tional Accelerator Laboratory), J. P. Hartnett (University of Illinois), A. H. Kassof (International Research and Exchanges Board, New York), M. F. Hawthorne (University of California, Los Angeles), N. Holonyak, Jr. (University of Illinois), I. M. London (Harvard University and Massachusetts Institute of Technology), I. J. Bennett (New York University), E. Ginzton (Varian Associates), L. Knopoff (University of California, Los Angeles), S. Udenfriend (Roche Institute), and, ex officio, D. Pines (University of Illinois), R. Roy (Pennsylvania State University), and A. Rich (Massachusetts Institute of Technology). C. Trumbull of NAS provided staff support.

7. The author of this article served as rapporteur for the Kaysen study, and was involved in several of the other recent evaluations, but the analysis presented here was written after the publication of the reports and is entirely a private, individual endeavor.
8. See *Background Materials on U.S.-U.S.S.R. Cooperative Agreements in Science and Technology* (Committee on Science and Technology, U.S. House of Representatives, Washington, D.C., 1975). There were 13 agreements earlier, but in 1977 the agreement on artificial heart research and development was combined with the agreement on medical science and public health.
9. For further information contact the Commission on International Relations, National Academy of Sciences, 2101 Constitution Avenue, NW, Washington, D.C. 20418. Identities of respondents, unknown to the administrators of the poll, cannot, of course, be supplied.
10. In 1963 a Louis Harris national poll indicated that only 34 percent of the American public favored exchanges of scientists and engineers with the Soviet Union and 54 percent opposed them. These data are too old, however, to be helpful today; furthermore, we do not know why such a large percentage was opposed to exchanges (for example, low opinion of Soviet science, or fear of giving away American scientific knowledge) [see R. F. Byrnes (*4*, p. 47)].
11. See, for example, *An Analysis of Export Control of U.S. Technology—A DOD Perspective* (Office of the Director of Defense Research and Engineering, Washington, D.C., 1976), and *U.S.-Soviet Commercial Relations: The Interplay of Economics, Technology Transfer, and Diplomacy* [Committee on Foreign Affairs, U.S. House of Representatives (prepared by J. P. Hardt and G. D. Holliday), Washington, D.C., 1973].
12. See Report No. 6 (*5*). Reports on progress in the bilateral agreements were given 13 February 1978 at the American Association for the Advancement of Science meeting in Washington, D.C. See *Abstracts of Papers of the 144th National Meeting* (AAAS Publication 78-2, Washington, D.C., 1978), pp. 96–97. Other reports on the bilateral agreements were presented at the International Studies Association Meeting in Washington, D.C., on 25 February 1978. See *19th Annual Convention Program* (International Studies Association, Washington, D.C., 1978), p. 231.
13. The National Science Board policy has been interpreted as prohibiting collaborative research on the interacademy program, but not joint symposia. Many joint symposia have been held in fields such as condensed matter, radio astronomy, partial differential equations, biological pyridoxal catalysis, nucleic acids, biological membranes, and protein chemistry. One of the most successful symposia series is described in C. Herring and D. Pines, "Theory of condensed matter; the joint symposia," *Phys. Today* **28**, 46 (November 1975).
14. This work was conducted while the author was professor of history at Columbia University.

Peptides in the Brain: The New Endocrinology of the Neuron

Roger Guillemin

Brain Peptides Controlling Adenohypophysial Functions

In the early 1950's, data from several groups in the United States and Europe showed that the endocrine secretions of the anterior lobe of the hypophysis—well known by then to control all the functions of all the target endocrine glands (thyroid, gonads, adrenal cortex) plus the overall somatic growth of the individual—were regulated by some integrative mechanism located in neuronal elements of the ventral hypothalamus (*1*). Because of the peculiar anatomy of the junctional region between ventral hypothalamus (floor of the third ventricle) and the parenchymal tissue of the anterior lobe of the pituitary (Fig. 1), the mechanisms involved in this hypothalamic control of adenohypophysial functions were best explained by proposing the secretion of products from (uncharacterized) neuronal elements of the ventral hypothalamus. Such products would somehow reach the adenohypophysis by way of the capillary vessels that appeared to join the floor of the hypothalamus to the pituitary gland. The concept of neurosecretion, or the ability of some hypothalamic neurons to secrete proteins related to the posterior pituitary hormones, had been proposed earlier by E. Scharrer and B. Scharrer (*2*).

The concept of a humoral hypothalamic control of adenohypophysial functions was ascertained by means of simple experiments with combined tissue cultures of fragments of the pituitary gland and of the ventral hypothalamus (*3*). Attempts to characterize the hypothetical hypothalamic hypophysiotropic factors started then. Simple reasoning and early chemical confirmation led to the hypothesis that these unknown substances would be small peptides. After several years of studies in several laboratories in the United States, Europe, and Japan, it became clear that characterizing these substances would be a challenge of originally unsuspected proportions. Entirely novel bioassays would have to be devised for routine testing of a large number of fractions generated by the chemical purification schemes, and enormous amounts of hypothalamic fragments (from slaughterhouse animals) would have to be obtained if we were to have available a sufficient quantity of starting material to attempt a meaningful program of chemical isolation. The early pilot studies had indeed shown the hypothalamic substances to be extremely potent and, on the basis of simple assumptions, to be present in each hypothalamic fragment only in a few nanogram quantities.

Essentially one, then two groups of investigators approached the problem with enough constancy and resolution to stay with it for the 10 years that it took to provide the first definitive solution, that is, the primary structure of one of the hypothalamic hypophysiotropic factors. My own group, then at Baylor College of Medicine in Houston, Texas (with an episode at the Collège de France in Paris), organized the collection over several years of more than 5 million sheep brains, handling in the laboratory more than 50 tons of hypothalamic fragments. Schally and his collaborators, after he had left my laboratory at Baylor, collected also very large numbers of porcine hypothalamic fragments. Late in 1968, from 300,000 sheep hypothalami, Burgus and I isolated 1.0 milligram of the first of these hypothalamic hypophysiotropic peptides, the thyrotropin-releasing factor (TRF), the molecule by which the hypothalamus regulates through the pi-

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tuitary the functions of the thyroid gland (*4*).

The following year, after overcoming more technical difficulties, we established the primary structure of ovine TRF by mass spectrometry as that of the deceivingly simple tripeptide (pyro)Glu-His-Pro-NH_2. The material of porcine origin was shown by Schally and his collaborators to be identical. The synthetic replicate, rapidly available in unlimited quantities, was shown to be highly potent in all vertebrate species and particularly in man; it is now widely used throughout the world in a highly sensitive test of pituitary function and as an early means of detection of pituitary tumors in man.

The isolation and characterization of TRF was the turning point which separated doubt—and often confusion—from unquestionable knowledge in this field. It was of such heuristic significance that I can say that neuroendocrinology became an established science on that event.

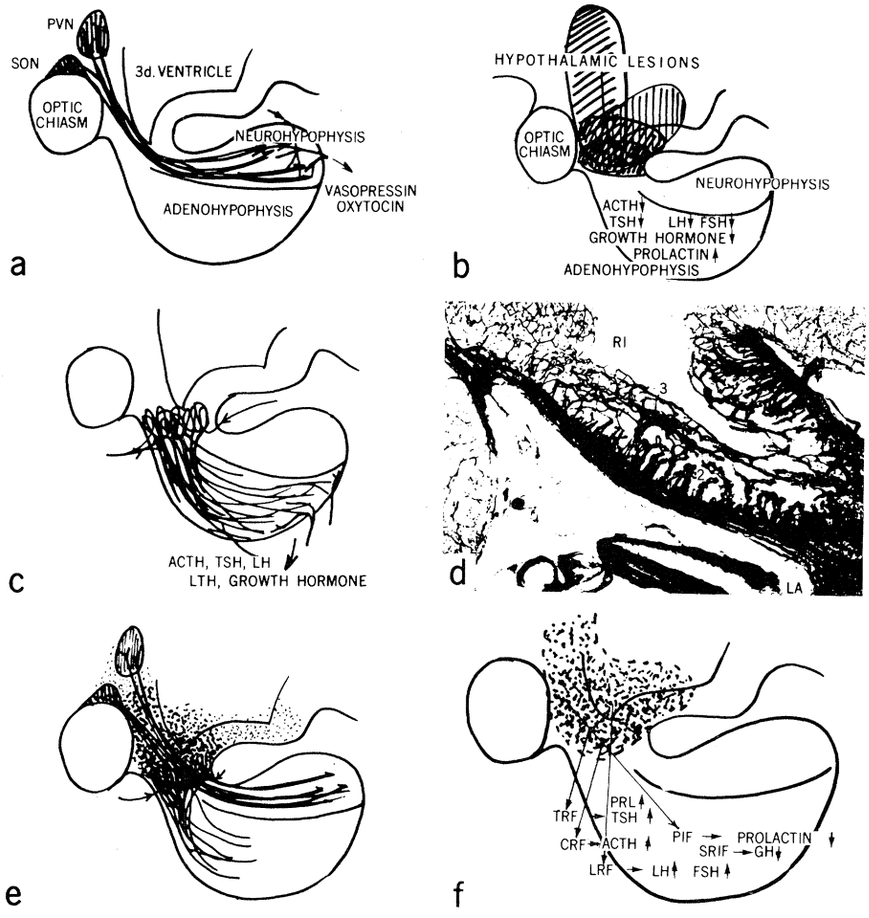

Fig. 1. (a) Diagrammatic representation of the pituitary gland and the innervation of the neurohypophysis by nerve fibers from the nucleus paraventricularis (*PVN*) and supra-opticus (*SON*). (b) Localized lesions in the hypothalamus produce changes in the pituitary secretion of the various adenohypophysial hormones (increase ↑, or decrease ↓). (c) Diagrammatic representation of the hypothalamo-hypophysial portal system. (d) Photomicrograph of the hypothalamo-hypophysial portal system after injection with an opaque dye. (e) Diagrammatic representation of the hypophysiotropic area. (f) Changes in pituitary secretion of various adenohypophysial hormones (increase ↑, or decrease ↓).

