm. Tumors used in previous animal studies of tumor-induced anorexia (*2*) did not meet these criteria. We report a rodent tumor system that appears suitable for the study of anorexia.

To evaluate tumors that might provide models of cancer anorexia, we studied the records of the tumor bank maintained by the Mason Research Institute (*3*). One tumor that appeared to arrest weight gain despite its small size was the Leydig cell tumor of Wistar rats, LTW(m). For comparison, a tumor of the Wistar rat not associated with weight loss, the breast carcinosarcoma MT/W9a-B, was also selected for study.

The first experiment was performed to study the effect of these tumors on the weight of male rats (*4*). Beginning 18 days after implantation there was an obvious arrest of weight gain in the rats with LTW(m) tumor, while the rats with breast tumor were relatively unaffected. Between days 18 and 35, seven control rats gained 26 ± 2 g (mean ± standard error); seven breast tumor–bearers gained 34 ± 4 g; and eight LTW(m) tumor–bearers lost 2 ± 2 g ($P < .001$). When the rats were killed the LTW(m) tumors weighed 1.5 ± 1.2 g and the breast tumors weighed 6.3 ± 10.1 g. In another experiment, eight control rats gained an average of 57 g while eight tumor-bearing rats gained only 9 g over 28 days ($P < .01$). Final tumor weight was 1.0 ± 0.1 g. A biochemical profile (*5*) revealed no major differences between normal and tumor-bearing rats except for significantly lower alkaline phosphatase in tumor bearers (141 ± 82 versus 241 ± 31 mU/ml). Glucose levels in both groups were slightly elevated, probably due to the ether used to anesthetize the rats. All animals were sub-

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jected to necropsy and histological study (6), which disclosed no metastases.

The next experiment was performed to study food intake and the reversibility of the phenomenon. Reduction in weight gain was again found to occur about 2 weeks after tumor implantation (Fig. 1). Removal of the tumor caused reversal of weight loss beginning within 3 days of surgery. Recovery occurred whether the tumors were removed after 26 or 43 days. When the tumor was reimplanted into those animals that had resumed weight gain following resection, anorexia and weight loss recurred. The alterations in weight associated with implantation and removal of the tumor were accompanied by parallel alterations in food intake.

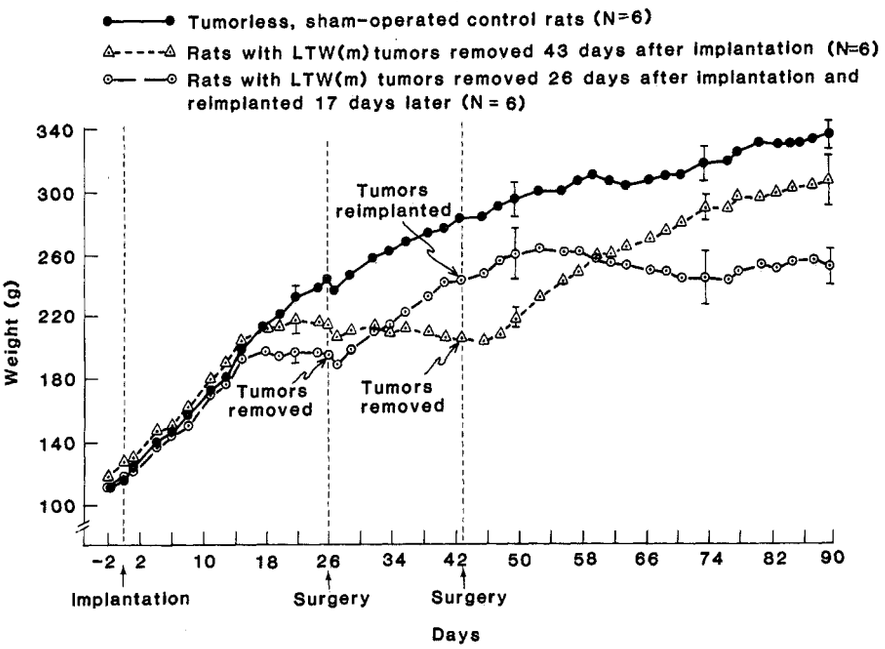

Fig. 1. Mean body weight of male Wistar Furth rats following implantation and removal of LTW(m) tumors. On day 0, two groups of six rats were implanted with tumors. Six controls received sham implants. On day 26, six of the tumor-bearing animals were anesthetized with ether and their tumors were surgically removed. The other 12 rats underwent sham surgery. On day 43 the remaining six tumor-bearing animals had their tumors removed, while the six that had undergone tumor removal on day 26 were reimplanted with LTW(m) tumors. Body weights include the weight of the tumor.

