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ABSTRACT

The effect of intravenous administration of serum containing antibodies to somatostatin (AS-SRIF) raised in the sheep was tested in conscious male dogs to study the role of somatostatin (SRIF) in the short term regulation of secretory bursts of growth hormone (GH). The experiments utilized trained animals previously cannulated under local anesthesia, with blood sampled at intervals as short as 1 min during initial manipulations, and otherwise normally at 5 min intervals. Bivariate GH vs time data (by RIA) was converted into GH secretion rates vs times using a single compartment model based upon previously determined near constant metabolic clearance rate (MCR) and apparent distribution volume (V) for dogs of about the same size.

Five dogs that had normal GH secretion characteristics (established in resting control experiments) reliably responded to a bolus injection of AS-SRIF with a high amplitude surge in GH secretion within one minute of the manipulation. Secretion rates calculated for the AS-SRIF provoked spikes were up to 25-fold those observed in spontaneous secretory bursts during resting control experiments in the same animals. Two of 5 naive dogs exposed to a bolus injection of control goat serum showed GH spikes with lags of 5 and 6 minutes.

In both groups of animals receiving sera, whether control or AS-SRIF, a rise in total glucocorticoids (by fluorometry) suggested that injection of the sera had been stressful to some

degree. Since the GH response to stress in dogs remains inadequately characterized, this issue was studied further with 3 separate experimental designs: (i) injection of control goat sera in previously sensitized dogs, thereby producing anaphylaxis which represents a more severe stress than either type of serum injected into naive animals; (ii) hypotension associated with a state of transient hypovolemia produced by blood withdrawal; and (iii) inhalation of ether by face mask. All animals in the anaphylaxis and hypovolemia experiments reliably produced GH spikes but secretory surges of GH comparable to spontaneous spikes were not obtained with ether, which had a hypertensive effect, as observed in recordings of mean arterial pressure.

A comparison of AS-SRIF, anaphylaxis and hypovolemia spikes showed the shortest lags, largest peak responses and largest amount of extra GH secretion in AS-SRIF experiments, proceeding progressively to longest lags and smallest peak and total extra GH secretion in the spikes resulting from hypovolemia (blood withdrawal).

These results suggest that decline in the concentration of SRIF impinging on the somatotrophs is at least in part causative of GH secretory episodes. It is possible that intervention with AS-SRIF lowers SRIF in the milieu of the somatotrophs in a more coordinated fashion that occurs physiologically, resulting in larger spikes than normally seen. The observation that transient hypotensive stresses provoke GH secretory bursts but a hypertensive one does not, may suggest a role for disturbances of hypothalamo-hypophyseal portal flow in setting off some bursts of GH secretion.

CHAPTER I

INTRODUCTION

1. Historical note

The neurohypophyseal portion of the hypothalamus (which forms the base of the third ventricle) is funnel shaped, which gave rise to the term infundibulum and to Vesalius' hypothesis that the cerebrospinal fluid drained mucus ("pituita") from the brain into the pituitary gland, and then somehow to the nose. In the early part of the twentieth century, however, observations in patients with pituitary tumors (notably Fröhlich's case of adiposo-genital syndrome) suggested that the anterior pituitary, although housed in the cranium, is not a neural structure, but an organ with endocrine function.

The mechanism of the relationship between the hypothalamus and hypophysis began to gel in the late thirties through the union of two ideas: Ernst & Berta Sharrer's proposal that the structural peculiarities of the cells of some hypothalamic nuclei suggested secretory activity, and experiments done by Harris that showed the hypophyseal portal blood supply as the vascular link that is the essential conduit of information between hypothalamus and anterior pituitary. For detailed historical review see Anderson and Haymaker (1974) and Meites et al. (1975). More recent progress is reviewed in many monographs and articles (Glick et al., 1965; Porter, 1977; Guillemin, 1978; McCann, 1979; DeGroot et al., 1979;

Vale et al., 1980; Reichlin, 1981).

2. Normal pattern of GH secretion

An important feature of pituitary hormone secretion, now documented for all adenohypophyseal hormones, is that release from the gland *in vivo* does not occur in a steady state fashion as doctrines of homeostasis implied some years ago, but rather in an intermittent, episodic manner (Martin, 1979). The secretion of growth hormone (GH) has been shown to be strikingly episodic not only in rats (Schalch and Reichlin, 1966; Martin et al., 1974; Tannenbaum and Martin, 1976; Willoughby et al., 1976), goats (Tindal et al., 1978), rhesus monkeys (Jacoby et al., 1974) and humans (Glick et al., 1965) but also in the dog (Takahashi et al., 1981).

Plasma concentrations of GH measured at frequent intervals throughout the day and night reveal that rat GH secretion is governed by an ultradian rhythm with a periodicity of approximately 3.3 hours with most peak concentrations in excess of 200 ng/mL (Tannenbaum & Martin, 1976) while individual surges of GH secretion in man may reach plasma levels of 40 to 60 ng/mL (Takahashi et al., 1968; Martin, 1976). Experiments done in the daytime on unstressed dogs (Cowan et al., 1981; Cowan et al., 1984) show mean peak plasma GH concentrations of ≈ 6 ng/mL with mean interpeak interval of about 3.9 hours.

3. Factors affecting GH secretion

Development of radioimmunoassays for GH (Schalch and Reichlin, 1966; Birge et al., 1967; Peake et al., 1968; Tsushima et al., 1971; Lovinger et al., 1974; Hampshire et al., 1975) have permitted studies which demonstrate that secretion of GH is modified by a wide variety of external stimuli and endogenous neural rhythms. The more important naturally occuring events that trigger GH release are exercise, physical and emotional stresses, high protein intake and carbohydrate rich meals (during the descending limb of blood glucose levels) (Reichlin, 1981). An important endogenous modification of GH release is the surge of secretion that occurs within an hour or two of falling asleep (Takahashi et al., 1968; Finklestein et al., 1972).

Factors which have a stimulatory or inhibitory influence on growth hormone secretion in primates are presented in Tables I and II. However, there are species differences among mammals in the GH response to a number of external stimuli (Reichlin, 1974). The response to stress is one such example and will be dealt with later in this chapter.

Several studies have been undertaken to determine the basis of physiological variations in GH secretion. Factors such as sleep

TABLE I

Factors that stimulate growth hormone secretion in primates†

Physiologic	Pharmacologic	Pathologic
1. Episodic, spontaneous	1. Insulin hypoglycemia	1. Acromegaly
2. Exercise	a. 2-Deoxyglucose	a. TRH
3. Stress	2. Amino acid infusions	b. LRH
a. Physical	a. Arginine	c. Glucose
b. Psychological	b. Leucine	d. Arginine
4. Sleep	c. Lysine, etc.	2. Pyrogens
5. Postprandial glucose decline	3. Small peptides	3. Protein depletion
	a. ADH	4. Fasting and starvation
	b. α MSH	
	c. ACTH (1-24)	
	d. Glucagon	
	4. Monoaminergic stimuli	
	a. Epinephrine, α receptor stimulation	
	b. L-dopa	
	c. Apomorphine	
	d. 2-Bromocryptine	
	e. Clonidine	
	f. 5-hydroxytryptophan	
	g. Fusaric acid (dopa-β-hydroxylase inhibitor)	
	h. Propranolol	
	i. Melatonin	
	5. Nonpeptide hormones	
	a. Estrogens	
	b. Diethylstilbestrol	
	6. Potassium infusion	
	7. Dibutyryl-cAMP	

†Adapted from Reichlin, S. Neuroendocrine aspects of control of specific pituitary tropic hormones, In: Textbook of Endocrinology by R.H. Williams (ed.) VI edition 1981, pp. 610-622. W.B. Saunders, Philadelphia. Based on material from Martin, J.B., Brazeau, P. et al., Neuroendocrine organization of growth hormone regulation in the Hypothalamus. Reichlin, S., Baldessarini, R.J. et al. (eds.) 1978, pp. 239-357. Raven Press, New York.

TABLE II

Factors that inhibit growth hormone secretion in primates*+

Physiologic	Pharmacologic	Pathologic
1. Postprandial hyperglycemia	1. Melatonin	1. Acromegaly
2. Elevated free fatty acids (?pharmacologic)	2. Serotonin antagonists	a. L-dopa
3. Elevated GH levels	a. Methysergide	b. Apomorphine
	b. Cyproheptadine	c. Phentolamine
	3. Phentolamine	d. 2-bromocriptive
	4. Chlorpromazine	2. Hyperthyroidism
	5. Morphine	3. Hypothyroidism
	6. Zn-tetracosactin	
	7. Progesterone	
	8. Theophylline	

*In many instances, the inhibition can only be demonstrated as a suppression of GH release induced by a pharmacologic stimulus.

+Adapted from Reichlin, S. Neuroendocrine aspects of control of specific pituitary tropic hormones, In: Textbook of Endocrinology by R.H. Williams (ed.) VI edition 1981, pp. 610-622. W.B. Saunders, Philadelphia. Based on material from Martin, J.B., Brazeau, P. et al., Neuroendocrine organization of growth hormone regulation, in The Hypothalamus. Reichlin, S., Baldessarini, R.J. et al. (eds.) 1978, pp. 329-357. Raven Press, New York.

(Lucke et al., 1976; Takahashi et al., 1981), exercise (Hansen, 1973), and stress account for some of the fluctuations, but available data indicates that many of the surges are spontaneous and occur randomly through the day and night, apparently unrelated to any identifiable extrinsic or internal event (Willoughby et al., 1976; Tindal et al., 1978). Moreover, as reviewed by Reichlin (1974) and Martin (1976) physiologic changes in metabolites such as glucose, amino acids and free fatty acids have limited effects on GH spikes. Hyperglycemia only temporarily suppresses daytime surges of GH secretion, and nocturnal GH rises are not affected by either fasting or hyperglycemia, although more frequent GH secretory pulses do occur during fasting.