Purification, Isolation, and Characterization of TRF

The characterization of the molecular structure of TRF was achieved in an unconventional manner (*5*). In January 1969, with the latest supply of highly purified ovine TRF available—1.0 mg obtained from 300,000 sheep hypothalamus fragments—amino acid analysis of hydrolyzates of this preparation in 6*N* hydrochloric acid revealed only the amino acids, glutamic acid (Glu), histidine (His), and proline (Pro), in equimolar ratios and accounting in weight for 81 percent of the preparation [theoretical contribution for a tripeptide monoacetate is 86 percent (*6*)]. Furthermore, the ultraviolet, infrared, and nuclear magnetic resonance spectra obtained with that preparation of TRF were consistent with those of a polypeptide and, upon close examination, most of the characteristics of those spectra could be accounted for by the structural features of the amino acids found in the hydrolyzates of TRF. The solubility properties and the lack of volatility were consistent with those of a polypeptide; also, the lack of effect of classical proteolytic enzymes could be related to the particular amino acids observed. With the analyses of the more highly purified material unmistakably showing the amino acids to account for the total weight of the preparation, an earlier hypothesis that TRF could be a heteromeric polypeptide was therefore abandoned in favor of the possibility that it might be a cyclic or a protected peptide, a view compatible with failure to detect an NH_2-terminus (*6–12*) or a COOH-terminus (*5, 11*), and with resistance of the biological activity to proteases.

We then reexamined, as Schally had done in 1968, derivatives of synthetic polypeptides containing equimolar ratios of these amino acids with a view to finding possible models for the methodology to be used in the characterization of ovine TRF. We tested for TRF activity six tripeptide isomers containing L-His, L-Pro, and L-Glu synthesized upon our request by Gillessen *et al.* (*13*) (containing only the peptides involving the α-carboxyl group of glutamic acid). The tripeptides proved to be devoid of TRF activity, confirming the earlier results of Schally *et al.* (*10, 11*). Our response to these negative results differed, however, from that of Schally *et al.* (*11*), in that I proposed treating each of the six tripeptides with acetic anhydride in an effort to protect the NH_2-terminus as in natural TRF.

The acetylation mixture from one, and only one of the peptides, namely H-Glu-His-Pro-OH, yielded biological activity qualitatively indistinguishable from that of natural TRF. It was active in vivo and in vitro in assays specific for TRF, and its action in vivo was blocked by prior injection of the animals with thyroxine (*14*). The specific activity of the material obtained was lower (about 1×10^{-3}) than that of purified natural TRF. The nature of several possible reaction products was considered: mono- or diacetyl derivatives, polymers of Glu-His-Pro, and cyclic peptide derivatives, or derivatives containing pyroglutamic acid [(pyro)Glu] as the NH_2-terminus. Subsequently we reported (*14, 15*) that the major product by weight of this procedure was indeed (pyro)Glu-His-Pro-OH. The material was isolated from the reaction mixture and its structure was confirmed by mass

spectrometry of the methyl ester, by comparing it with authentic (pyro)Glu-His-Pro-OH (*13*), by thin-layer chromatography and infrared spectroscopy and by its intrinsic biological activity in vivo.

This was the first demonstration of a fully characterized synthetic molecule, on the basis of the known composition of natural TRF, reproducing the biological activity of a hypothalamic releasing factor.

Because of the differences between the specific biological activities of (pyro)Glu-His-Pro-OH and natural ovine TRF and the different behavior of these two compounds in various chromatographic systems, it was evident that TRF was not (pyro)Glu-His-Pro-OH as such. From knowledge of the primary structures of other biologically active polypeptides (vasopressins, oxytocin, gastrins, for example), Burgus proposed that a likely candidate for the structure of the natural material would be (pyro)Glu-His-Pro-NH_2, and synthesis of this material was approached through the simple procedure of methanolysis of the methyl ester, (pyro)Glu-His-Pro-OMe (*15–17*).

The ester, prepared by treatment of the pure synthetic (pyro)Glu-His-Pro-OH with methanolic HCl, was purified by partition chromatography and identified as (pyro)Glu-His-Pro-OMe on the basis of its behavior on thin-layer chromatography, its infrared spectrum, and by mass spectrometry (*16*, *17*). Its biological activity in vitro and in vivo now approached half of the specific activity of isolated ovine TRF. Ammonolysis of the methyl ester in methanol produced a material which upon partition chromatography gave a small yield of a substance, presumably (pyro)Glu-His-Pro-NH_2, occurring in a Pauly-positive zone separated from the starting material, which had a specific activity in vivo or in vitro statistically identical to that of ovine TRF. Among the derivatives tested, the properties of native ovine TRF most closely matched that of the amide, failing to separate from the synthetic compound in four different systems of thin-layer chromatography when run in mixtures. The infrared spectra of several of the more highly purified preparations of the amide, including (pyro)Glu-His-Pro-NH_2 now prepared by total synthesis (*13*), were almost identical to that of ovine TRF, showing only minor differences in two regions of the spectra. These new observations, together with the demonstration that the specific activity of (pyro)Glu-His-Pro-NH_2 was not statistically different from that of natural ovine TRF, led us to reconsider (*16*, *18*) an earlier hypothesis (*15*) that ovine TRF may have a secondary or tertiary amide on the COOH-terminal proline, rather than correspond to the primary amide of the tripeptide (pyro)Glu-His-Pro.

Availability of large amounts of the synthetic tripeptides made possible a series of experiments with Desiderio, of Horning's group at Baylor, to modify the design of the direct probe of the then available low resolution mass spectrometer and simultaneously to obtain volatile derivatives of the peptides that would give clear mass spectra on only a few micrograms of the peptides. Once this was achieved, we obtained evidence that the native ovine TRF preparation originally obtained in late 1968 was essentially homogeneous and had unquestionably the structure (pyro)Glu-His-Pro-NH_2. Both synthetic (pyro)Glu-His-Pro-NH_2 (*16–18*) and the highly purified ovine TRF (*6*) were introduced by direct probe into a low resolution mass spectrometer as the methyl or trifluoroacetyl derivatives (Fig. 2). All preparations gave volatile materials in the temperature range of 150° to 200°C ($\leq 10^{-6}$ torr). Several mass spectra taken throughout the range of the thermal gradient (seven in the case of the isolated ovine TRF) showed fragmentation patterns corresponding to a single component. Although none of the spectra revealed a molecular ion, fragments arising from the structures (pyro)Glu, methyl-(pyro)Glu, His, methyl-His, Pro, Pro-NH_2, $CONH_2$, (pyro)Glu-His, and His-Pro-NH_2 were observed. The low resolution mass spectra of the corresponding derivatives of synthethic (pyro)Glu-His-Pro-NH_2 and TRF were essentially identical. Fragments arising from unsubstituted (pyro)Glu or His were observed in the spectra of both types of derivatives.

The elemental compositions of these fragments were confirmed by high resolution mass spectroscopy of the methyl derivatives (*16*, *18*).

Thus, the structure of ovine TRF as isolated from the hypothalamus was established as (pyro)Glu-His-Pro-NH_2 (Fig. 3). However, we did point out (*18*) the possibility that, as opposed to the isolated material, the native molecule of TRF might occur as Gln-His-Pro-NH_2

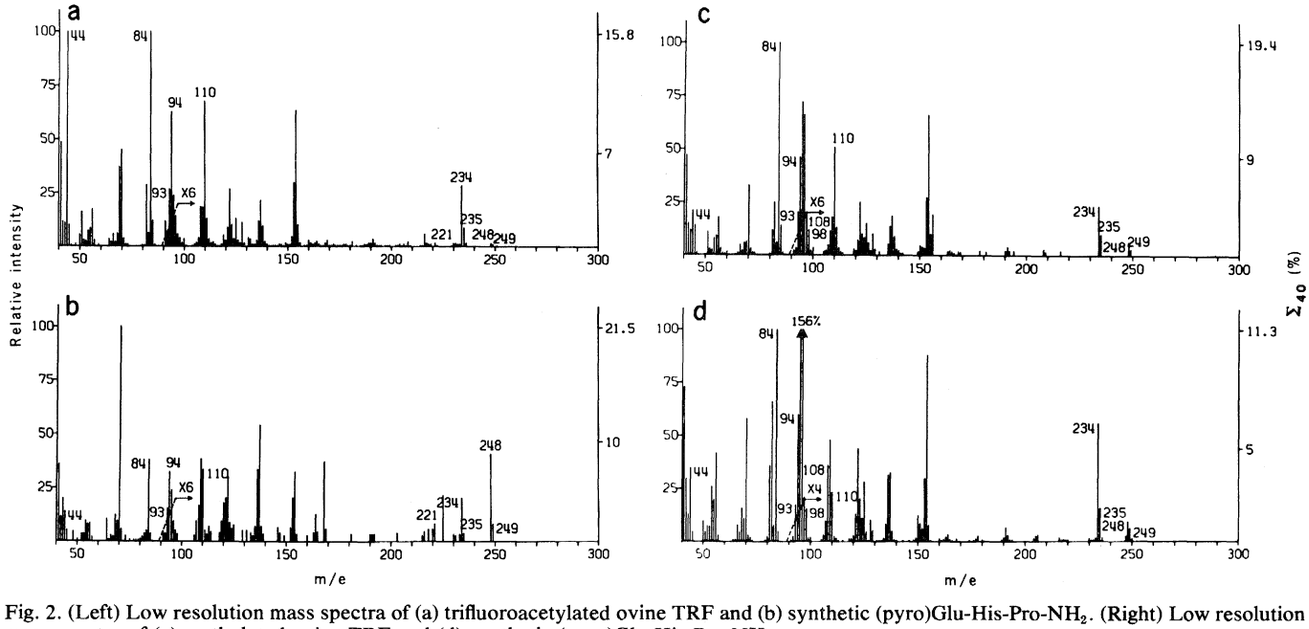

Fig. 2. (Left) Low resolution mass spectra of (a) trifluoroacetylated ovine TRF and (b) synthetic (pyro)Glu-His-Pro-NH_2. (Right) Low resolution mass spectra of (c) methylated ovine TRF and (d) synthetic (pyro)Glu-His-Pro-NH_2.