In a final experiment, 22 parabiotic pairs of rats were formed (7) and, 6 weeks later, the LTW(m) tumor or the MT/W9a-B tumor was implanted into one member of each pair. Additional pairs received sham implants. Both the weight of the parabiotic pairs and the weight gain of the individual members were measured. When a tumor was implanted subcutaneously into one member of a parabiotic pair, the weight gain of the paired rats was analogous to that observed in single tumor-bearing rats (Fig. 2A). Within 16 to 18 days the rate of weight gain slowed, and eventually the weight of the pairs declined. In contrast, MT/W9a-B tumors did not arrest the weight gain of parabiotic rats.

Separation of the pairs at the end of the experiment permitted measurement of the weight gain of each partner from the time parabiosis was established to the time of slaughter (Fig. 2B). Weight gain of rats bearing breast tumor (164 ± 13 g) was not significantly different from the weight gain of animals with sham implants (178 ± 8 g). Animals with

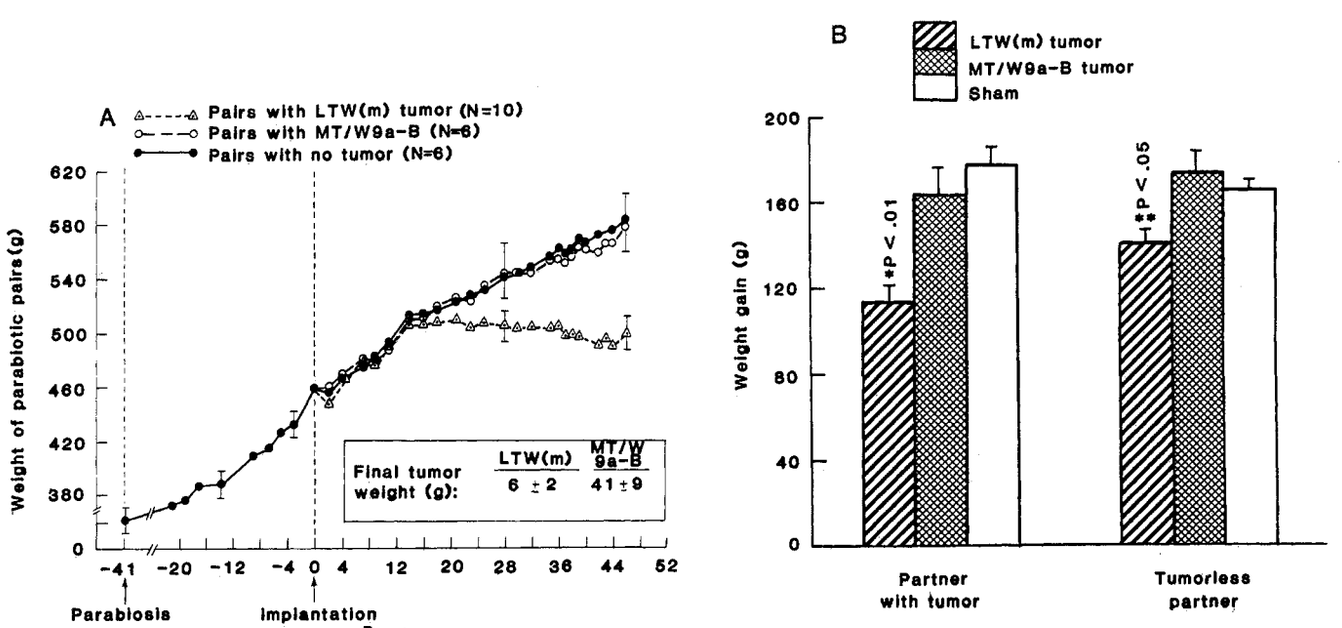

Fig. 2. (A) Mean body weight of male Wistar Furth rats in parabiosis, with LTW(m) or MT/W9a-B tumor in one partner or no tumor in either partner. Body weights include the weight of the tumor. Each rat was weighed just before parabiosis surgery. Following surgery, the pairs were allowed to heal for approximately 6 weeks before tumor implantation. Such rats cross-circulate 1 to 2 percent of their blood volume per hour. On day 0, LTW(m) tumor was implanted subcutaneously into one partner in ten pairs. MT/W9a-B tumor was implanted into six pairs, and six pairs received no tumor. On day 46 all pairs were killed and separated, and each rat was weighed. (B) Mean weight gain of individual rats in the parabiotic pairs. Weight gains exclude the weight of the tumor. Data are for ten pairs with LTW(m) tumor, six pairs with MT/W9a-B tumor, and six pairs without tumor.

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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