The profile of the secretory bursts and their non-suppressibility by potential metabolic regulators of GH secretion suggest that the surges are the result of primary activation of GH secretion induced by neural mechanisms (Martin et al., 1978).

4. Control of GH secretion: role of somatostatin

The secretion of growth hormone is regulated by a complex interaction of various influences and there is evidence that this control is achieved by at least two hypothalamic hormones: GH-releasing factor (GRF), which has been characterized only recently (Brazeau et al., 1981; Guillemin, 1982), and GH release inhibiting factor (somatostatin or somatotropin release inhibiting factor; SRIF) which was first isolated and structurally identified about a decade ago. These hormones are believed to be synthesized in,

and released from, neurons in the medial basal hypothalamus. Also, there is evidence that monoamines, notably dopamine and norepinephrine (Müller et al., 1970), and serotonin (Arnold and Fernström, 1980) affect GH secretion, but they act at a neural level to modulate the release of the hypothalamic hormones, perhaps acting as neurotransmitters in monoaminergic neuron systems (Martin, 1976).

As evidenced by experiments employing the techniques of pituitary stalk section (Wehrenberg et al., 1980), hypophysectomy and extrasellar pituitary transplants (Schalch and Reichlin, 1966), integrity of the "hypothalamo-hypophyseal unit" is essential to normal growth Hormone secretion. Using stereotactic surgery, experimental evidence to support the role of particular hypothalamic areas has been obtained in several species. In the squirrel monkey, small lesions of the median eminence and midline basal hypothalamus block insulin induced (Abrams et al., 1966) and stress mediated (Brown et al., 1971) GH release. Lesions of the ventromedial nucleus (VMN) in young female rats result in growth retardation and a fall in plasma and pituitary GH levels (Frohman and Bernardis, 1968). This hypothesis is strengthened further by consideration of the effects obtained with electrical stimulation. Both unilateral (Frohman et al., 1968) and bilateral (Martin, 1972) stimulation of the mediobasal hypothalamus in pentobarbital anesthetized rats elicits GH secretion within 5-15 min after the onset of pulsed square waves. A similar GH surge has been described after hypothalamic stimulation in sheep (Malven, 1974) and in

unanesthetized rabbits (McIntyre and Odell, 1974). However, as these workers noted, rise in plasma GH levels induced by hypothalamic stimulation invariably occured after termination of the stimulus as a post inhibitory rebound surge of secretion. Also, stimulation of the medial preoptic area of the hypothalamus caused inhibition of GH release (Martin et al., 1975; Willoughby and Martin, 1978). Before the characterization of specific hypothalamic hormones effecting GH secretion, these findings would only have pointed to a supra hypophyseal dual control mechanism serving both in a facilitatory and inhibitory way.

Direct evidence for the existence of an extractable hypothalamic factor effecting pituitary release of GH was first reported in 1964 by Deuben and Meites. Later, in 1972, the Q shaped cyclic tetradecapeptide somatostatin was isolated from ovine hypothalami (Brazeau et al., 1973). It has subsequently been synthesized by several groups in linear and cyclic forms (Brazeau

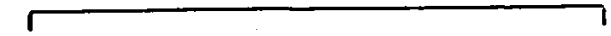


Fig. 1. Structure of somatostatin

et al., 1973; Coy et al., 1973; Yamashiro and Li, 1973). The synthetic peptide has the same biological activity as the natural compound (Yamashiro and Li, 1973; Vale et al., 1975).

Somatostatin has been identified at several sites in the central nervous system. Krülich and coworkers (1968) demonstrated high SRIF concentrations in rat median eminence by bioassay. The

availability of radioimmunoassays for SRIF (Arimura et al., 1975; Patel and Reichlin, 1978) and immunohistochemical evidence (Pelletier, 1980) indicates that although found in measurable quantities in other parts of the brain and spinal cord, somatostatin is present in highest concentrations in the hypothalamus. Outside the nervous system, immunoreactive somatostatin has a widespread distribution (Efendic et al., 1978; Efendic and Luft, 1980; Arimura and Fishback, 1981).

Somatostatin is a powerful inhibitor of GH secretion in vitro and in vivo, in animals (Kasting et al., 1981; Cowan et al., 1981; Cowan et al., 1984) and humans (Ward et al., 1975; Christensen et al., 1978), though no effect on basal GH levels has been demonstrated in man (Mortiner et al., 1974) or dog (Cowan et al., 1984). This lack of effect may be either due to assay insensitivity for hormonal changes within the normal range (although this possibility was excluded in Cowan's dog experiments) or may reflect that maximal endogenous somatostatinergic tone is present in the basal state. However, basal secretion is suppressed in the rat (Brazeau et al., 1974; Martin, 1974) and from isolated pituitary tissue (Stachura, 1976) as is the GH response to several stimuli. These include exercise (Prange-Hansen et al., 1973), insulin induced hypoglycemia (Hall et al., 1973), arginine (Siler et al., 1973; Mortiner et al., 1974), L-dopa (Siler et al., 1973; Lovinger et al., 1974), electrical stimulation of the ventromedial nucleus (Martin, 1974), sodium pentobarbital (Brazeau et al., 1974), isoprenaline and

chlorpromazine (Kato et al., 1974), dibutyryl cyclic AMP (Borgeat et al., 1974 Peracchi et al., 1976), and hyperthermia (Zierden et al., 1975). In addition, the elevated GH levels seen in hepatic and renal disease are lowered (Pimstone et al., 1975). GH surges during sleep (Parker et al., 1974; Lucke et al., 1976) and when alert (Ward et al., 1975) are also inhibited.

Somatostatin suppresses the elevated basal GH levels of acromegaly (Hall et al., 1973; Yen et al., 1974) as also the spontaneous GH surges and those in response to feeding (Besser et al., 1974) in these patients. The GH response to TRH seen in some acromegalics is blocked by somatostatin (Gomez-Pan et al., 1975). There is evidence that secretion of the monomeric and therefore most biologically active form of GH is preferentially blocked by SRIF (Benker et al., 1975).

Infusion of exogenous growth hormone inhibits endogenous GH secretion in the rhesus monkey (Sakuma and Knobil, 1970) as does the injection of GH into the lateral ventricle of the rat brain (Tannenbaum, 1980). This influence of GH on it's own secretion may be mediated by SRIF as hypophysectomy and the consequent decrease in circulating GH lowers somatostatin like immunoreactivity in the rat hypothalamus (Molitch and Hlyviyak, 1980) particularly in the medial preoptic area (Terry and Crowley, 1980). Moreover, intraventricularly injected GH stimulates somatostatin release into rat hypophyseal portal blood (Chihara et al., 1981) and manipulations increasing or decreasing plasma GH

show that an inverse relationship exists between hypothalamic SRIF concentration and release, and circulating GH levels (Berelowitz et al., 1981). Also, an intraventricular injection of SRIF into the third ventricle of rats induces the release of GH and may possibly act by suppressing the release of somatostatin from somatostatin containing neurons (Lumpkin et al., 1981).

The large body of accumulated evidence strongly suggests that somatostatin is at least in part responsible for the control of GH secretion, but it's precise role in the regulation of the high amplitude secretory bursts of GH needs to be further elucidated. Certainly, decline in GH biosynthesis in pituitary homografts (Zanini et al., 1979) and a decrease in plasma GH levels in pituitary stalk sectioned animals (Wehrenberg et al., 1980) and those with extrasellar pituitary transplants (Schalch & Reichlin, 1966) suggest the existence of a suprahypophyseal facilitatory influence as well, and this issue will be examined further in the Discussion.

5. Effect of stress on GH secretion

It has been demonstrated that endocrine systems respond specifically and in discrete patterns to different stressors (Mason, 1971). As reviewed by Lenox and associates (1980), brain neurochemical systems also respond to stress.

Various forms of stress provoke an increase in plasma growth hormone levels in humans (Glick et al., 1965; Schalch, 1967) and rhesus monkeys (Meyer and Knobil, 1967), though the stress of

transportation has been shown to suppress GH secretion in cattle (Reynaert et al., 1976).

Studies on the effects of different stressful stimuli on the release of GH in small rodents, both rats (Takahashi et al., 1971) and mice (Schindler et al., 1972), have been found to differ markedly from those seen in the primate, and confirm the original observations of Schalch and Reichlin (1968) and Garcia and Geschwind (1968) that a variety of stress stimuli depress plasma GH in the rat. They further demonstrate the importance of distinguishing between the effects of the blood sampling procedure per se (e.g. handling of animals, catheterization, type of anesthesia) and the influence of the experimental stimulus being examined. The apparent exquisite sensitivity of the rat's hypothalamic-pituitary GH secretory system to stress undoubtedly contributes to the marked fluctuations in plasma GH values observed in this species and the non parametric distribution of these levels. Even in rats, minimizing the stress of handling by prior training or "gentling" results not only in a significant increase in the mean plasma levels of fed animals but also in a distribution approaching a parametric pattern for these values (Takahashi et al., 1971).