(where Gln is glutaminyl) either free or conjugated to another structure such as a protein, which would not be necessary for biological activity in vivo or in vitro. We and others are still looking for a hypothetical prohormone of TRF.

The structure of porcine TRF was shown by Schally and his collaborators to be identical with that of (pyro)Glu-His-Pro-NH_2; mass spectrometry was also the method of ultimate proof used by Nair *et al.* (*19*).

It was rapidly shown that TRF shows no evidence of species specificity for its biological actions, (pyro)Glu-His-Pro-NH_2 being readily active in humans (*20*, *21*).

Purification, Isolation, and Characterization of LRF

In the early 1960's, several investigators reported experimental results that were best explained by proposing that crude aqueous extracts of hypothalamic tissues contained substances that specifically stimulated the secretion of luteinizing hormone (LH), and that were probably polypeptides (*22–24*). The active substance was named LH-releasing factor or LRF. Preparations of LRF, active at 1 microgram per dose in animal bioassays, were soon obtained by gel filtration and ion-exchange chromatography on carboxymethylcellulose (*11*, *25*). In spite of the vagaries of the various bioassay methods available, several laboratories reported preparations of LRF of increased potency. Several of these early reports, however, contained contradictory statements regarding purification and separation of LRF from a follicle-stimulating hormone (FSH) releasing factor (*11*, *25–27*).

Two laboratories independently reported in 1971 the isolation of porcine LRF (*28*) and ovine LRF (*29*), both groups concluding that LRF from either species was a nonapeptide containing, on the basis of acid hydrolysis, His, 1; Arg, 1; Ser, 1; Glu, 1; Pro, 1; Gly 2; Leu, 1; Tyr, 1. Earlier results with the pyrrolidone-carboxylpeptidase prepared by Fellows and Mudge (*30*) had led us to conclude (*31*) that the NH_2-terminal residue of LRF was Glu in its cyclized pyroglutamic [(pyro)Glu] form, as in the case of hypothalamic TRF [(pyro)Glu-His-Pro-NH_2]. The total amount of the highly purified ovine LRF that we had isolated from side fractions of the TRF program and that was available for amino acid sequencing was about 80 nanomoles (as measured by quantitative dansylation).

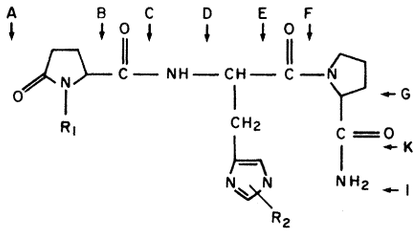

Fig. 3. The primary structure of TRF (ovine) with the fragmentation points of mass spectrometry indicated (*A* to *G*). R_1 and R_2 represent the methyl derivative prepared for mass spectrometry; in the native molecule $R_1 = R_2 = H$.

It is to the credit of Schally's group that porcine LRF was first reported (*32*) to contain one residue of tryptophan (Trp) in addition to the other amino acids earlier observed by acid hydrolysis. On the basis of experiments conducted on about 200 nmole of peptide and which included enzymatic hydrolysis with chymotrypsin and thermolysin, and analysis of the partial sequences of their decapeptide by Edman degradation-dansylation and selective tritiation of COOH-terminals, Matsuo *et al.* (*33*) proposed the sequence (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 for porcine LRF as that best compatible with the partial sequence data. They also stated that synthesis of that particular sequence had given a material with biological activity. A few weeks later, we reported the synthesis by solid-phase methods of the decapeptide (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2; after isolation from the reaction mixture it had quantitatively the full biological activity in vivo and in vitro of ovine LRF (*34*).

Shortly thereafter, we reported (*35*) the amino acid sequence of ovine LRF obtained on 40 nmole of peptide by analysis of hydrolysis products after digestion with chymotrypsin or pyrrolidone-carboxylpeptidase. For this analysis we used Edman degradation followed by determination of NH_2- and COOH-terminals by a quantitative ^{14}C-dansylation technique. Confirmation of the positions of some of the amino acid residues obtained by combined gas chromatographic–mass spectrometric analysis of phenylthiohydantoin (PTH) derivatives (*36*, *37*) resulting from Edman degradations was described. We also reported results obtained by degradation of the synthetic decapeptide, since they confirmed and clarified some peculiarities observed upon enzymatic cleavage of the native peptide (*38*).

The amino acid sequence of ovine LRF was found to be identical to that of the material of porcine origin.

Of considerable interest was the observation that the synthetic replicate of LRF, now available in large quantities, stimulated the secretion of both LH and FSH in vivo and in vitro. This confirmed the earlier results obtained with the minute quantities of the isolated ovine or porcine LRF (*28*, *29*) and cast doubt on earlier reports (*11*, *39*, *40*) that LRF free of FSH-releasing activity had been obtained.

There is now no solid evidence of an FSH-releasing factor existing as a specific entity, discrete from the decapeptide LRF [but see (*41*)]. All analogs of LRF that have been synthesized release LH and FSH with the same ratio of specific activity when this is related to the activity of LRF in the particular assay involved. Also, the two gonadotropins (LH and FSH) occur primarily in the same secretory granules of the same pituitary cell (*42*).

Later on, Schally's group (*43*) and our group (*44*) confirmed the primary structure of, respectively, porcine and ovine LRF, using larger quantities of native material.

Purification, Isolation, and Characterization of Somatostatin

Although it has been generally accepted that control of the pituitary secretion of growth hormone (GH) is exerted by a hypothalamic hypophysiotropic releasing factor, no such factor has yet been isolated. The "GH-RH" characterized by Schally *et al.* (*45*) as H-Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala-OH (where Val is valine; Leu, leucine; Ser, serine; Ala, alanine; and Lys, lysine), was found to be a decapeptide fragment of the NH_2-terminal of the β-chain of porcine hemoglobin (*46*) that was inactive in stimulating secretion of immunoreactive GH, as was a tetrapeptide reported by Yudaev *et al.* (*47*).

In searching for this still hypothetical somatotropin-releasing factor in the crude hypothalamic extracts used in the isolation of TRF and LRF, we regularly observed that their addition in minute doses ($\leq$ .001 of a hypothalamic fragment equivalent) to the incubation fluid of dispersed rat pituitary cells in monolayer cultures (*48*) significantly decreased the resting secretion of immunoreactive GH by the pituitary cells. This inhibition was related to the dose of hypothalamic extract added and appeared to be specific. It was not produced by similar extracts of cerebellum, and the crude hypothalamic extracts that inhibited secretion of GH simultaneously

stimulated secretion of LH and thyrotropin. The inhibition of GH secretion could not be duplicated by addition to the assay system of Arg^8-vasopressin, oxytocin, histamine, various polyamines, serotonin, catecholamines, LRF, or TRF. We decided to attribute this inhibitory effect on the secretion of GH to a somatotropin release inhibiting factor, which we later named somatostatin.

Inhibition of secretion of GH by crude hypothalamic preparations had been reported by others (*49*), but the active factor had not been characterized. Starting with the chloroform-methanol-glacial acetic acid extract of about 500,000 sheep hypothalamic fragments (*35*, *38*) that we had used in characterizing the gonadotropin releasing factor, we attempted to isolate the factor that inhibited somatotropin release. The extract (2 kilograms) had been partitioned in two systems; the LRF concentrate was subjected to ion-exchange chromatography on carboxymethylcellulose. At that stage, a fraction with GH release inhibiting activity was observed well separated from the LRF zone; it was further purified by gel filtration (Sephadex G-25) and liquid partition chromatography (*n*-butanol, acetic acid, and water, 4:1:5 by volume). Thin-layer chromatography and electrophoresis of the final product showed only traces of peptide impurities. the yield was 8.5 mg of a product containing 75 percent of amino acids by weight; we will refer to this material by the name somatostatin which was actually given to it only after it had been fully characterized.

