most prominent shrubs are about 2 m tall. Species of Leguminosae and *Dodonaea viscosa* comprise the bulk of the woody vegetation, but the xerophytic nature of the slope is accentuated by scattered specimens of *Trichocereus peruvianus* and *Opuntia maxima*, both tree-like cacti. This species composition is in marked contrast to both the heavily cultivated valley bottom a few hundred meters downslope from the cave and the bright green slopes of the Cordillera Blanca on the other side of the valley. These areas must once have supported a dense, broad-leaved forest which has long since disappeared. On the other hand, the species composition of the vegetation of the dry slopes of the Cordillera Negra, although it is obviously disturbed by grazing animals and firewood gatherers, probably deviates less from its original condition. Since the time of the conquest, many herbaceous plants have been eliminated by the sheep and goats that were introduced and some species, such as *Agave*, have been added. In many ways, the slope of the Cordillera Negra is reminiscent of the habitats for wild beans in Mexico.

The common beans are present in sufficient numbers (approximately 30 specimens) in highly reliable contexts to leave no doubt that they belong with the cultural features of stratum II. Dark red-brown and dark red beans are present. Some are mottled. Some specimens are rounded, and others are flatter, more elongated kidney beans. The more rounded variety is generally darker in color and sometimes mottled. We were most fortunate in recovering five separate rounded beans and two pod fragments, one a stem end with three beans in place, in unit 146 which has been reliabily dated 7680 ± 280 years before the present (B.P.). Other examples were recovered nearer the bottom of stratum II, but none was found in the lowest portion which has been dated at more than 10,000 ± 300 years B.P. We are convinced that the cultivation of beans was known in the Callejón de Huaylas by about 6000 B.C. Previously, the oldest record for cultivated common beans was 7000 years B.P. at Tehuacán (*2*) in Mexico and 4700 ± 80 years B.P. (*3*) in South America.

The idea that the beans recovered from Guitarrero Cave were cultivated is beyond doubt. Wild or wild-type beans collected in Mesoamerica (*4*) and South America (*5*) are consistently small in size and are usually tan or gray, often with darker flecking or brindling. The common beans of Guitarrero Cave are fully as large as those recovered in more recent strata. They have thin seed coats, they are dark, and they are within the size range and form of contemporary cultivars; they are sometimes mottled, but they are without trace of the brindling so common in wild populations.

Furthermore, the fragments of pod found in this stratum do not have the heavy, inner fibrous layer characteristic of wild bean pods. This layer, instrumental in twisting the pod valves tightly in opposite directions, has been selected against in cultivation to prevent the loss of beans before or during harvest (*6*). The pod fragment with included beans shows no tendency to separate and curl, although pods of other leguminous species from the same deposit still retain the ability to curl with drying. Common beans were probably cultivated in the valley bottom along the Río Santa rather than on the dry slopes near the cave.

In the same stratum of the Guitarrero Cave deposit, we recovered four specimens of lima beans (*Phaseolus lunatus*). One of these seeds, probably reddish in color, although the color is largely obscured by an incrustation, was found under fairly reliable conditions. The other three seeds, one solid black and two tan with black markings, were found in an area with somewhat looser fill which might be an indication of disturbance. Like the common beans, all of these seeds are similar in size and shape to those recovered in more recent strata in the Guitarrero deposits. They were fully domesticated, cultivated lima beans that are of Peruvian, as distinct from the Mesoamerican, type, and in spite of the less secure circumstances under which they were found, they lend further support to the proposition that the people of Guitarrero Cave practiced cultivation of common and lima beans between 5500 and 8500 B.C.

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Hypothalamic Polypeptide That Inhibits the Secretion of Immunoreactive Pituitary Growth Hormone

Abstract. *A peptide has been isolated from ovine hypothalamus which, at* 1×10^{-9}*M, inhibits secretion in vitro of immunoreactive rat or human growth hormones and is similarly active in vivo in rats. Its structure is*

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

The synthetic replicate is biologically active.