Stimuli that have been used experimentally to demonstrate the depressive effect of stress on rat plasma GH include ether (Schalch and Reichlin, 1968; Takahashi et al., 1971), insulin induced hypoglycemia and intraperitoneal hypertonic glucose (Takahashi et al., 1971), blood withdrawal (Rice and Critchlow, 1976),

tibial fracture and rubber band tourniquets (Takahashi, 1978), exposure to cold and immobilization stress (Lenox et al, 1980). In fact these workers have shown an inverse relationship between plasma GH and corticosterone in stressed rats and have suggested the likelihood of a final common pathway at the level of the hypothalamus in the mediation of decline in plasma GH. Arimura and coworkers (1976) and Terry and coworkers (1976) demonstrated that antisera to somatostatin prevent the stress induced decrease in circulating GH levels in the rat, and their conclusion regarding the role of SRIF is supported by the finding of Rice and Critchlow (1976), who in experiments utilizing brain lesions found that the preoptic region of the hypothalamus, now known to be the source of hypothalamic somatostatin, was essential for the GH response to stress in this species.

Although the effect of stress on the release of GH has been characterized with various stressors in both rat and man, more needs to be known in this regard with respect to the dog. Insulin induced hypoglycemia and 5-hydroxytryptophan, both of which cause stressful behaviour in dogs, elicit an increase in plasma GH and glucocorticoid levels (Dorsa and Connors, 1979). An earlier study (Lovinger et al., 1974) suggested that surgical stress had no effect on canine GH levels, but their contention is not very informative since they assayed for GH only 5 min before and 5 min after the acute surgical stress (adrenal vein cannulation). Even though scant, the available work does imply that stress in general, does not decrease GH secretion as it does in the rat, and dogs may more

resemble primates in this respect.

6. Techniques employed in studying the GH control system

As reviewed briefly earlier in this chapter, actions of the hypothalamo-hypophysiotropic hormones were extensively studied even before these hormones were isolated. In order to understand their action, experiments were performed using crude extracts from hypothalamic tissue or by suppressing or stimulating production or release of a hormone by stereotactically placed lesions or electrical stimulation of specific areas of the brain. Although the use of extracts and surgical techniques has yielded much information about the neurochemical mechanisms and neural areas involved, their usefulness in clarifying the actual physiological conditions effecting and controlling release of an adenohypophyseal hormone is limited. Information gleaned by utilizing hypothalamic extracts is of dubious significance because of the possible presence of impurities or more than one active neuroendocrine fraction; likewise, surgery can distort the physiological system by interrupting fibres conveying impulses or disturbing normal portal flow. The stressful effects of surgical intervention, substances released by tissue damage attendant on surgical trauma, and anesthetics, independently or in conjunction with each other, can modify normal GH secretion.

The availability of pure preparations of releasing or inhibiting hormones is particularly fortuitous in this respect. Used as antigens to generate specific antisera, they have not only made

it possible to establish specific radioimmunoassays for these hormones, but also blocking the action of an endogenous hormone by neutralization with it's antiserum permits the elucidation of it's physiological role in vivo.

Some studies utilizing antisera to somatostatin are available where the antisera have been raised variously in rabbits, sheep and monkeys, and all published material is from studies done on rats. Following treatment with serum containing antibodies to SRIF, increased basal GH values are observed in apparently unstressed rats (Ferland et al., 1976; Terry and Martin, 1981) and abolition of episodic GH bursts by stress induced SRIF secretion is reversed (Arimura et al., 1976; Terry et al., 1976; Tannenbaum et al., 1978) although it has little or no effect on the amplitude of GH surges in unstressed rats (Terry and Martin, 1981).

Most of the in vivo data on episodic secretion to date are essentially bivariate time vs. concentration data with distribution of GH in a circulating pool that damps out changes. Since the event being studied is the release of GH from the pars distalis, the calculation of secretion rates is a much more sensitive and appropriate determination than an evaluation of changes in GH concentration with time. Radioactive tracers can be used in secretion rate calculations, but infusion of anything more than true trace amounts may interfere with endogenous hormone production or release, and unless the tracer is incorporated into an amino acid of the hormone molecule, labelling may alter it's biological

activity or clearance. An approach based upon predetermined model elements, on the other hand, can be utilized providing that the metabolic clearance rate (MCR) determined for a given substance is constant for that species and does not vary with changes in the circulating concentration (C) of that substance (or that such variation be predictable). Also, the apparent volume of distribution (V) for that substance, in that species, must be constant (or must vary in a reliable and predictable way with concentration).

The choice of a single compartment model with constant MCR to convert bivariate GH concentration vs. time data into continuous secretion may seem simplistic, especially in light of the multi-exponential disappearnce of labelled GH in the dog (Kramer et al., 1973), human (Glick et al., 1964) and rat (Peake et al., 1968). However, as discussed in relation to the GH system by Cowan and coworkers (1984), as a first approximation even for very rapid departures from steady state, this approach has been entirely successful for ACTH (Cowan, 1978) and despite the handling of labelled GH (or the label) noted above, it seems likely that in physiological situations it is handled nearly linearly. Normalizing data from the literature rather arbitrarily on a per weight basis, it is noted that neither species nor concentration of GH alters it's MCR very much, being: in man 3.25 mL Kg^{-1} min^{-1}; and in male and female mice 5.25 mL Kg^{-1} min^{-1} (Taylor et al., 1969; Frohman and Bernardis, 1970; Althen and Gerrits, 1976; Sinha et al., 1979). Cowan's value of 3.99 ± 0.30 mL Kg^{-1} min^{-1} for dogs, lying as it

does between the value for men and swine and the values for rodents, seems unsurprising; and the narrow range of all these values suggests that in the working range a load term model might well use a near constant MCR. As for the single compartment, and hence single value for apparent distribution volume, this is suited to the study of only relatively fast events. For concentrations changing rapidly, the single fast compartment is dominant, and for slower changes in concentration, the VxdC/dt term is of no consequence compared to the MCRxC term, so that any errors resulting from the single-compartment approach would be negligible. The value of V calculated for the dog is 57.9 ± 5.5 mL Kg^{-1} (Cowan et al., 1984). An example of the effect of using this approach to calculate continuous secretion rates from bivariate concentration vs. time data on one experiment in this study is depicted in Figure 2.

7. Physiological context of this study

Observations made upon the effects of the addition of somatostatin to isolated pituitary tissue or the intravenous administration of somatostatin *in vivo* may represent pharmacological rather than physiological effects. Normally, very small amounts of somatostatin reach the pituitary via the hypothalamo-hypophyseal portal circulation (Chihara et al., 1979; Gillioz et al., 1979; Schusdziarra et al., 1980; Kasting et al., 1981). Likewise, observations on the effects of SRIF in pathological conditions such as acromegaly cannot be used to draw conclusions regarding the physiological role of SRIF as tumor cells may not respond in the same manner as normal tissue. The use of anti SRIF

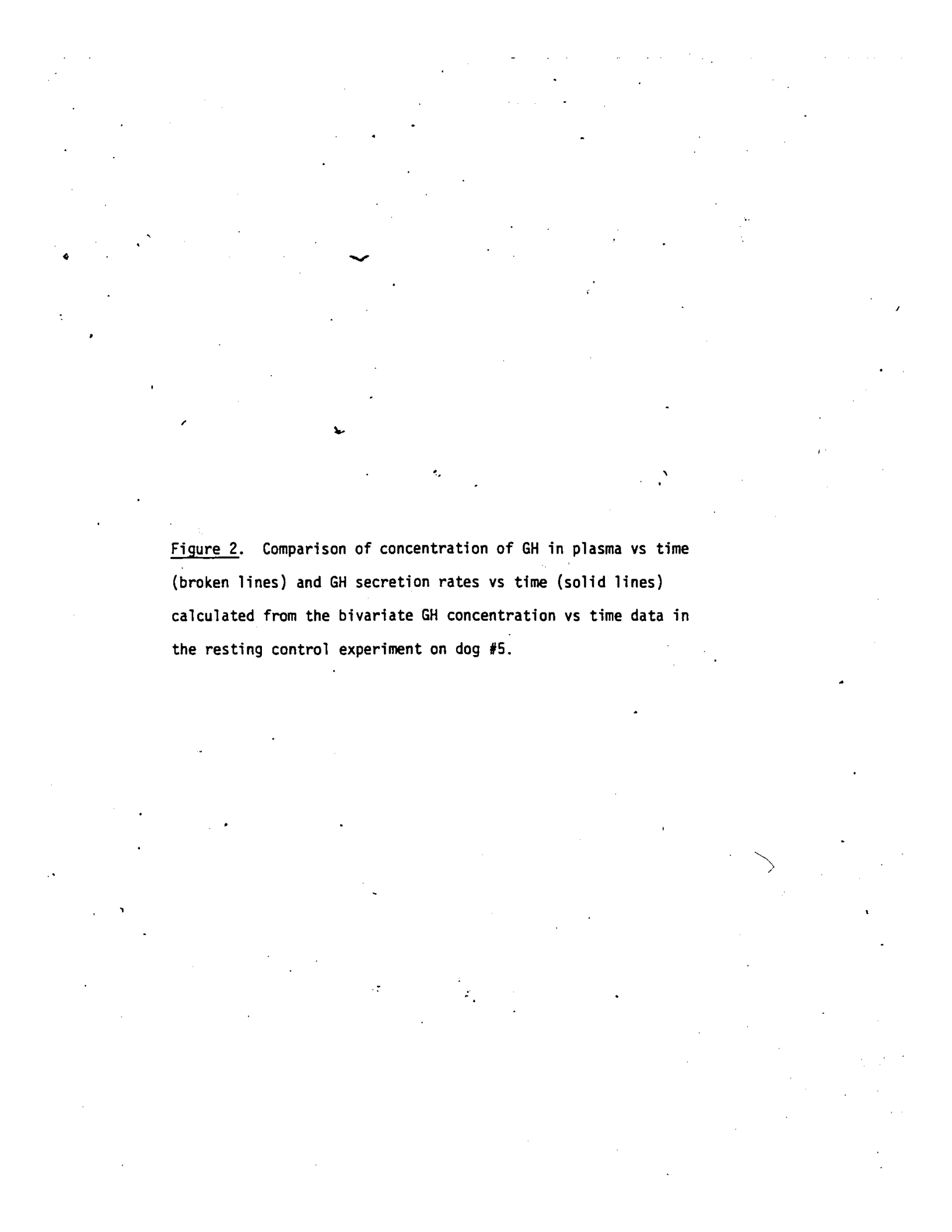
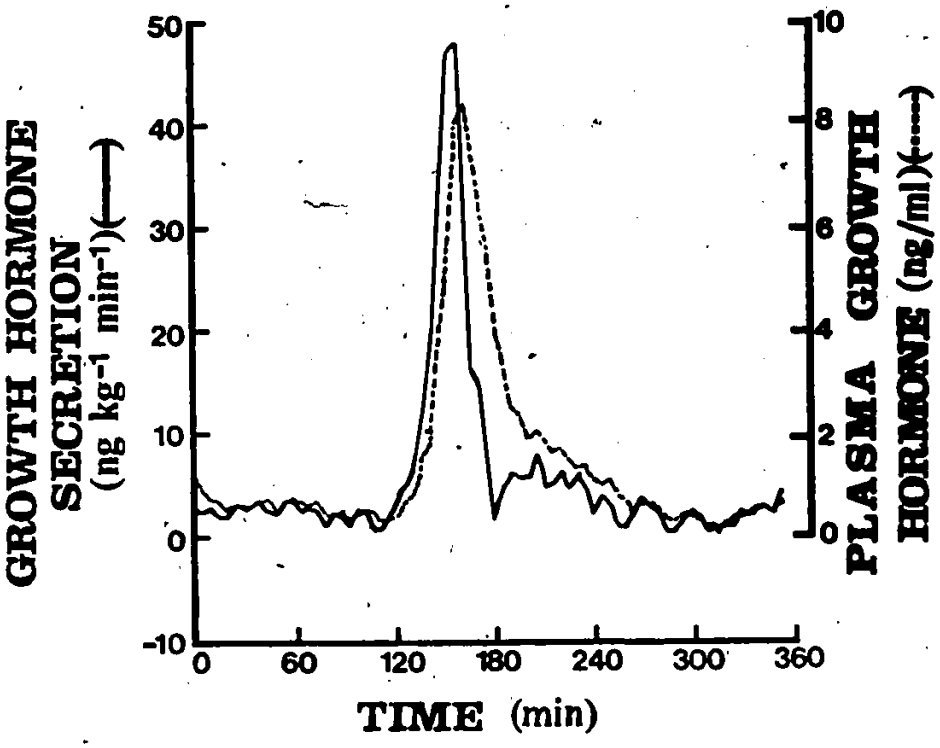