Analysis of amino acids obtained from somatostatin after acid hydrolysis in 6*N* HCl–0.5 percent thioglycollic acid gave the molar ratios Ala, 0.9; Gly, 1.1; Cys (cysteine), 0.2; Cys-SS-Cys, 1.0; Lys,

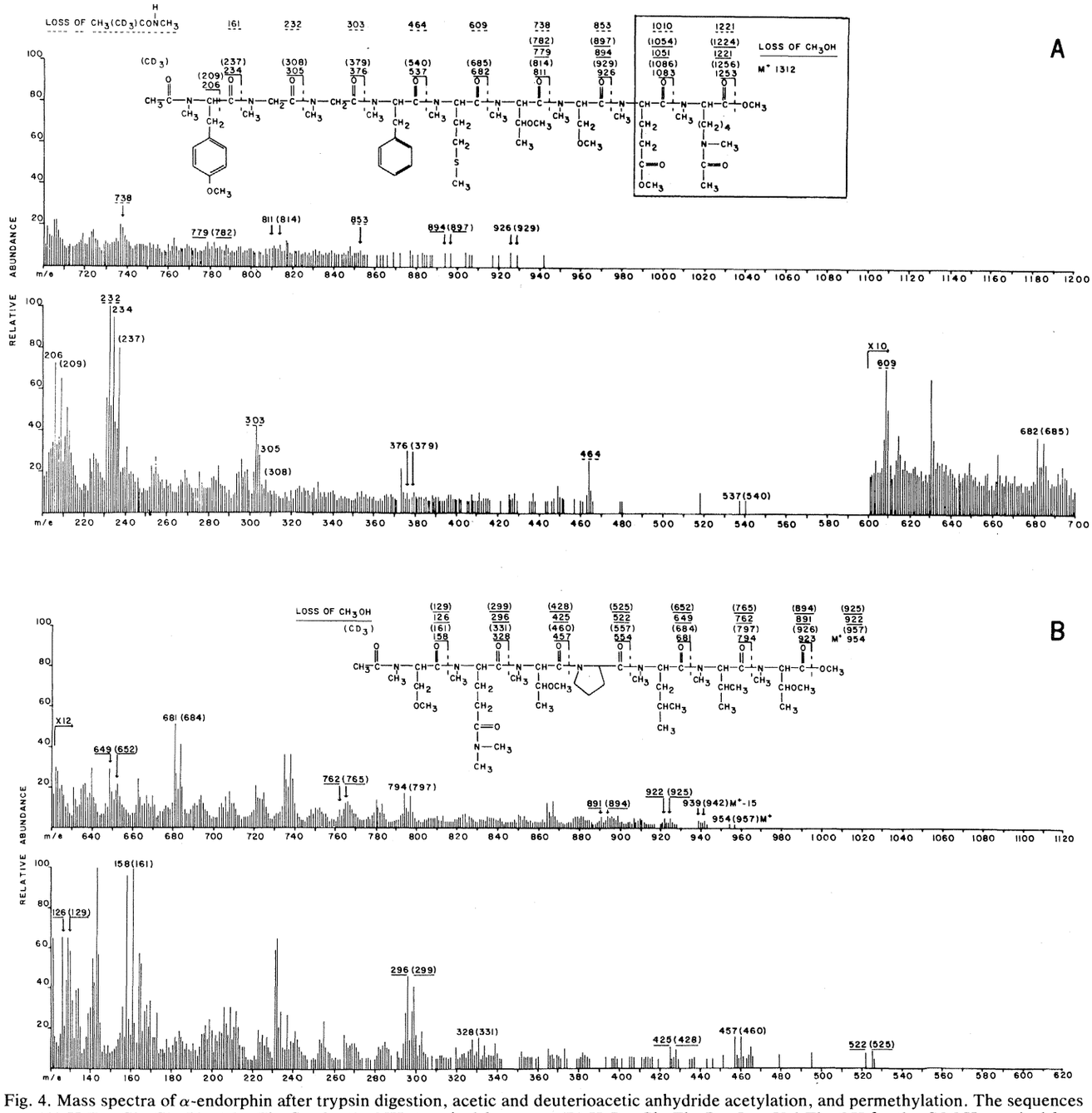

Fig. 4. Mass spectra of α-endorphin after trypsin digestion, acetic and deuterioacetic anhydride acetylation, and permethylation. The sequences are: (A) H-Tyr-Gly-Gly-Phe-Met-Thr-Ser for the NH_2-terminal fragment; (B) H-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH for the COOH-terminal fragment.

2.0; Asp (aspartic acid), 1.0; Phe (phenylalanine), 3.3; Trp, 0.5; Thr (threonine), 2.0; Ser, 0.8; and NH_3, 1.1. Enzymic hydrolysis gave the ratios Ala, 0.9; Gly, 0.9; Lys, 2.0; Phe, 3.4; and Trp, 0.9; Asn (asparagine), Thr, and Ser were not well resolved, giving a total of about 3.6 moles per mole of peptide. Edman degradation of the carboxymethylated trypsin digests of somatostatin and mass spectrometry led to the final demonstration of the following primary structure for somatostatin: H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH, in the oxidized form (*50*).

This peptide, which was soon synthesized by the Merrifield method (*51*), was biologically active in vivo and in vitro (*52–54*) and, unexpectedly, the reduced form was as active as the oxidized form.

Endorphins: Opiate-Like Peptides of Brain or Pituitary Origin

The demonstration some years ago of (synaptosomal) opiate receptors in the brains of mammals (*55*) led to the search of what have been termed the endogenous-ligands of these opiate receptors. The generic name endorphins (from endogenous and morphine) was proposed for these (then hypothetical substances) by Eric Simon and will be used here. Some time in the summer of 1975 I became interested in these early observations largely because of the possibility that, like morphine, the endorphins might stimulate the secretion of GH. The isolation of these endogenous ligands of the opiate receptors proved to be relatively simple and was achieved in less than 3 months.

Dilute acetic acid–methanol extracts of whole brain (from ox, pig, and rat) contained substances, presumably peptidic in nature, with naloxone-reversible, morphine-like activity in the bioassay with the myenteric plexus–longitudinal muscle of the guinea pig ileum. Our findings were in agreement with the earlier results of Hughes (*56*), Terenius and Wahlstrom (*57*), Teschemacher *et al.* (*58*), and Pasternak *et al.* (*59*). In searching for an enriched source of endorphins in concentrates remaining from the isolation of corticotropin-releasing factor, TRF, LRF, and somatostatin, I recognized that acetic acid–methanol extracts of porcine hypothalamus-neurohypophysis contained much greater concentrations of the morphine-like activity than extracts of whole brain. From such an extract of approximately 250,000 fragments of pig hypothalamus-neurohypophysis we isolated several oligopeptides (endorphins) with opioid activity (*60–62*). For the isolation procedure we used successively gel filtration, ion exchange chromatography, liquid partition chromatography, and high-pressure liquid chromatography (*60–62*). By that time, had appeared the evidence for the isolation and primary structure of methionine-5-enkephalin (Met^5-enkephalin) and leucine-5-enkephalin (Leu^5-enkephalin) (*63*). Hughes *et al.* (*63*) had also observed that the amino acid sequence of Met^5-enkephalin was identical to the sequence Tyr^{61}-Met^{65} of β-lipotropin (β-LPH), a polypeptide of ill-defined biological activity, isolated and characterized in 1964 by C. H. Li *et al.* (*64*).

The primary structure of α-endorphin was established (*60, 62*) by mass spectrometry and classical Edman degradation of the enzymatically cleaved peptide and is H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH (Fig. 4, A and B). The primary structure of γ-endorphin was similarly established by mass spectrometry and by Edman degradation: γ-endorphin has the same primary structure as α-endorphin with one additional Leu as the COOH-terminal residue in position 17.

Thus it became apparent that Met^5-enkephalin is the NH_2-terminal pentapeptide of α- and γ-endorphin which have, respectively, the same amino acid sequences as β-LPH(61–76) and β-LPH(61–77). β-LPH(61–91), a fragment of β-LPH isolated earlier on the basis of its chemical characteristics (*64, 65*) was shown also to have opiate-like activity (*61, 66, 67*) and was named β-endorphin (*64*). Using the same starting material from which we originally isolated α- and γ-endorphin, we have recently isolated two peptides characterized by their having the same amino acid composition as β-endorphin [β-LPH(61–91)] and δ-endorphin [β-LPH(61–87)].

Experimental and Clinical Studies with TRF and LRF

After large quantities of TRF and LRF were obtained by synthesis, they were studied extensively in the laboratory and in clinical medicine (*20, 21, 68, 69*). It was soon discovered that synthetic TRF and LRF, both characterized only from tissues of ovine and porcine origin, and the synthetic replicate of somatostatin, as characterized from ovine brains, were biologically fully active in all species of vertebrates studied, including man (Figs. 5 and 6).

In 1972, our laboratory reported the synthesis of the first partial agonist-antagonist analogs of LRF (*70*), all having a deletion or a substitution of His^2 or Trp^3 in the (otherwise identical) amino acid sequence of LRF. These were antagonists of low activity and of no practical value as clinically significant inhibitors of LRF. They showed, however, that analogs as competitive antagonists of the decapeptide LRF could be prepared, and to this day the most potent antagonist analogs of LRF have the deletion or substitution of His^2 or Trp^3.

It was originally observed by Tashjian *et al.* (*71*) that TRF stimulates the secretion of prolactin by the cloned line GH_3 of pituitary cells. This observation was confirmed by others and found to apply also to normal pituitary tissues in vitro and in vivo, including the human pituitary. TRF can thus be considered to participate in the control of the secretion of thyrotropin and of prolactin. Of the many analogs of TRF which have been synthesized and studied biologically, only one has a significantly increased specific activity over that of the native compound. Described by our group and synthesized by Rivier *et al.* (*72*) a few years ago, it is the analog [3*N*-methyl-His]-TRF. Its specific activity is approximately ten times that of the native molecule, on the secretion of thyrotropin as well as of prolactin. Of the several hundreds of TRF analogs synthesized, none has been found to be even a partial antagonist. They are all agonists with full intrinsic activity but variable specific activity.