Physiological, experimental, and clinical observations (*1*) have led to the concept that the hypothalamus controls and regulates the secretion of pituitary growth hormone (somatotropin). It has been generally accepted that this control would be exerted by a hypothalamic hypophysiotropic releasing factor, as is now proven to be the case for the secretion of thyrotropin (TSH) (*2, 3*) and the gonadotropin, luteinizing hormone (LH) (*4*). The nature of the postulated hypothalamic releasing factor for growth hormone, however, remains elusive, mostly due to the difficulties and ambiguities of the various assay systems used so far in attempts at its characterization [for review see (*5*)].

Searching to demonstrate the presence of this still hypothetical somatotropin releasing factor in the crude hypothalamic extracts used in the isolation of TRF (thyrotropin releasing factor) and LRF (luteinizing hormone releasing factor), we have regularly observed that their addition in minute doses (≥ .001 of a hypothalamic frag-

ment equivalent) to the incubation fluid of dispersed rat pituitary cells in monolayer cultures (*6*) significantly decreases the resting secretion of immunoreactive growth hormone (*7*) by the pituitary cells. This inhibition is related to the dose of hypothalamic extract added and is specific (see Table 1).

It is not produced by similar extracts of cerebellum, and the crude hypothalamic extracts that inhibit secretion of growth hormone simultaneously stimulate secretion of LH and TSH. The inhibition of growth hormone secretion could not be duplicated by addition to the assay system of [Arg^8]-vasopressin, oxytocin, histamine, various polyamines, serotonin, catecholamines, LRF, or TRF. For operational facility, we decided to attribute this inhibitory effect on the secretion of growth hormone to a "somatotropin-release inhibiting factor" or SRIF.

Inhibition of secretion of growth hormone by crude hypothalamic preparations has been reported (*8*); however, the active factor possibly involved has not been characterized.

Our results on the inhibition by the hypothalamic extracts of the in vitro secretion of growth hormone by the pituitary monolayer cultures were so consistent and so easily quantitated that we decided to attempt the isolation of the postulated SRIF with the in vitro method as an assay system. Characterization of a natural inhibitory factor of hypothalamic origin was of interest in view of current efforts at designing synthetic polypeptides as antagonists of LRF (*9*).

Table 1. Effects of ovine hypothalamic extracts, purified preparations, pure native and synthetic SRIF on the secretion of growth hormone by rat pituitary cells in monolayer cultures; *N*, number of cell culture dishes; rGH, rat growth hormone by radioimmunoassay; CMC, carboxymethyl cellulose; N.S., not significant.

Additions	Doses	*N*	rGH (ng/hr)	*P*†
Saline		4	421.7 ± 49.1	
Hypothalamic extract	0.001 fragment/ml	3	231.0 ± 13.6	< .01
Hypothalamic extract	0.01 fragment/ml	3	118.7 ± 15.8	< .01
Hypothalamic extract	0.1 fragment/ml	3	91.3 ± 9.9	< .01
CMC fraction (185–200)	20 ng/ml	3	196.3 ± 52.5	< .01
CMC fraction (185–200)	200 ng/ml	3	83.6 ± 17.1	< .01
Saline		5	354.8 ± 23.9	
Hypothalamic extract	0.002 fragment/ml	5	190.7 ± 23.4	< .01
Hypothalamic extract	0.01 fragment/ml	3	120.0 ± 22.7	< .01
Hypothalamic extract	0.05 fragment/ml	3	50.7 ± 16.4	< .01
Native ovine SRIF*	0.2 n*M*	3	304.0 ± 45.5	N.S.
Native ovine SRIF	1.0 n*M*	3	210.7 ± 17.0	< .01
Native ovine SRIF	5.0 n*M*	3	70.7 ± 16.8	< .01
Native ovine SRIF	25.0 n*M*	4	52.5 ± 8.4	< .01
Synthetic SRIF‡	0.2 n*M*	3	420.0 ± 23.1	N.S.
Synthetic SRIF	1.0 n*M*	4	205.0 ± 23.8	< .01
Synthetic SRIF	5.0 n*M*	3	110.0 ± 5.8	< .01
Synthetic SRIF	25.0 n*M*	3	100.0 ± 6.1	< .01
Synthetic SRIF	3.3 μM	3	29.3 ± 14.6	< .01

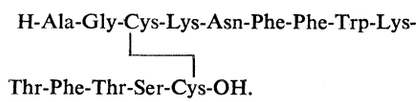
* From data obtained by a four-point assay of synthetic versus native SRIF in best fit data (interval of doses = 5), the potency ratio of synthetic SRIF to native SRIF was 0.90. † From analysis of variance and multiple comparison test of Dunnett. ‡ Concentration of native or synthetic SRIF (triacetate) based on a calculated molecular weight of 1818.