Figure 2. Comparison of concentration of GH in plasma vs time (broken lines) and GH secretion rates vs time (solid lines) calculated from the bivariate GH concentration vs time data in the resting control experiment on dog #5.


GROWTH HORMONE SECRETION (ng kg⁻¹ min⁻¹)(——)
PLASMA GROWTH HORMONE (ng/ml)(----)
50
40
30
20
10
0
-10
10
8
6
4
2
0
0
60
120
180
240
300
360
TIME (min)

antibodies in intact animals which allows for "non-surgical" decline in circulating somatostatin, therefore presents an appropriate model. Dogs are a useful animal for the purpose of this study because they are easily available, have a blood volume sufficient for serial sampling in experiments lasting several hours, and adapt well to handling and experimental conditions; also, sensitive RIAs for canine GH are available. The differences of GH secretion between sexes (Birge et al., 1967; Reynaert et al., 1976; Tindal et al., 1978) is eliminated as a source of variation by using only male animals in the study.

As reviewed earlier in this chapter, the GH response to stress in the dog has not been well characterized. This study presents an opportunity to evaluate the secretory characteristics of GH due to the effects of specific stressors.

8. Statement of the problem. Somatostatin is an inhibitor of growth hormone secretion in vitro and in vivo, and blocks the augmented GH released in response to several stimuli. A modest constant infusion of SRIF abolishes the spontaneous GH secretory spikes normally seen in dogs (Cowan et al., 1981; Cowan et al., 1984), but does not lower basal secretion. This suggests that during basal GH secretion, a surfeit of SRIF impinges on the somatotrophs, as extra SRIF does not further lower secretion; but for secretory bursts to occur, very little SRIF must be present, as exogenous SRIF blocks these bursts.

These indirect indications that decline in concentration of somatostatin plays a major role in the initiation of secretory episodes of GH needs to be clarified by a more direct approach to the problem.

Thesis question: Does exogenous manipulation which artifically reduces endogenous SRIF impinging on the somatotrophs evoke GH secretory bursts?

Since the injection of foreign sera are stressful to animals to some degree, the GH response to stress in the experimental animal used in this study needed to be examined.

Corollary thesis question: Do stresses evoke GH secretion in dogs?

CHAPTER II

MATERIALS AND METHODS

1. Animals

Ten random source normal adult male mongrel dogs, 6.9 - 17.3 Kg, were used in the study. On arrival at our Animal Care facility, they were subjected to a period of "conditioning". During this time their nutritional status was assessed, hematological profiles evaluated and stool tests were done for gastrointestinal parasites. Their immunization status was ascertained to include vaccinations for Distemper, Adenovirus type I & II, Leptospirosis, Parainfluenza, Parvovirus and Rabies, and any deficiencies in this regimen were made up.

The dogs were quartered in standard cages under conditions of constant temperature (20-25^{0} C) and relative humidity (45-55%) on a 12 hour light, 12 hour dark cycle with lights on at 7:00 a.m. The animals received a normal diet of dry dog food and water ad libitum, with routine daily exercise.

Prior to being used in experiments, the dogs were trained along the lines of a simple reward-punishment principle, to lie on a comfortable pad in the laboratory for up to 6 hours at a time. At the end of each training session, they were rewarded with a can of dog food. Such training was continued during periods between experiments on the same dog.

2. Experimental Designs

Group I: Effects of sheep serum containing antibodies to SRIF on GH secretion.

To determine the effect of antibodies to SRIF (contained in sheep serum) in dogs, they were subjected to resting control experiments, to experiments in which the antibody to SRIF was injected systemically, and to experiments in which control goat sera not containing antibodies to SRIF were used. The essential features of the 3 kinds of experiments in this group are as follows:

a) Resting Control Experiments. Blood from conscious, resting dogs was sampled at 5 min intervals for 6 hours for GH (by RIA) and at 30 or 60 min intervals for determination of total glucocorticoids (as an indicator of stress). Sampling was with saline replacement, and total blood removal was less than 140 ml. Although adequate control GH data on dogs was available from previous studies (Ref: Cowan et al., 1984), these experiments were necessary to screen animals to be used for experiments described below in (b). Because of a limited supply of antibodies to SRIF, and in order to give comparatively larger doses of this material, small animals had to be used. The purpose of these experiments then, was to exclude endocrine abnormalities, (i.e. to make sure that the dogs were small because they were small, and not because they were dwarfs!) 5 dogs (6.9 - 11.5 Kg) that had been selected in this way were used in the anti-SRIF experiments.

b) Anti SRIF Experiments. These 6 hour experiments were similar to controls, but 3 hours after the start of the experiment, 8.5 mls of

sheep serum containing antibodies to SRIF was administered as a bolus systemically. Starting 5 min before this injection and for 30 min following it, blood was sampled at 1 min intervals to improve the resolution of the measured responses. In 4 of 5 such experiments, two additional blood samples for glucocorticoids were also taken at 20 and 40 min after the manipulation to get a more precise time course of any stress caused by it.

c) Experiments Using Control Goat Sera in Naive Animals. These experiments were performed to determine whether the effects observed in the anti SRIF experiments were due to the antibodies to SRIF or a result of the administration of foreign serum per se. 5 naive animals (dogs that had no history of previous exposure to foreign serum) were treated with a bolus injection of plain goat serum*. The sera used in these animals were from 3 different goats (for details see Table III).

The first of these five experiments was of 6 hours duration with sampling intervals and timing of injection identical to the experiments described in (2) above. The remaining four experiments lasted 2 hours each**; there was a control period of 45 min, and blood sampling

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*For dose of goat serum used in these experiments, refer to Table IV.
**Explanation needs to be given regarding the sequence of experiments and the change made in their duration. After the anti-SRIF experiments had been completed and plasma from these experiments sent for GH RIA, it was decided to proceed with experiments using control goat serum. 4 "sensitized" animals that had received anti-SRIF containing sheep serum (described later in the second group of experiments done) and one "naive" animal were available at the time and the old 6 hour protocol was followed in these experiments. However, after the results of the GH assay were received a few weeks later, it was observed that the effect of the anti SRIF serum became evident within one minute following its injection. It was therefore decided that shorter 2 hour experiments with plain goat sera would suffice for comparison. The protocol with respect to intervals in

was continued for an hour and 15 min, after injection of the plain goat serum.

GH secretory responses to plain goat sera in naive (non-sensitized) dogs were either nonexistent or small in comparison to the effects of anti-SRIF antiserum (see Results), but clinical observations from the dogs (see Appendix) and glucocorticoid data suggested that both plain sera and that which contained antibodies to SRIF had been stressful to the animals to some degree. The GH response to stress in dogs had not so fare been clearly characterized, and to study this issue further, a second group of experiments was undertaken.