In contrast, antagonist as well as potent agonist analogs of LRF have been prepared by a number of laboratories. There are now available preparations of a series of what we may accurately call "super-LRF's," analogs which have as much as 150 times the specific activity of the native compound. In fact, in ovulation assays, they may have 1000 times the specific activity of the native peptide. All the agonist analogs or super-LRF's possess structural variations around two major modifications of the amino acid sequence of native LRF. They all have a modification of the COOH-terminal glycine, as originally reported by Fujino *et al.* (*73*). The Fujino modification consists of deletion of Gly^{10}-NH_2 and replacement by primary or secondary amide on the (now COOH-terminal) Pro^9. In addition to the Fujino modification, they have an additional modification at the Gly^6 position by substitution of one of several D-amino acids as originally discovered in our laboratories (*74*). The most potent of the LFR-analog agonists prepared are [D-Trp^6]-LFR; des-

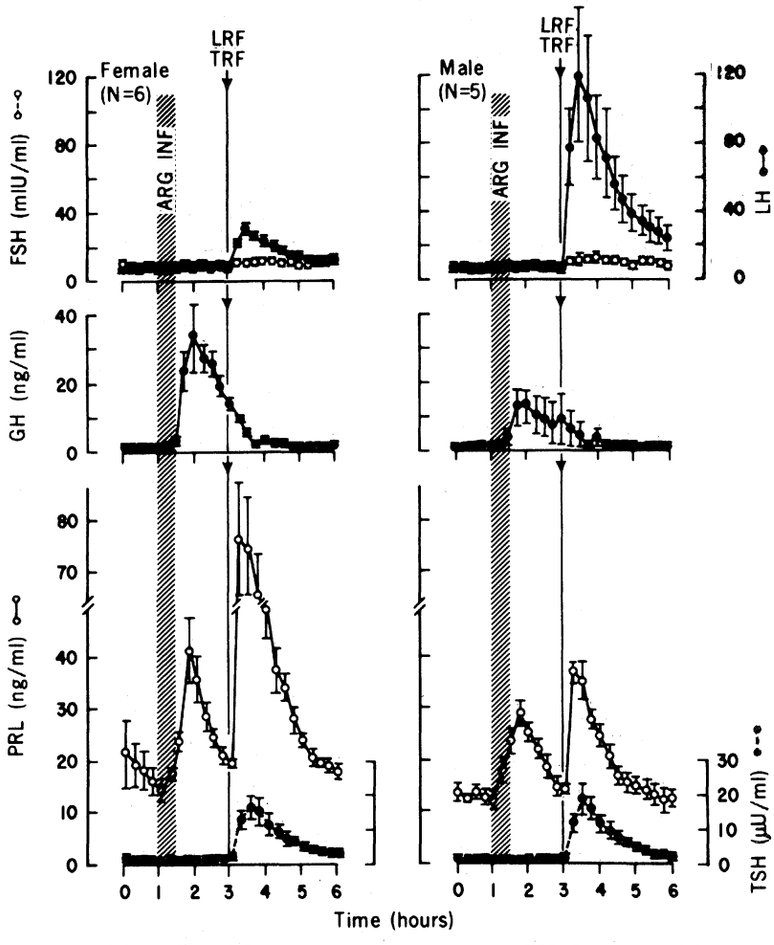

Fig. 5. Testing of the ability of the anterior pituitary to secrete GH, thyrotropin (*TSH*), prolactin (*PRL*), LH, and FSH in normal human subjects. Stimulation of the secretion of GH is achieved by intravenous administration of arginine; stimulation of the secretion of thyrotropin, prolactin, LH, and FSH is produced by intravenous injection of a solution in saline of synthetic TRF (250 μg) and synthetic LRF (150 μg). Note that arginine infusion stimulates secretion of GH and prolactin. All pituitary hormone plasma concentrations were measured by radioimmunoassays. [From Yen *et al.* (*68*)]

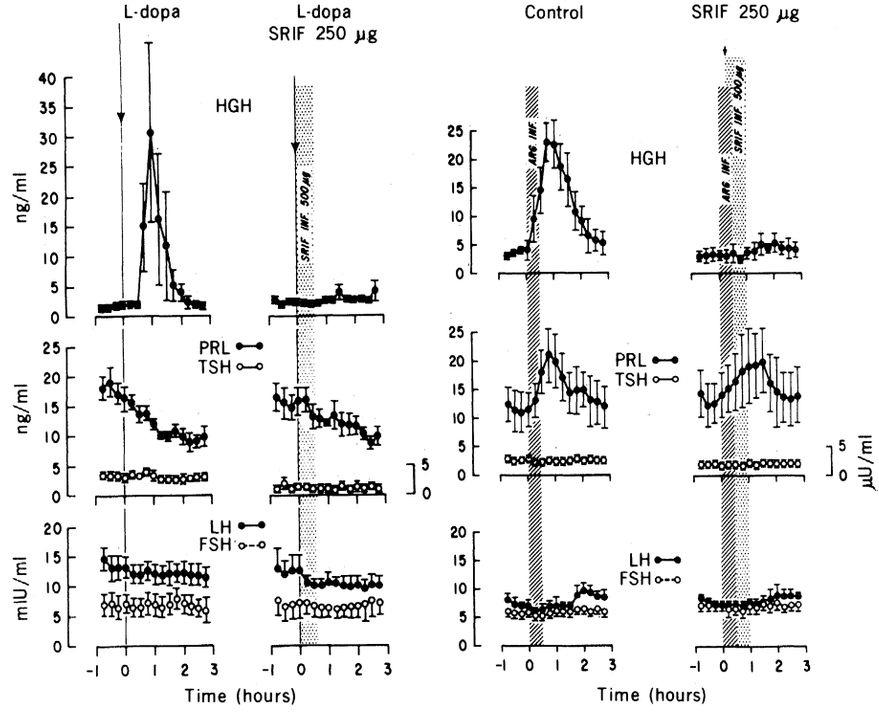

Fig. 6. Effects of the administration of synthetic somatostatin in normal human subjects. There is complete inhibition of the increase in GH secretion normally produced by infusion of arginine or oral administration of L-dopa, when somatostatin is administered prior to or concurrently with the stimulating agent. Plasma concentrations of pituitary hormones were measured by radioimmunoassays. [From Yen *et al.* (*68*)]

Gly^{10}-[D-Trp^{6}-Pro^{9}-N-ethyl]-LRF; [D-Leu^{6}, Pro^{9}-N-ethyl]-LRF.

In an assay in vitro in which the peptides stimulate release of LH and FSH by surviving adenohypophysial cells in monolayer cultures, these analogs of LRF have a specific activity 50 to 100 times greater than that of the synthetic replicate of native LRF. There is no evidence of dissociation of the specific activity for the release of LH from that of FSH. All agonist analogs release LH and FSH in the same ratio (in that particular assay system) as does native LRF. Probably because of their much greater specific activity, when given in doses identical in weight to the reference doses of LRF, the super-LRF's are remarkably long-acting in vivo. While the increased secretion of LH (or FSH) induced by LRF is returned to normal in 60 minutes, identical amounts in weight of [D-Trp^{6}-des-Gly^{10}]-N-ethyl-LRF lead to statistically increased concentrations of LH for up to 24 hours in several species, including man. These analogs effectively stimulate ovulation (*75*) and, according to Marks and Stern (*76*), are considerably more resistant than the native structures to degradation by tissue enzymes.

Injection into laboratory animals of large doses of the super-LRF's (that is, several micrograms per animal as opposed to several nanograms, which would be in the physiological range) has been shown by several groups to have profound antigonadotropic effects, both in males and females; moreover, when such large doses of the super-LRF's are injected in the early days of pregnancy in rats, they consistently lead to resorption of the fetus (*77*). These results are best explained by the current concepts of negative cooperativity between the peptidic ligands involved and their receptors at the several target-organ sites.

All of the antagonist LRF analogs found by our group (*70*) or by others have a deletion or a D-amino acid substitution of His^{2}. For reasons not clearly understood, addition of the Fujino modification on the COOH-terminal (*73*) does not increase the specific activity of the antagonist analogs. Administered simultaneously with LRF the antagonist analogs inhibit LRF in weight ratios ranging

from 3:1 to 15:1. The most potent of these antagonists inhibit activity of LRF not only in vitro, but also in various tests in vivo. They inhibit the release of LH and FSH induced by a single dose of LRF; they also inhibit endogenous release of LH-FSH and thus prevent ovulation in laboratory animals.

Biological Activity of Somatostatin

It is now recognized that somatostatin has many biological effects other than the one on the basis of which we isolated it in extracts of the hypothalamus, that is, as an inhibitor of GH secretion (*52*). Somatostatin inhibits the secretion of thyrotropin, but not prolactin, normally stimulated by TRF (*78*); it also inhibits the secretion of glucagon, insulin (*79*), gastrin, and secretin by acting directly on the secretory elements of these peptides. I have shown (*80*) that somatostatin also inhibits the secretion of acetylcholine from the (electrically stimulated) myenteric plexus of the guinea pig ileum, probably at a presynaptic locus, thus explaining at least in part the reported inhibitory effects of somatostatin on gut contraction, in vivo and in vitro (see Fig. 7).

In addition to being found in the hypothalamus (see Fig. 7), somatostatin also occurs in neuronal elements and axonal fibers in multiple locations in the central nervous system, including the spinal cord (*81*), and in discrete secretory cells of classical epithelial appearance in all the parts of the stomach, gut, and pancreas (*82, 83*) in which it was first recognized as having an inhibitory effect.