Table 2. Effects of extracts of ovine hypothalamus or cerebellum and synthetic SRIF on growth hormone in plasma in rats treated with sodium pentobarbital. Animals were anesthetized with ether; the various substances (treatments) were injected intravenously (0.2 ml); this injection was immediately followed by intravenous administration of 2.5 mg of sodium pentobarbital per 100 g of body weight (*14*); blood was withdrawn 15 minutes later for radioimmunoassay of rat growth hormone (rGH) (*7*); *N*, number of rats per treatment; *P*, as in legend for Table 1. Both experiments were designed in completely randomized blocks.

Treatment	Doses	*N*	rGH (ng/ml)	*P*
Saline		21	74.1 ± 6.3	
Hypothalamic extract	0.3 fragment	21	54.5 ± 7.8	< .05
Hypothalamic extract	1.0 fragment	21	32.1 ± 5.7	< .01
Cerebellum extract	1.0 fragment equiv.	21	73.8 ± 8.8	N.S.
Saline		10	65.6 ± 8.4	
Hypothalamic extract	1.0 fragment	10	41.2 ± 5.3	< .05
Synthetic SRIF	0.1 μg	10	70.8 ± 7.2	N.S.
Synthetic SRIF	10 μg	10	32.0 ± 7.5	< .01

The starting material was the chloroform–methanol–glacial acetic acid extract of about 500,000 sheep hypothalamic fragments (*4*) used in the program of characterization of the releasing factors for the gonadotropins. The extract (2 kg) had been partitioned in two systems; the LRF concentrate was subjected to ion-exchange chromatography on carboxymethyl cellulose. At that stage, a fraction with SRIF-activity was observed well separated from the LRF zone; it was further purified (*10*) by gel filtration (Sephadex G-25) and liquid partition chromatography (*n*-butanol, acetic acid, water, 4 : 1 : 5). 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, which is approximately 2 percent of that calculated on quantitative estimates of the amount of total SRIF activity in the original extract. The low yield was not of primary concern, because the early purification stages were designed specifically for the isolation of LRF and the amount of SRIF obtained as a side fraction was considered adequate for its characterization. Samples of SRIF gave a color reaction with ninhydrin, Ehrlich reagent, but not with Pauly reagent. The biological activity was totally destroyed by hydrolysis in 6*N* HCl as well as by digestion with chymotrypsin. Quantitative amino acid analyses of SRIF after acid hydrolysis (*11*) gave the following residues: Ala, 1; Gly, 1; Thr, 2; Lys, 2; Phe, 3; Ser, 1; Cys, 2; Trp, 1; Asp, 1; NH_3, 1 (*12*). Amino acid analysis, after total enzymatic digestion with papain and leucine aminopeptidase, showed the same amino acid ratios except that Asp occurred as Asn.

The sequence of SRIF was determined by stepwise Edman degradation performed on the intact carboxymethylated peptide, as well as on the unresolved products of tryptic and chymotryptic digests of the peptide. The products of Edman degradation were evaluated by the subtractive method by means of amino acid analysis, determination of the successive amino terminals with [^{14}C]dansyl chloride and mass spectrometry of 3-phenyl-2-thiohydantoin (PTH) derivatives when applicable. The primary structure of isolated ovine SRIF is



The sequence -Asn-Phe-Phe-Trp-Lys- was confirmed in an acetylated, permethylated tryptic digest of SRIF by direct mass spectrometry (*10*).