Group II: Effects of Various Stressors on GH Secretion: Anaphylaxis Hypovolemia, Ether.

d) Exposure of "sensitized" animals to control goat sera. A total of six such experiments were performed. The first four of these were done on dogs that had previously received anti-SRIF-containing sheep serum, one week earlier in 3 cases and four months earlier in the fourth dog. The design for these was similar to the anti-SRIF experiments except that 10 mL of plain goat serum was injected 3 hours after the

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blood sampling and timing of manipulation established for these experiments was maintained for all subsequent 2 hour experiments. Samples for GH assay were taken every 5 min, with sampling intervals reduced to 2 min during the period from 10 min before the manipulation to 30 min after it. For the estimation of glucocorticoids, blood samples were taken at about 20 min intervals with 2 such samples during the control period of an experiment. Blood pressure was continuously monitored in four (the second to the fifth) of these 5 experiments. Total blood withdrawal never exceeded 75 mL, with fluid replacement as described under Experimental and Surgical Procedures.

start of the experiment. Two of these first 4 dogs which received the control goat serum only a week after the AS-SRIF serum showed severe anaphylaxis; one of them (dog #5) actually died before the experiment was complete. More time (about 2 months in each case) was allowed to elapse before second exposure in the additional 2 dogs, with an attempt to permit a degree of decline in titre of antibodies to constituents of foreign serum to occur. Both these were 2 hour experiments with continuous monitoring of arterial blood pressure.

e) Hypovolemia Experiments. Four such experiments were done. Forty-five min after the onset of these 2 hour experiments, blood was rapidly withdrawn into sterile siliconized heparinized glass syringes, with the aim of producing a transient hypotensive state (lasting 30 min) with a mean arterial pressure of 60 mmHg. This procedure took 3½ - 10 min and the blood withdrawn was collected in sterile siliconized heparinized glassware which was immediately sealed with parafilm. (A total of 1000 i.u. of heparin was added per dL of blood and was found sufficient to prevent coagulation). Vital signs were monitored. Twenty-five to 30 min from the time that blood withdrawal had been completed, the blood was reinfused rapidly into the animal. Before reinfusion the blood was filtered to remove gas bubbles and any small clots that may have formed.

f) Ether Inhalation Experiments. Four such experiments were done. Forty-five min after the beginning of each of these experiments, ether was administered to the animal by means of a face mask, taking care that liquid ether didn't come in direct contact with skin, mucous membranes

or eyes of the animal. The mask was continuously held in place for 10 min and then removed. During this time the animal usually struggled vigorously and had to be restrained. Continuous monitoring of blood pressure and sampling intervals similar to other two hour experiments were used.

3. Experimental and Surgical Procedures

On the day of an experiment, the animal was released from it's cage at 7:30 a.m. and allowed to move about freely in an outdoor run for a few minutes. The dog was weighed before being taken to the experiment room.

Insertion of polyethylene cannulae for blood sampling (usually saphenous vein to inferior vena cava, Clay Adams PE 190) was done under sterile conditions using lidocaine hydrochloride local anaesthesia (L.A.), normally the day before an experiment. From time to time, cannulae were maintained for aweek and reused. About half an hour before an experiment, the *in situ* cannula was re-exposed under L.A. and flushed with 5-mL of heparin in isotonic saline to confirm patency and free flow.

In experiments where arterial pressure was to be monitored, the femoral artery and vein were cannulated through a medial longitudinal incision in the thigh (PE 190, 205 or 240 depending on the size of the blood vessel). These cannulations were carried out on the morning of the experiment, with the experiment starting an hour after completion of the surgical procedure.

Fluid replacement, after withdrawal of a blood sample, was achieved with normal saline, with about one and a half times the volume of saline to the volume of blood withdrawn. Periodically, the cannula was flushed with weakly heparinized normal saline (20 USP units heparin/mL of normal saline) to maintain free flow through the cannula during sampling.

Observations concerning the animal's general condition, vital signs etc. were recorded in each experiment, and these have been commented upon in the Appendix.

At the end of the day's experimental procedures, the surgical wound was closed with 00 silk and a furacin dressing applied. The animal received a can of dog food and water ad libitum before being returned to its cage.

4. Characteristics of the AntiSomatostatin Antiserum (AS-SRIF)

About 43 mL of this material was obtained from Dr. Fernand Labrie of L'Universite Laval, Quebec, in a frozen state. It had been raised in a sheep, and had been proven effective in Sprague-Dawley rats, 0.5 mL i.v. being sufficient to elevate basal plasma GH in the rat substantially for a period of at least an hour. The antiserum was prepared by the method described by Arimura and co-workers (1975) and had been used previously in studies done on rats (Ferland et al., 1976; Norstedt et al., 1983).*

The antiserum was divided into aliquots of 8.5 mL. Although normalized by weight this constituted a smaller dose for a dog than had been used for tests in rats, dividing in this way allowed for testing it's effects on 5 dogs.

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*This serum contained a high titre of antibodies to SRIF. At a dilution of 1/80,000 it was capable of producing a binding curve with SRIF with an IC50 of 16 pg/tube (16 pg/mL). It did not cross react with LHRH, TRH, substance P, vasopressin, oxytocin, β-endorphin, met-enkephalin or neurotensin.

The antiserum was maintained at -70° C in a freezer. On the day that it was required for injection, it was removed from the freezer about 3 hours prior to it's use, and allowed to thaw gradually, first in the lower compartment of the refrigerator, and then to room temperature. Ten min before injection, it was loaded into a sterile disposable 10 mL syringe.

5. Characteristics of the Goat Sera

Three batches of this material, from three different goats, were obtained from Qualicum Scientific Ltd. Ottawa, Ontario. The sera had been prepared in the following way: blood collected from a goat under sterile conditions was defibrinated and centrifuged and the supernatant was passed through a crude .22 Millipore filter. The filtrate (serum) was hermetically sealed in a sterile glass bottle and frozen, and was delivered to us in this state.

The sera were stored in 10 mL aliquots at -70° C and thawed for use in the same fashion as the serum containing antibodies to SRIF.

6. Monitoring Mean Arterial Pressure (M.A.P.)

The femoral artery, exposed through a longitudinal incision on the superomedial aspect of the dog's thigh was cannulated with a polyethylene tube (Clay Adams PE 190, 205 or 240, depending upon the size of the blood vessel). The cannula was connected to a Statham P23Db pressure transducer that had been hooked on to a Grass Model 7 Polygraph through a low level 7P1 D.C. preamplifier.

This apparatus had been calibrated about two hours before each experiment, using a mercury manometer. Pressure tracings were recorded at a paper speed at 25 mm/min, with M.A.P. obtained by electronic integration (low frequency filter).

7. Handling of Samples

Blood samples for GH assay were collected in 1.5 mL heparinized labelled polypropylene test tubes that were kept on ice. They were spun in pairs at 4° C in an Eppendorf microfuge (15,600 g) for 3 min. There was never a gap of more than about 5 min between sample collection and centrifuging. The plasma was pipetted into numbered, color coded 1.5 mL polypropylene test tubes and kept in the freezer compartment of the refrigerator.

At the end of an experiment, the samples were transferred to a -70° C deep freeze. Later, they were packed in dry ice and sent to the Department of Physiology, Queen's University where they were once again stored at -70° C. On the day that the samples were to be assayed, they were taken out of the freezer and allowed to thaw at room temperature. They were then assayed for growth hormone in triplicate by RIA.

The samples taken for measurement of corticosteroids were treated in the same manner except that after they had been centrifuged, plasma was collected in labelled, color coded glass counting vials. On the day that they were to be assayed, they were removed from the deep freeze, and allowed to thaw at room temperature and assayed for glucocorticoid content in our laboratory.

8. Radioimmunoassay for Growth Hormone

GH was measured in triplicate, usually at one dilution, by a double antibody RIA using reagents provided by the NIAMDD Pituitary Hormone Distribution Programme with the antibody to canine GH provided by Dr. A.F. Parlow. This assay is similar in important respects to the

one described by Lovinger et al. (1974) but differs from this in that antiserum to cGH is raised in monkeys (AFP-214-121577) instead of guinea pigs.

Results were expressed as nanograms per mL (ng/mL) of canine GH (AFP-1983-B). Samples from one experiment were measured within one assay, together with a known pooled sample and a sample of hypophysectomized plasma which consistently gave values near zero, and always below 0.4 ng/mL. Values above the low standards (0.5 ng/mL) were sufficiently reliable and repeatable that 0.5 ng/mL was considered as a reliable lower limit for assay sensitivity for individual samples, with even lower limits for consistent runs of sequential samples. The intra-assay coefficient of variation was less than 10%.

9. Assay for glucocorticoids

Samples were assayed in duplicate using a micro-modification of the fluorimetric method of Silber, Busch and Oslapas (1958), on an Aminco-Bowman Spectrophotofluorometer with an excitation wavelength of 465 nm and emission wavelength of 525 nm. Standards containing 2 µg/mL of cortisol and corticosterone (Sigma) and blanks (distilled water) were run at the beginning and end of each assay. All the glassware used had been pre-washed in absoluté alcohol.

Three mL of methylene chloride (Baker), introduced with an automatic dispenser, were added to 500 λ of plasma (300 λ for some experiments) that had been aliquoted into conical bottomed glass tubes. The tubes were vigorously agitated for 20 sec each, then centrifuged at 1000 g for two min. Using a vacuum line, the top layer (aqueous phase) was

removed. From each tube, 2 mL were pipetted into a fresh set of round bottom glass test tubes. No NaOH wash was used, since only plasma from male animals were assayed.

The fluorescence evoked by this 2 mL of steroid containing methylene chloride in 2 mL of a sulphuric acid-ethanol mixture was measured at 60 minutes. Initiation of the fluorescence reaction was staggered so that all samples could be read at 60 ± 1 min.