Somatostatin does not inhibit indiscriminately the secretion of all polypeptides or proteins. For instance, it does not inhibit the secretion of prolactin (*78*), the gonadotropins LH or FSH, calcitonin, or adrenocorticotropin (ACTH) in normal animals or from normal pituitary tissues in vitro; it also does not inhibit the secretion of steroids from adrenal cortex or gonads (*52*). Although somatostatin will inhibit hormone secretion from abnormal tissues such as pituitary adenomas, gastrinomas, and insulinomas, it is not expected to diminish tumor growth, because of its locus of action relating to that of adenosine 3′,5′-monophosphate (*53*).

The powerful inhibitory effects of somatostatin on the secretion not only of GH but also of insulin and glucagon have led to studies of a possible role of somatostatin in the management or treatment of juvenile diabetes (Figs. 8 and 9) and have proved useful in studying the physiological and pathological effects of these hormones on human metabolism. Such studies, showing that somatostatin lowers plasma glucose concentrations in normal man despite its inhibitory effect on insulin (*84–86*) have provided the first clear-cut evidence that glucagon has an important physiological role in human carbohydrate homeostasis. Somatostatin itself has no direct effect on either hepatic glucose production or peripheral glucose utilization, since the decrease in plasma glucose concentrations can be prevented by exogenous glucagon (*85*).

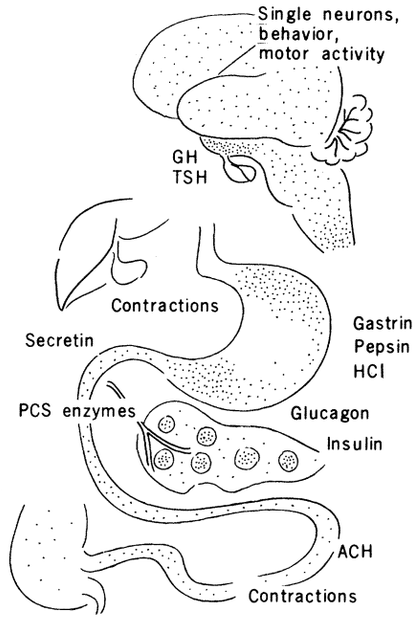

Fig. 7. Multiple locations of somatostatin and multiple effects of somatostatin.

In juvenile-type diabetics, somatostatin diminishes fasting hyperglycemia by as much as 50 percent in the complete absence of circulating insulin (*85*). Somatostatin impairs carbohydrate tolerance in normal humans given oral or intravenous glucose by inhibiting insulin secretion; however, carbohydrate tolerance after ingestion of balanced meals is improved in patients with insulin-dependent diabetes mellitus through the suppression of excessive glucagon responses (*85*). The combination of somatostatin and a suboptimal amount of exogenous insulin (which by itself had prevented neither excessive hyperglycemia nor hyperglucagonemia in response to meals) completely prevents plasma glucose concentrations from rising after meal ingestion in insulin-dependent diabetics (*85*). Through its suppression of glucagon and GH secretion, somatostatin has also been shown to moderate or prevent completely the development of diabetic ketoacidosis after the acute withdrawal of insulin from patients with insulin-dependent diabetes mellitus (*85*).

In view of its ability to inhibit the secretion of various hormones, somatostatin may eventually be of therapeutic use in certain clinical conditions such as acromegaly, pancreatic islet cell tumors, and diabetes mellitus. However, the multiple effects of somatostatin on hormone secretions and its short duration of action make its clinical use impractical at present (*86*); moreover, its long-term effectiveness and safety have not been established. For this reason, attempts are being made to synthesize "improved" analogs of somatostatin having a longer duration of activity than the native form. Other analogs have been sought that might have dissociated biological activity on one or more of the multiple recognized targets of somatostatin. The first such analog, found by the group at the Wyeth Research Laboratories, was [des-Asn^5]-somatostatin. This analog has approximately 4, 10, and 1 percent of the activity of somatostatin in inhibiting, respectively, the secretion of GH, insulin, and glucagon (*87*). Although such an analog was not of clinical interest, it showed that dissociation of the biological activities of the native somatostatin on three of its receptors could be achieved. Some of the most interesting analogs with dissociated activities and of potential clinical use have been prepared in our laboratories; they are [D-Ser^{13}]-somatostatin, [D-Cys^{14}]-somatostatin, and [D-Trp^8,D-Cys^{14}]-somatostatin. When compared to somatostatin, this last compound has approximately 300, 10, and 100 percent of the activity of somatostatin in inhibiting the secretion of, respectively, GH, insulin, and glucagon (*88, 89*).

Biological Studies with the Endorphins

All the morphinomimetic peptides that have been isolated from natural sources on the basis of a bioassay or displacement assay for ^{3}H-labeled opiates on synaptosomal preparations, and that have been chemically characterized, have been related to a fragment of the COOH-terminus of the molecule of β-LPH, starting at Tyr^{61} (where Tyr is tyrosine). In the case of Leu^5-enkephalin, the relation still holds for the sequence Tyr-Gly-Gly-Phe; no β-LPH with a Leu residue in position 65 has been observed.

β-Lipotropin has no opioid activity in any of the tests above. Incubation of β-LPH at 37°C with the supernatant prepared by centrifugation at $10^5 g$ of a neutral sucrose extract of rat brain generates

opioid activity, thus suggesting the presence of peptidases in the rat brain that could cleave β-LPH and result in a peptide with opioid activity. Thus, β-LPH may be a prohormone for the opiate-like peptides (*61*). This implies that the biogenesis of endorphins may be similar to that of angiotensin, with cleaving enzymes being available in the central nervous system (CNS) or in peripheral blood. There is also good evidence from immunocytochemistry (*90*) and biosynthesis studies (*91, 92*) that β-endorphin exists as such and as part of a larger precursor in discrete pituitary cells. Indeed, we have recently shown (*93*) that β-endorphin and ACTH are secreted simultaneously in vivo or in vitro under all circumstances tested thus far (Fig. 10).

In all biological assays tested, β-endorphin is considerably more active on a molar ratio than either enkephalin; the duration of activity of β-endorphin is also much longer than that of the enkephalins. A very large number of analogs of the enkephalins has been prepared by total synthesis, in a number of laboratories. Several of these, involving substitution with D-amino acids, have much greater specific activities than the native molecule (*94, 95*).

Considerable efforts are being devoted to clarifying the biosynthetic origin of Leu^5-enkephalin. Of interest are results observed with analogs of α-, β-, γ-, and δ-endorphins in which a Leu residue has been substituted for methionine in position 5 from the NH^2-terminus (*95, 96*). [Leu^5]-β-endorphin and [Leu^5]-γ-endorphin are more potent than their native congeners in the brain synaptosome assays, though not in the guinea pig ileum assay. One may thus speculate that the brain variety of endorphins might contain a Leu residue in position 5. Proof of such a hypothesis would require isolation and characterization of such molecules. To this date, no [Leu^{65}]-β-lipotropin has been identified. Leu^5-enkephalin might come from an allele of β-lipotropin of brain origin. It is also quite possible that Leu^5-enkephalin of brain origin derives from a larger molecule with no relation to β-lipotropin (other than the common tetrapeptide Tyr-Gly-Gly-Phe). Recent studies in collaboration with Bloom and Rossier (*97, 98*) have indeed shown remarkable dissociation in the distribution of neurons containing either β-endorphin or enkephalins.

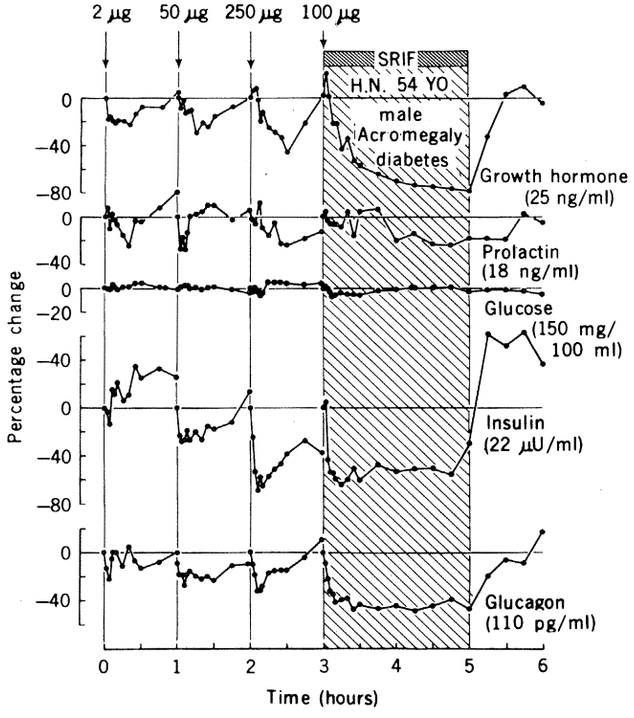

Fig. 8. Effect of multiple doses of somatostatin decreasing the plasma concentrations of GH, insulin, and glucagon in a patient with acromegaly and diabetes. [From Yen *et al.* (*68*)]