The linear tetradecapeptide was synthesized by solid-phase methodology and purified by gel filtration in presence of 2-mercaptoethanol (*10*). After purification, the synthetic peptide had the biological activity of the native SRIF (Tables 1 and 2); at concentrations $\geqslant 1$ n*M*, native or synthetic SRIF inhibits the secretion of growth hormone from monolayer cultures of dispersed cells of rat adenohypophysis. In one experiment, native SRIF, at a concentration of 20 n*M*, inhibited significantly the spontaneous secretion of growth hormone by enzymatically dispersed cells derived from the pituitary gland of a patient with confirmed active acromegaly (*13*). The biological results with native and synthetic SRIF do not help to resolve the question of the reduced or oxidized state of the Cys residues in the peptide when it is recognized by the pituitary receptors; both the isolation procedure and the conditions that are used in the bioassays might convert one form into the other.

With the exception of primates, no simple adequate laboratory animal model seems to exist, which would exactly duplicate in vivo what is known of the physiological regulation of the secretion of growth hormone in humans. However, we found that the crude hypothalamic extract was able to inhibit (Table 2) the elevation in the plasma of growth hormone, as determined by radioimmunoassay, induced in rats by intravenous injection of sodium pentobarbital (*14*). This effect is specific for the hypothalamic extract as it is not duplicated by similar extracts of sheep cerebellum. Synthetic SRIF inhibits the secretion of growth hormone in similarly prepared assay rats (Table 2).

Native or synthetic SRIF has no effect on the basal secretion of LH or FSH (follicle stimulating hormone) in vitro at concentrations at which it inhibits maximally the secretion of somatotropin.

The peptide SRIF has been isolated and characterized with the use of an in vitro assay method of considerable reliability (*6*); the effects observed in vivo proceed from a method the rationale of which is less clearly established. Thus a physiological role for SRIF remains to be demonstrated. Should SRIF be active in humans (*15*), its possible clinical significance, particularly in the treatment of acromegaly and in the management of juvenile diabetes, has not escaped our attention.

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10. The methodology involved is in preparation. See also (*2*) and J. Rivier *et al.*, *Chimia* **26**, 300 (1972).
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12. Abbreviations for amino acids are Ala, alanine; Asn, asparagine; Cys, cysteine; Gly, glycine; Lys, lysine, Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Asp, aspartic acid.
13. Radioimmunoassay for human growth hormone, by courtesy of Drs. S. Yen and T. Siler, University of California School of Medicine, San Diego. Fragments of a human acromegalic pituitary were obtained at time of surgical hypophysectomy, courtesy of Drs. Levin, Wilson, and Aubert, University of California School of Medicine, San Francisco.
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15. We propose to name the peptide described here *somatostatin*, from somato(tropin), a pituitary factor affecting statural growth, and stat(in), from the Latin "to halt, to arrest" (as in hemostat and bacteriostatic). This is in keeping with the efforts of several international nomenclature committees (with which the final decision should remain) aiming at creating trivial names for biologically active polypeptides rather than maintaining the use of acronyms.
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Exocytosis in the Adrenal Medulla Demonstrated by Freeze-Etching

Abstract. *Replicas of fractured chromaffin cells are indicative of a range of activities thought to characterize exocytosis, including attachment of secretory vesicles to the plasma membrane, fusion, extrusion of contents, and membrane retrieval. Exocytosis sites are abundant on stimulated cells but are infrequent when calcium is omitted from the system.*

There is now abundant evidence that secretion from exocrine, endocrine, and nervous tissue occurs by a process termed exocytosis [for reviews see (*1*)]. In exocytosis, it appears that the secretory vesicle fuses with the plasma membrane, creating a stoma which allows passage of the vesicle contents to the extracellular space. Biochemical studies of the adrenal medulla provide strong support for the concept, having demonstrated that the total soluble contents of chromaffin cells are secreted without concomitant release of cytoplasmic constituents (*2*). As well, profiles indicative of exocytosis have been shown by electron microscopy of thin sections (*3, 4*). However, difficulties are encountered in providing morphological evidence for exocytosis, probably owing to the infrequency with which the point of fusion of vesicle and plasma membrane coincides with the plane of a thin section. As a consequence, studies of thin sections cannot provide a visual representation of the extent of exocytosis in the adrenal medulla, a surface phenomenon resulting in the massive release of catecholamines. In the study reported here, we overcame this difficulty by applying freeze-fracture techniques to expose large areas of the plasma membrane of chromaffin cells; we used both stimulated and unstimulated adrenal glands.

Golden hamsters (weighing approxi-