Using a linear regression program on the Wang 600 mini-computer, total glucocorticoid concentration was calculated, with the assumption that B:F ratio$^{\Delta}$ was unity, as is roughly the case in dogs (Ganong, 1981). This assumption could introduce systematic errors of ± 6% in total glucocorticoids if there were a 20% shift in this ratio (due to different fluorescence of B and F).

10. Processing of Data

a) Continuous Secretion Rates for GH were obtained by transforming the bivariate GH concentration vs. time data using the single compartment approach previously validated for ACTH (Cowan, 1978) with clearances and volumes verified for GH (Cowan et al., 1984)$^{+}$ using the equation:

$$\text{Secretion rate } (Ra)_t = \left(GH_t \times MCR\right) + \left(\frac{dGH_t}{dt} \times V\right) *$$

$^{+}$For dogs, MCR and V were found to be 3.99 ± 0.30 mL·Kg^{-1} min^{-1} and 57.9 ± 5.5 mL/Kg, respectively.

*GH_t: Concentration of GH at time for which secretion rate is being calculated.
MCR: Metabolic clearance rate of GH in dog.
V: Apparent distribution volume of GH in dog.

$^{\Delta}$Corticosterone: cortisol ratio

The rates of change of GH concentration ($\frac{dGH_t}{dt}$ term) were obtained by fitting sliding parabolas through 3 points (including the points preceding and following the point for which secretion rate is being calculated). Secretion rates were plotted against time.

In experimental periods with "rapid" sampling (i.e. intervals between samples of 1 or 2 min instead of 5 min), assay errors contributed substantially to the $\frac{dGH_t}{dt}$ term, and from time to time obscured physiological influences on the derivative term. Data for these periods were smoothed further by using sliding parabolas fitted to 5 rather than 3 points, and the middle of such a curve used for calculation of $\frac{dGH_t}{dt}$. In this way time periods of 5-10 min were incorporated into the function fit, and 2 degrees of freedom remained for error analysis.

A different approach was used for the initial and final intervals of the experiments, where the slopes from 2 point straight lines were used.

b) Basal Secretion Rates were determined from the basal periods of curves obtained by graphing secretion rates vs. time data, with the basal secretion rate taken as the value which had an equal area of the curve above and below it.

c) Total Duration of the Peaks in GH secretion were taken to be the time at which the secretion rate crossed the mean basal secretion rate

in a monotonically increasing function, which subsequently rose to a peak value, to the time at which the secretion rate crossed the mean basal secretion rate, following a peak, in a monotonically decreasing function. Failing this criterion, points of slope reversal were used, i.e., the time at which the slope of the secretion rate changed from negative to positive at the onset of a peak or from positive to negative at the termination of a peak. Since the initiation and termination of a GH spike may not always be abrupt, as is especially the case with a gradual rise in GH secretion before the steep sloped increase in amplitude in the spontaneous episodes, the duration of the steep portion of the spike is separately assessed.

d) Duration of the Steep Component of a Peak was taken to be the central portion of the secretory burst during which the first derivatives of the secretion rate significantly exceeded those occuring during the normal excursions which characterized basal periods.

e) Integrals of the Peaks were determined by evaluating the area under each secretory spike, the integral representing the total secretion during a peak (ng/kg). These integrals were computed sums of histogram rectangles topped by right-angled triangles.

All calculations were done using programmes on the Wang-600-14-TP minicomputer.

Means ± S.E.M. were calculated for each kind of experiment for the maximum secretion rate during a peak, the total and steep duration of each peak and the total amount of GH secreted during

these peaks, and has been presented in the tables in the Results section.

TABLE III

Summary of animals and distribution of experimental designs

Dog #	Weight Range (Kg)	6 hr Resting	Anti SRIF	Goat Serum in Naive Animals*	Goat Serum in Sensitized Animals*	Hypovolemia	Ether
1	8.5	✓	✓	-	-	-	-
2	10.4-13.05	✓	✓	-	✓(G.S.1)	-	-
3	8.3-8.9	✓	✓	-	✓(G.S.1)	-	-
4	6.9-7.2	✓	✓	-	✓(G.S.1)****	✓	✓
5	8.6-8.9	✓	✓	-	✓(G.S.1)**	-	-
6	10.7-11.3	-	-	✓(G.S.1)	✓(G.S.2)	✓	✓
7	13.25-13.6	-	-	✓(G.S.2)	-	✓	✓
8	17.15-17.3	-	-	✓(G.S.2)	-	✓***	✓
9	7.6	-	-	✓(G.S.3)	-	-	-
10	10.4	-	-	✓(G.S.3)	-	-	-

*G.S.1, 2 and 3 represent goat sera from the first, second and third batch, respectively.

**Dog died about 30 min following injection of goat serum. This experiment will be separately dealt with qualitatively in the Discussion.

***For dogs #4, 6 and 7 the ether and hypovolemia experiments were done on the same day with ether exposure first and a rest period of 2 hours between the two experiments. In the case of dog #8, because of problems with the arterial cannula after the ether experiment, the hypovolemia and goat serum experiments were done on the same day with the goat serum experiment starting 2 hours after the end of the hypovolemia experiment.

****Additionally, this dog was exposed to goat serum (G.S.2) once again after an interval of 2 months following this experiment (which used G.S.1). Results from the extra experiment not noted in this table are examined in the Discussion.

TABLE IV

Doses of sera injected as a bolus in different types of experiments*

Dog #	Sheep Serum Containing Antibodies to SRIF (mL/Kg)	Control Goat Sera in Naive Dogs (mL/Kg)	Control Goat Sera in Previously Sensitized Dogs (mL/Kg)
1	1.0	-	-
2	0.74	-	0.77
3	0.96	-	1.12
4	1.20	-	1.45
5	0.97	-	(1.12)**
6	-	0.86	0.93
7	-	0.74	-
8	-	0.58	-
9	-	1.12	-
10	-	1.06	-
Mean ± S.E.M.	0.97 ± 0.07	0.87 ± 0.10	1.07 ± 0.15

*Dose normalized for weight of each dog on the day of the experiment.

**Not included in mean, as this experiment is incomplete (see Discussion).

CHAPTER III

RESULTS

1. Growth Hormone Secretion

These data have been described and presented in tabular form in this chapter. Graphical representations of each of the experiments are appended at the end of this thesis.

a) Resting Control Experiments. GH secretion characteristics observed in 5 resting dogs are summarized in Table V. All animals had one or two spontaneous secretory bursts in 6 hours, superimposed on a basal secretion which was remarkably uniform throughout an experiment and showed relatively little variation in subsequent experiments done on the same animals.

These dogs show very similar characteristics in their secretion of GH when compared to previous work (Cowan et al., 1984). The peak/basal secretion ratio $\frac{22.3}{3.1} = 7.3$, peak GH secretion rate, the total amount of GH in excess of basal secreted during the episodes and total spike duration are all comparable. The duration of the steep portion of the spike is somewhat longer than the value of 25.1 ± 1.2 min found in the previous study, because of the bias introduced by the large value obtained for dog #5 superimposed on a small "N".

b) Anti SRIF Experiments. High amplitude GH secretory surges were elicited in all 5 animals that received a bolus injection of serum

TABLE V

Resting control experiments*

Dog #	Basal GH Secretion Rate (ng/kg·min)	Peak GH Secretion Rate in Spike (ng/kg·min)	Total Duration of Spike (min)	Steep Portion of Spike (min)	Number of Peaks During Expt.	Total Extra GH Released Due to Spike** (ng/kg)
1	3.6 (4.0)	17.2 (18.9)	45 (30)	25 (22)	1 (1)	399.6
2	4.8 (4.8)	12.8 (12.4;16.0)	50 (30,N.A.)	35 (22,N.A.)	1 (2)	223.6
3	2.5 (2.5)	19.4; 10.3	27.5; 26	25; 22.5	2	227.5; 101.2
4	2.5 (2.2)	25.2	50	27.5	1	428.7
5	2.2 (2.5)	48.6	64	46	1	1137.2
Mean ± S.E.M.	3.1 ± 0.5	22.3 ± 5.7***	43.8 ± 6.0	30.2 ± 3.6	1.2 ± 0.2	419.6 ± 151.9***

*Parentheses represent GH baselines and spikes occuring during control periods of anti-SRIF experiments.

**Calculated as the integral of the GH secretion rate curve during the spike, less basal secretion for that period.

***Values represent mean spike, not mean dog, as each spike in Dog #3 is taken as a datum (n = 6).

containing antibodies to SRIF. There was no observable lag between injection of the serum and onset of GH response in 4 dogs while in 1 dog there was a delay of 1 min before the beginning of a secretory episode.

The maximal secretion rates during the spikes were about 4-25 times larger and the total extra GH released during the spikes up to 25 fold that released in the spontaneous secretory episodes occuring during resting control experiments in the same animals. Also, unlike spontaneous spikes, the duration of the steep component was not substantially different from the total duration of these spikes. The results from these experiments are displayed in Table VI.

Both dogs #1 and 2 had spontaneous GH spikes during the control periods of their anti-SRIF experiments although one of the spikes in dog #2 could not be completely assessed as the experiment ended before the spike was completed. These episodes have been shown in Table V. In dog #5, the injection of the antiserum coincided with the terminal part of a spontaneous peak, and this aspect is commented upon in the Discussion.

c) Experiments Using Control Goat Sera in Naive Animals. Of the 5 dogs so treated, a GH secretory response was observed only in 2 cases, and then occuring with delays of 5 and 6 minutes following the injection of control goat serum. Where a spike did occur, the peak GH secretion rates and total extra GH secreted were in the same range observed in spontaneous episodes. The difference between the duration of the steep component of the spike and the total duration was 12 and 8 min.