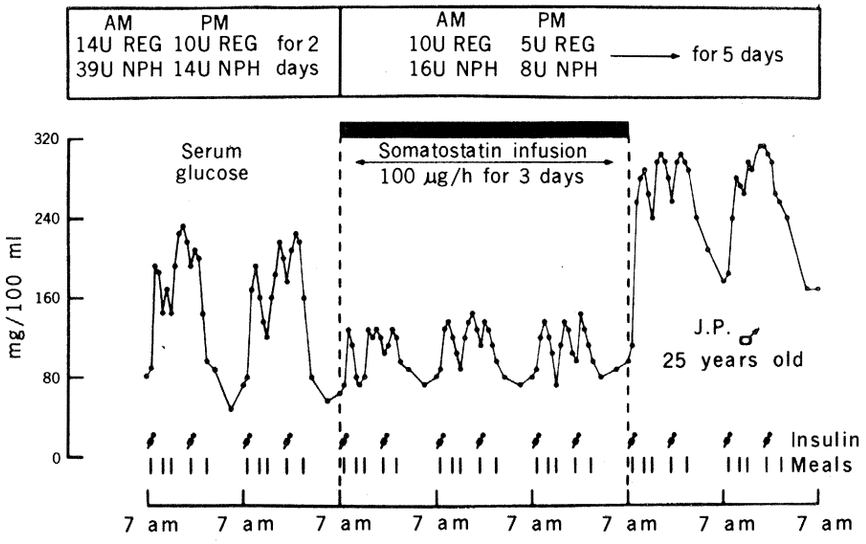

Fig. 9. Juvenile diabetic; improved control of glycemia during infusion of somatostatin and reduced amounts of insulin. *REG*, regular; *NPH*, neutral protamine Hagedorn. [From Gerich *et al.* (*82*)]

The effects of the opiate-like peptides on neuronal activity, together with the biochemical and histochemical evidence for their existence in the brain, are consistent with the hypothesis that these peptides are neurotransmitters in the CNS. Indeed, iontophoretic studies have

Table 1. Summary of neuronal effects of opioid peptides and morphine. In each category the total number of cells tested and the percentage of this total that was inhibited or excited is shown. [From Nicoll *et al.* (*105*)]

Region	Cell type	Met-enkephalin			β-Endorphin			Normorphine		
		N	Percentage		*N*	Percentage		*N*	Percentage	
			Excited	Inhibited		Excited	Inhibited		Excited	Inhibited
Cerebellum	Purkinje	34	18	21	13	23	23	5	20	60
Cerebral cortex	Unidentified	58	1	79	44	25	49	27	26	52
Brainstem	Lat. Ret. Nuc. +	113	3	47	35	23	45	20	10	75
Caudate nucleus	Unidentified	83	0	83	35	10	86	20	9	73
Thalamus	Unidentified	15	0	100	5	0	100	4	0	100
Hippocampus	Pyramidal	19	90	5	14	86	7	12	92	0

shown that the enkephalins can modify the excitability of a variety of neurons in the CNS. Most neurons tested were inhibited by these peptides (*101–103*), although Renshaw cells responded with an excitation (*104*). Recently, investigators have explored systematically the sensitivity of neurons to the endorphins or have made systematic regional surveys of neurons responsive to the peptides (*105*).

A surprising finding in the study of Nicoll *et al.* (*105*) was the potent excitatory effects of the peptides and normorphine on hippocampal pyramidal cells (Table 1). The regional specificity of this excitatory action could be clearly demonstrated with the same electrode by recording from cells in the overlying cerebral cortex and the underlying thalamus during a single penetration. No tachyphylaxis occurred in response to either the excitatory or inhibitory action of the peptides in any of the regions examined, even though the peptides were often applied repeatedly to the same cell for periods in excess of 1 hour. When the specific opiate antagonist naloxone was administered either by iontophoresis from an adjacent barrel of the microelectrode or by subcutaneous injections, both the excitations and the inhibitions were antagonized.

The pharmacological properties of endorphins have thus far been screened by tests in vitro or in vivo previously used to characterize opiate agonists and antagonists.

When injected into the cerebrospinal fluid, endorphins affect several behavioral and physiological measures, as well as responses to noxious agents, and each of the peptides exhibits different dose-effect profiles on these measures. β-Endorphin induces a marked catatonic state lasting for hours (*106*) at molar doses 1/100 those at which Met⁵-enkephalin transiently inhibits responses to noxious agents (*107–109*).

In terms of molar dose-effectiveness on the various parameters examined, β-endorphin is clearly the most potent substance tested, suggesting that its regulation could have etiological significance in mental illness. Rats given seven daily intracisternal injections of 14.9×10^{-9} mole of β-endorphin continued to show the full set of responses and duration of action. The catatonic state induced by β-endorphin was not observed with the other endorphin peptides, even at considerably higher doses. At very high doses of α-endorphin, γ-endorphin, or Met⁵-enkephalin, transient losses of corneal reflexes were observed, and α-endorphin seemed more potent in this regard than either γ-endorphin or Met⁵-enkephalin. No significant depressions of responsiveness to tail-pinch or pinprick stimuli were observed with Met⁵-enkephalin, α-endorphin, or γ-endorphin, but such effects (*66, 108, 110*) could have been missed by the 5-minute interval after injection and before testing began. In contrast to the syndrome induced by β-endorphin, rats given γ-endorphin showed consistent increases in rectal temperature (about 2.0 ± 0.2°C at 30 minutes after injections of 281×10^{-9} mole), and sometimes exhibited some degree of hyperresponsivity to sensory testing and handling, although there were individual variations in this response.

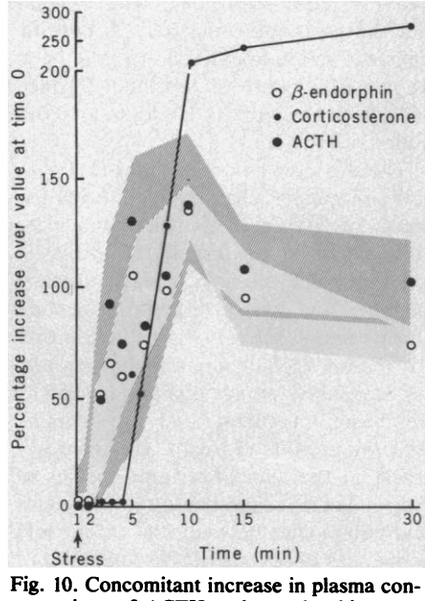

Fig. 10. Concomitant increase in plasma concentrations of ACTH and β-endorphin upon acute exposure to stress applied at time zero. (Peptides measured by radioimmunoassay.) Solid line shows plasma corticosterone concentrations measured by fluorometry.

All of our observations suggest that normal variations—either qualitative or quantitative—in the homeostatic mechanisms regulating the postulated (*61*) conversion of β-LPH as a prohormone to its several endorphin cleavage products could constitute a system fundamentally involved in maintaining "normal" behavior; alterations of the mechanisms normally regulating the homeostasis of β-lipotropin and endorphins could lead to signs and symptoms of mental illness. Such a potential psychophysiological role of endorphins could be tested through the therapeutic administration of available opiate antagonists. This has already been attempted in several clinical centers throughout the world; results obtained have been interpreted differently and, in my own mind, are too preliminary to warrant any conclusions. The ultimate identification of endorphin-sensitive behavioral events and specific treatment of their dysfunctional states may require the development of more specific "anti-endorphins" than those now available; other naturally occurring brain peptides, such as substance P, have already been reported to be endorphin antagonists in some assay systems (*111*).

Endocrine and Paracrine Secretions of the Brain: Hormones and Cybernins

Although TRF, LRF, and somatostatin, all originally isolated from extracts of the hypothalamus, actually occur throughout the CNS, including the spinal cord, this does not imply that these peptides are randomly distributed. Several groups have shown that each of these

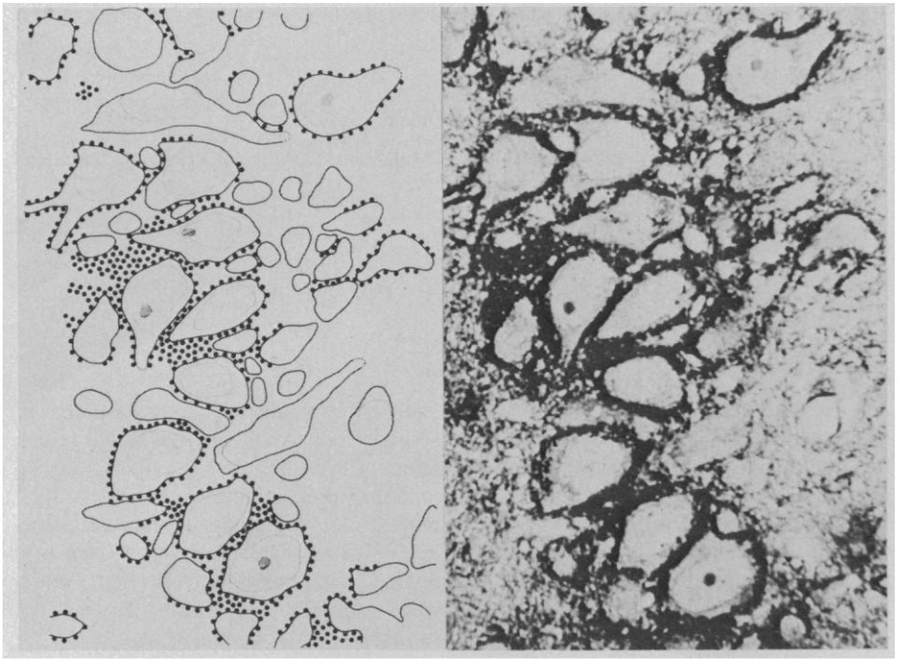

Fig. 11. Presumptive nerve terminals are somato-dendritic terminals around pyramidal cells of the hippocampus of the rat brain. Immunocytochemistry with antiserums to somatostatin. [From Petrusz *et al.* (*119*)]

compounds has a unique distribution pattern, and they have been separately identified by immunocytochemistry in axonal tracts and neuronal bodies in well-characterized anatomical formations of the CNS (*112*).