TABLE VI

Injection of antibodies to SRIF in sheep serum

Dog #	Basal GH Secretion Rate (ng/kg·min)	Peak GH Secretion Rate in Spike (ng/kg·min)	Total Duration of Spike (min)	Steep Portion of Spike (min)	Lag* (min)	Total Extra GH Released Due to Spike** (ng/kg)
1	4.0	89.9	28	24	1	1141.2
2	4.8	45.1	13	13	0	282.7
3	2.5	494.7	30	27	0	5089.9
4	2.2	597.3	30	25	0	4924.4
5	2.5	324.2	35	35	0	5945.1
Mean ± S.E.M.	3.2 ± 0.5	310.2 ± 108.5	27.2 ± 3.5	24.8 ± 3.5	0.2 ± 0.2	3476.7 ± 1149.9

*Lag: delay between injection of antiserum and onset of GH response.

**Calculated as the integral of the GH secretion rate curve during the spike, less basal secretion for that period.

The experiment on dog #6 was a 6 hour experiment (instead of the two hour experiments in all the other animals in this group) and a spontaneous spike was seen more than an hour before the goat serum injection. In dog #10, the manipulation was done a few minutes following the end of a spontaneous GH burst. Dog #7 gave peculiar results with a constant upward drift of the baseline from the beginning of the experiment, reaching high values towards the end of the experiment, though no stimulated effect was observed as a result of the goat serum injected. The result from this group of experiments are shown in Table VII.

Note needs to be made regarding the basal secretion rate measurements for the experiments lasting two hours. Because of short control periods, estimation of basal secretion is difficult in some of these experiments. However, the lack of precision in this measurement has no important prejudice on other parameters being evaluated; for example, in this group, a shift in the baseline value of 50% would introduce an error of only ± 10% in the calculated total extra GH released during small spikes such as those which occur in dogs #6 and 10 with this design. For larger spikes the error would be correspondingly smaller.

d) Exposure of "Sensitized" Animals to Control Goat Sera. In all of these "sensitized" dogs receiving an intravenous bolus of control goat serum, GH secretory surges start within 14 min following injection, with mean peak secretion rates and amount of extra GH released during the spikes substantially larger than control in one case (dog #4) and

TABLE VII

Exposure of naive dogs to control goat sera

Dog #	Basal GH Secretion Rate* (ng/kg·min)	Peak GH Secretion Rate in Spike (ng/kg·min)	Total Duration of Spike (min)	Steep Portion of Spike (min)	Lag**(min)	Total Extra GH Released Due to Spike*** (ng/kg)
6	1.8	17.5	34	22	5	281.6
7	****	NONE	-	-	-	0
8	3.9	NONE	-	-	-	0
9	3.2	NONE	-	-	-	0
10	3.6	39.9	39	31	6	721.3

*Because of short control periods in these two hour experiments, reliable basal secretion rate measurements are difficult.

**Lag: Delay between injection of goat serum and onset of GH response.

***Calculated as the integral of the GH secretion rate curve during the spike, less basal secretion for that period.

****Irregular

somewhat larger than control in the remaining experiments. The duration of the steep portion of the spike was only a little smaller than the total spike duration. Data from these experiments is shown in Table VIII. These animals responded markedly to the second dose of foreign serum, and when interval between the two exposures was short, often displayed severe anaphylaxis and decline in mean arterial pressure (recorded during experiment on dog #6). The clinical features observed during these experiments are detailed in the Appendix.

Dog #2 displayed a spontaneous burst of GH secretion which terminated 15 min prior to goat serum injection and another spike towards the end of the experiment.

e) Hypovolemia Experiments. In these experiments, all 4 dogs subjected to transient hypovolemia with decreased mean arterial pressure, showed a spike in GH secretion 2-40 min following onset of blood withdrawal. The occurance of the GH secretory episode did not correlate with the reinfusion of blood as in 3 of 4 cases it started before blood replacement was begun, occuring following completion of reinfusion only in dog #6.

The maximal GH secretion rates during the peaks tended to be in the range of those observed in spontaneous spikes or slightly higher and the difference between total duration and duration of steep portion of the spikes was not large. Results from this group of experiments are summarized in Table IX. The manipulation to onset of GH response clearly shows substantial delays with a mean lag of 25.4 min. The total extra GH released during these "hypovolemia

TABLE VIII

Exposure of previously sensitized dogs to control goat sera (stress #1).

Dog #	Basal GH Secretion Rate (ng/kg·min)	Peak GH Secretion Rate in Spike (ng/kg·min)	Total Duration of Spike (min)	Steep Portion of Spike (min)	Lag* (min)	Total Extra GH Released Due to Spike** (ng/kg)
2	5.6	52.7	21	21	14	363.5
3	< 2***	78.8	19	19	8	693.9
4	2.0	333.4	38	25	2	3038.5
6	3.8	98.7	28	28	0	1357.1
Mean ± S.E.M.	3.1 ± 1.0	140.9 ± 64.9	26.5 ± 4.3	23.3 ± 2.0	6.0 ± 3.2	1363.3 ± 595.4

*Lag: Delay between injection of goat serum and onset of GH response.

**Calculated as the integral of the GH secretion rate curve during the spike, less basal secretion for that period.

***Value taken as 1.0 for statistical purposes.

stimulated GH spikes" is in the same range as the value for spontaneous episodes.

Due to slow flow through the cannula used to withdraw blood in dog #8, the desired decline in blood pressure was not achieved in the usual 5-10 min required during experiments on other dogs, but took 25 min, and then only a drop to a M.A.P. of 80 mmHg was achieved. Reinfusion in this animal was correspondingly delayed 15 min more than in other experiments, but these differences did not prevent GH secretory surges, as two such episodes were observed in this dog before the experiment was completed.

f) Ether Inhalation Experiments. Results from this group are summarized in Table X. Only 2 of the 4 dogs showed substantial increase in GH secretory rates with lags of 7 and 0 min from start of ether administration by face mask and onset of GH response. When a period of increased secretion was observed, it did not have durations comparable to secretory spikes seen in the other experiments though maximal secretion rate values in these two cases were similar to those observed in spontaneous spikes. Also, the extra GH released during such episodes was small and only one of them was about the lowest value for a control spike from this study. Ether may effect the release of GH from the pituitary gland by some mechanism other than that which occurs usually. Speculation on these responses is made in the Discussion.

Of note in this group is the effect of ether on mean arterial pressure which doubled from resting values of about 100 mmHg to 200 mmHG

TABLE IX

Hypovolemia (Stress #2)

Dog #	Basal GH Secretion Rate* (ng/kg·min)	Peak GH Secretion Rate in Spike (ng/kg·min)	Total Duration of Spike (min)	Steep Portion of Spike (min)	Lag**(min)	Total Extra GH Released Due to Spike*** (ng/kg)
4	2.6	70.1	37.5	27	2	1042.8
6	2.0	56.5	27	23	28	724.8
7	3.5	19.4	25+	15+	40	400+
8	4	39.7/18.3	25/20	20/20	12/45	407.9: 152.8
Mean + S.E.M.	3.0±0.4	40.8±10.2****	27.4±3.7****	22.5±1.5****	25.4±8.2****	482.1±193.1*****

*Because of short control periods in these two hour experiments, reliable basal secretion rate measurements are difficult.

**Lag: Delay between start of blood withdrawal and onset of GH response.

***Calculated as the integral of the GH secretion rate curve during the spike, less basal secretion for that period.

****Values represent mean spike, not mean dog, as each spike in dog #8 is taken as a datum (n = 5).

*****Since the experiment terminated before the spike was completed in dog #7, values for the total and steep durations and the total extra GH released in this spike cannot be included in the statistics (n = 4).

N.B. Dogs #4, 6 and 7 were done in the afternoon, with hypovolemia initiated at least 4 h after ether in the a.m. Dog #8 was done in the morning. Variations in stress response due to diurnal variation or preceding stress are not unheard of, particularly in the secretion of glucocorticoids. However, such effects seem unlikely here since
(a) Basal GH secretion is constant all day; (b) The somatotrophs are a storage gland; and
(c) The response in dog #8 is not different from that in the other dogs.

TABLE X

Ether inhalation (Stress #3)

Dog #	Basal GH Secretion Rate* (ng/kg·min)	Peak GH Secretion Rate in Spike** (ng/kg·min)	Total Duration of Spike** (min)	Steep Portion of Spike** (min)	Lag*** (min)	Total Extra GH Released Due to Spike** (ng/kg)****
4	*****	NONE	-	-	-	0
6	≈ 1	29.2	15	15	7	249.1
7	≈ 4	NONE	-	-	-	0
8	≈ 4	14.5	12	12	0	69.4

*Because of short control periods in these two hour experiments, reliable basal secretion rate measurements are difficult.

**The durations of these excursions above baseline and total extra GH released during such episodes in these experiments raise the possibility that these events may not be comparable to GH spikes occuring spontaneously or following manipulations in other experiments in this study.

***Lag: Delay between start of 10 min period of ether inhalation and onset of GH response.

****Calculated as the integral of the GH secretion rate during the episode, less basal secretion for that period.

*****Irregular.

or more and some hypertensive effect was sustained during ether inhalation, declining towards normal gradually after removal of the face mask.

Statistical Comparisons of key features of GH spikes occuring with different experimental designs are displayed in Table XI. Using a linear regression program on the Wang 600 minicomputer, no dose-response relationship was found between the volume of serum injected and amount of extra GH released in any of the experimental designs. It should however, be noted (Table IV) that the range of doses of the sera (normalized by weight) was quite small.