This information led a few years ago to the solution of one of the most puzzling dilemmas in this field, and this solution had far-reaching consequences. It was difficult to reconcile the short biological half-life of somatostatin (less than 4 minutes) when injected in the bloodstream, and its effects on the secretion of glucagon and insulin, with the hypothesis that hypothalamic somatostatin was involved in the physiological control of the secretion of pancreatic glucagon and insulin. Luft in Stockholm and I independently wondered whether somatostatin could be delivered to the endocrine pancreas by means other than the general circulation, possibly by nerve fibers, known to innervate the islets of Langerhans. It was then demonstrated by means of immunocytochemistry that the endocrine pancreas in all vertebrates studied contains a discrete population of cellular elements containing somatostatin (*82*, *83*). The somatostatin-containing cells belong to the D cells of the pancreas, long known to morphologists to be different from the A cells containing glucagon and the B cells containing insulin, but for which no specific secretory products had previously been recognized. Moreover, in the early studies a large number of secretory cells containing immunoreactive somatostatin were found throughout the gastrointestinal tract, and it has now been shown that somatostatin can inhibit the secretion of gastrin, secretin, cholecystokinin, pepsin, and HCl by acting directly at the level of the gastric mucosa (*113*). TRF has recently been reported in extracts of the stomach and of the duodenum. Neurotensin and substance P have also been located in the hypothalamus and throughout the gastrointestinal tract in specific cells and in crude extracts—as had been known in the case of substance P since 1931 from the work of Gaddum and von Euler.

There is now evidence that other peptides originally characterized from extracts of tissues of the gastrointestinal tract occur in the brain, for example, gastrin-cholecystokinin, vasointestinal peptide, and gastric inhibitory peptide [for a review, see (*114*)]; this is also true for the endorphins and enkephalins and for several of the small peptides such as bombesin, caerulein, and physalamine that were isolated years ago from extracts of the skin of several species of frogs. There are remarkable analogies and homologies between the amino acid sequences of several of these peptides of CNS and gastrointestinal origin, as well as those isolated from frog skin.

These peptides have been found by immunocytochemistry in essentially two types of cells: (i) in axonal and dendritic processes of neurons in brain, spinal cord, spinal ganglia, and the myenteric plexus; and (ii) in typical endocrine cells, for example, in the pancreatic islets of Langerhans, the enterochromaffin cells of the gut, and the adrenal medulla. Neuroblastomas have been reported to contain high concentrations of the vasointestinal peptide (*115*).

An interesting concept formulated some 10 years ago by Pearse (*116*) brings together these rather startling observations. Pearse observed that neurons and some endocrine cells producing polypeptide hormones shared a set of common cytochemical features and ultrastructural characteristics. He thus formulated the APUD theory, APUD being an acronym for amine precursor uptake and decarboxylation. Pearse postulated that these endocrine cells were derived from a common neuroectodermal ancestor, the transient neural crest, and that other endocrine cells would eventually be found sharing these common properties when further studies were made of the adult endocrine tissues derived from the neural crest. Recent observations, particularly those of Le Douarin on topical chimeras with chromosomal markers, have led Pearse to modify the original APUD concept in a remarkable manner: It is now postulated that all peptide hormone-producing cells are derived from the neural ectoderm (*114*), as are all neurons. With such ontogenic commonality, it is thus less surprising to recognize the presence of gastrointestinal peptides in the brain, and of brain peptides in the gastrointestinal tract.

Corticotropin, β-endorphin, and GH of pituitary origin, cholecystokinin, secretin, and gastrin of gastrointestinal origin are well-recognized hormones that satisfy all definitions of the word (*117*), particularly as it implies their action on distant target cells. The release of TRF, LRF, or somatostatin by hypothalamic neurons at the level of the hypothalamo-hypophysial portal vessels, and their hypophysiotropic activities, can also be considered as hormonal in nature; however, the distances traveled by the hypophysiotropic peptides to their target pituitary cells are measured by a few millimeters. Furthermore, there is no generally accepted evidence that the hypothalamic hypophysiotropic peptides enter the general circulation for any length of time or in physiologically meaningful concentrations.

When we consider these same peptides in parts of the brain other than the hypophysiotropic hypothalamus, the situation is even more restrictive. Studies

with both the optic and the electron microscope, combined with data from immunocytochemistry, indicate very punctual localizations which imply similarly punctual roles—that is, to be played over distances measured in angstroms. Scharrer (*118*) has for some time described what she calls peptidergic synapses. Moreover, recent studies with antibodies to somatostatin have yielded photographs that have been interpreted by Petrusz *et al.* (*119*) as showing the localization of immunoreactive somatostatin in multiple dendritic endings. Some of these photographs are spectacular (see Fig. 11). Their most heuristic interpretation is that each dark point is that of a dendritic contact either with another dendrite or abutting on a specialized locus of the axon or of the soma of recipient neurons. Clearly these recipient neurons do not seem to contain immunoreactive somatostatin. The cells of origin containing and sending the presumptive somatostatinergic terminals have not yet been characterized. Similar photographs have been observed in multiple locations in the brain and for several immunoreactive peptides. In this context, these peptides that we called hormones earlier do not fit the definition of a hormone any more; they seem to be candidates for the definition of neurotransmitters. Hökfelt (*112*) has concluded that some neurons may contain both peptides and one of the catecholamines, a classical neurotransmitter.

It is obvious that we are only beginning to learn of the physiological significance of these peptides in the brain. Their local punctual release and the short distance they travel make them fit what Feyrter called earlier the paracrine secretory system. Feyrter evolved his concept of paracrine secretion while studying with simple morphological tools the very cells of the gastrointestinal tract and of the pancreas that we know now to secrete the very peptides discussed in this lecture. I proposed earlier the generic name cybernin for these substances; the etymology of the word implying local information. It is difficult not to hypothesize that these peptides play some role in the functions of the brain. Once this simple proposal is made, and with the recognition that the existence of these substances in the CNS is not mentioned in any of the classical texts of neuropsychiatry, one cannot but be optimistic that the early observations summarized in this article will lead to profound reappraisals of the mechanisms involved both in the functions of the normal brain and in mental illness.

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NEWS AND COMMENT

Harvard Public Health Dean Hiatt Meets His Runnymede

The departmental barons of Harvard's School of Public Health rose up last summer and tried to depose their dean.

For 56 years the school has been run as a federation of departments under a lax administrative rule. That system is being challenged now by an aggressive dean—Howard Hiatt—appointed in 1972 by the then new president of Harvard, Derek Bok.

When Bok came into office, he decided that the School of Public Health needed radical improvement. He chose Hiatt to do that job, despite the objections of the public health professors, because Hiatt had already done a job of institutional renovation at Beth Israel Hospital in Boston, where he was chief of medicine from 1963 to 1972. Hiatt was trained at Harvard Medical School, whose faculty and students have always been disdainful of their counterparts at the School of Public Health. The latter reciprocate by insisting that only people with a "public health background" can understand public health. The feeling at Harvard is that Hiatt made little effort to conceal his low opinion of the school he was chosen to overhaul, an insult that has never been forgotten. The story is colored also by the belief, passed along by one professor, that some of Hiatt's severest critics are pretenders to the deanship.

In the last few years, Hiatt has been gathering up the reins that control funds, appointments, promotions, and curriculum and centralizing authority in the dean's office. This "meddling" in departmental affairs and Hiatt's alleged "intemperate behavior" have deeply embittered the older faculty.

While Hiatt was on vacation last June, 17 of the 34 tenured professors wrote to Bok demanding that Hiatt be fired for "administrative ineptitude." Hiatt's integrity was attacked as well. This confidential indictment (five pages, single-spaced) somehow fell into a reporter's hands, stirring up the kind of publicity Harvard most dearly wishes to avoid. It looked as though the dean really had lost his grip, the best evidence being the faculty revolt itself.

Hiatt came home and appealed to Bok for help. Briefs for and against were filed. Hiatt's friends argued that he was being undermined by a faculty that had never accepted his ideas, never made an effort to cooperate, and was desperately trying to stop Hiatt just as he was gaining the upper hand. According to these younger faculty members, Hiatt is trying to redefine the public health profession in ways that are alarming to the people who have made it their life's work. In Hiatt's vision, his friends say, a public health school should be a place where one studies health problems that confront a whole society and where one learns how to resolve government dilemmas on issues that are not clear-cut. For example, according to this view, a public health school should not simply do research to identify toxic substances in water; it should also help society decide whether or not it makes economic sense to remove those substances. Hiatt has brought economists, sociologists, businessmen, lawyers, and government officials to teach new courses in policy-making and management. The anti-Hiatt faction regards much of this as flimsy stuff, but insists that this is not the issue. The real problem, they say, is that Hiatt is not fit to do his job.

Bok Steps In

Bok listened to all of this for weeks, then decided firmly in the dean's favor. Just before the fall term, on 24 August, he delivered a stern lecture to the public health faculty, telling them he was not persuaded by the five-page indictment or by the unwritten complaints he had heard since June. He conceded that Hiatt was not a lovable dean, but said that "Those who are willing to take on the lonely, painful task of carrying out reform are rarely perfect diplomats nor can they be expected to have unswerving patience in dealing with their critics." He reminded the audience that although

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