2. Total Glucocorticoid Data

a) Resting Control Experiments (Table XII). The concentrations of glucocorticoids in plasma were not significantly elevated, indicating that the animals were not stressed to any important degree. Values measured ranged from 1.8 - 5.2 µg/dL and were consistent through each experiment.

b) Anti SRIF Experiments (Table XIII). That the injection of the antiserum had been stressful to the dogs is evidenced by increase in plasma glucocorticoids from 2-15 fold 20 min after injection. Peak values were sometimes achieved within 20 min following manipulation (dogs #3 and 4) or as late as 60 min. Also, an appreciable secondary rise after an hour is observed in dog #4.

c) "Naive" Exposure to Control Goat Sera (Table XIV). Rise in plasma glucocorticoid concentrations suggest a degree of stress from manipulation in all but one (dog #7) of these 5 dogs. It is interesting

TABLE XI

Comparison of key features of GH spikes occuring spontaneously or evoked by injections of sera

	Resting Control Experiments (n = 5)	Injection of Antibodies to SRIF in Sheep Serum (n = 5)	Injection of Control Goat Serum in Naive Dogs (n = 5)	Injection of Control Goat Serum in Sensitized Dogs (n = 4)
Lag (min)	-	0.2 ± 0.2[a]	5,6*	6.0 ± 3.2[a]
Peak GH Secretion Rate in Spike (ng/kg-min)	22.3 ± 5.7[b,c]	310 ± 109[b]	17.5,39.9*	141 ± 64.9[c]
Total Extra GH Released (Due to Spike) (ng/kg)	420 ± 152[d]	3477 ± 1150[d]	201 ± 141**	1363 ± 595
Steep portion of Spike (min)	30.2 ± 3.6	24.8 ± 3.5	22,31	23.3 ± 2.0

*Only two of five experiments with this design yielded a GH spike. Both of these spikes showed lags and peak GH secretion rates which lie outside the 95% confidence interval for those parameters in the AS-SRIF experiments.

**Only two of these five experiments yielded any extra GH, the values being 282 and 721 ng/kg. The other three experiments yielded no significant extra GH above basal secretion and are taken as zero for purposes of this global calculation of extra GH. This resulting mean extra GH is significantly less than that for AS-SRIF experiments ($p < 0.02$) or for sensitized dog experiments ($p < 0.05$).

a: $p < 0.05$

b: $p < 0.01$

c: $p < 0.05$

d: $p < 0.01$

by unpaired t-testing.

to note that no GH spikes occured in those 3 animals (dogs #7, 8 and 9) that did not show a decline in mean arterial pressure following injection of the serum, even though total glucocorticoid numbers in dogs #8 and 9 indicate that the procedure had been stressful.

d) Injection of Control Goat Sera in "Sensitized" Dogs (Table XV). Effect of injection of control goat sera on circulating glucocorticoid concentration was observed within 20 min in all dogs, with increases from about 2½ times (dog #2) to about 13-fold (dog #3) although the rise was much more gradual and less than 2-fold in dog #6.

e) Hypovolemia Experiments (Table XVI). Glucocorticoid values rose consistently following manipulation in all the dogs reaching peak concentrations at 20 min in 2 dogs and 40 min in the other 2 dogs, with peak values ranging from about double to somewhat more than 3-fold basal values.

f) Ether Inhalation Experiments (Table XVII). All dogs subjected to ether inhalation showed plasma glucocorticoids raised from double to 4-fold within 20 min after onset of the procedure. Dog #8 shows steadily rising values to the end of the experiment though somewhat increased GH secretion in this dog is observed only during the first 12 min after ether administration was started.

TABLE XII

Total glucocorticoids in plasma*† during resting control experiments (µg/dl)

Dog #	Time 0	30 min	60 min	90 min	120 min	150 min	180 min
1	5.09±0.20	5.17±0.21	3.20±0.58	2.52±0.35	2.59±0.09	1.90±0.38	2.99±0.18
2	2.91±0.02	3.03±0.45	3.94±0.57	3.44±0	3.03±0.41	2.86±0.20	2.66±0.31
3	2.95±0.19	-	2.52±0.08	-	2.12±0.11	-	2.38±0.12
4	2.47±0.03	-	1.88±0.08	-	2.28±0.05	-	2.49
5	3.27±0.15	-	3.21±0.3	-	3.07±0.02	-	2.67±0.12

Dog #	210 min	240 min	270 min	300 min	330 min	360 min
1	3.25±0.26	2.18±0.07	3.14±0	1.90±0.14	2.66±0.03	2.32±0.33
2	2.07±0.10	2.30±0.02	2.81±0.19	3.57±0.04	3.84±0.02	3.49±0.16
3	-	2.31±0.13	-	1.87±0.09	-	2.76±0.27
4	-	2.10±0.19	-	1.96±0.05	-	1.79±0.04
5	-	2.70±0.03	-	2.20	-	2.65±0.08

*Determination is fluorometric and calculation assumes equal amounts of cortisol and corticosterone in dogs.

†Results expressed as mean ± mean deviation from the mean.

TABLE XIII

Total glucocorticoids in plasma*† during experiments with injection of antibodies to SRIF in sheep serum (µg/dL)

Dog #	-180 min	-120 min	-60 min	0	20 min	40 min	60 min	120 min	180 min
1	2.67±0.7	3.56±0.57	4.75±1.37	2.43±1.13	-	-	15.18±0.28	-	10.10±0.73
2	5.53±0.48	2.73±0.30	2.00±0.15	3.08±0.19	6.51±0.69	8.66±0.06	11.20±0.44	10.16±0.37	5.82±0.19
3	-	-	2.43±0.18	2.22±0.21	33.93±0.6	25.15±0.38	26.43±0.30	24.78±0.45	15.07±0.05
4	-	-	4.53±1.64	5.26	22.47±0.6	9.50±0	15.61±1.89	20.22±2.38	19.27±2.5
5	-	-	0.53±0.24	1.21±0.19	8.28±0.29	19.15±1.26	12.65±0.15	13.99±1.09	6.78±1.16

*Determination is fluorometric and calculation assumes equal amounts of cortisol and corticosterone in dogs.

†Results expressed as mean ± mean deviation from the mean.

TABLE XIV

Total glucocorticoids in plasma*† during experiments with exposure of naive dogs to control goat sera (µg/dL)

Dog #	-15 min	0	20 min	40 min	60 min	75 min
6	3.02±0.07**	3.58±0.58	11.34 ± 0.58***		6.10±0.97	3.82±0.44****/ 4.94±0.78*****
7	2.71±0.29	4.71±0.14	4.31±0.14	3.50±0.29	2.52±0.04	2.71±0.29
8	1.95±0.32	0.68±0.03	2.98±0.55	6.45±0	7.23±0.05	7.06±0.22
9	2.33±0.52	3.67±0	6.53±0.5	3.89±0.05	4.36±0.58	7.21±0.14
10	4.25±0.79	2.52±0.05	18.8 ±4.50	23.3 ±0.70******	-	21.8 ±0.40

*Determination is fluorometric and calculation assumes equal amounts of cortisol and corticosterone in dogs.

**Time: -60 min; ***Time: 30 min; ****Time: 120 min; *****Time: 180 min; ******Time: 45 min

†Results expressed as mean ± mean deviation from the mean.

TABLE XV

Total glucocorticoids in plasma*† during experiments with injection of control goat sera in previously sensitized dogs (µg/dL)

Dog #	-180 min	-120 min	-60 min	0	20 min	40 min	60 min	120 min	180 min
2	3.81±0.18	1.80±0.27	3.07±0.26	2.13±0.30	5.80±0.31	5.94±0.09	4.75±0.18	3.54±0.09	2.61±0.06
3	-	-	1.16±0.44	1.06±0.53	14.30±1.89	17.14±0.12	20.65±0.85	12.05±0.85	5.47±0.58
4	-	-	8.65±0.12	6.49±0	32.13±0.80	26.91±0.05	24.07±0.85	21.99±0.12	21.92±1.75
6	-	-	3.76±0**	4.32±0.10	4.91±0.19	5.71±0.15	8.24±0.03	7.82±0.19***	

*Determination is fluorometric and calculation assumes equal amounts of cortisol and corticosterone in dogs.

**Time: -15 min

***Time: 75 min

†Results expressed as mean ± mean deviation from the mean.

TABLE XVI

Total glucocorticoids in plasma*† during hypovolemia experiments (µg/dL)

Dog #	-15 min	0	20 min	40 min	60 min	75 min
4	5.81±0.18	7.00±0.44	12.57±1.5	7.99±0.10	5.10±0.38	5.01±0.54
6	4.18±0.44	7.38±0.13	8.59±0.25	13.96±0.18	6.14±0.03	5.32±0.60
7	3.37±0.29	5.67±0.22	16.26±0.13	12.61±0.42	11.66±0.13	7.50±0.04
8	1.21±0.17	2.27±0.30	7.10±0.58	7.93±0.08	7.21±0.30	6.68±0.10

*Determination is fluorometric and calculation assumes equal amounts of cortisol and corticosterone in dogs.

†Results expressed as mean ± mean deviation from the mean.

TABLE XVII

Total glucocorticoids in plasma*† during experiments with ether inhalation (µg/dL)

Dog #	-15 min	0	20 min	40 min	60 min	75 min
4	5.04±0.32	4.21±0.2	8.46±0.06	5.77±0.72	6.20±0.29	5.78±0.96
6	5.38±0.11	3.85±0.12	8.00±0.29	4.81±0.54	5.20±1.04	3.86±0.16
7	2.67±0.07	3.20±0.46	7.46±0.13	3.79±0.26	3.40±0.39	2.87±0.26
8	1.80±0.40	0.54±0.17	2.22±0.25	3.35±0.18	4.79±0.23	8.26±0.08

*Determination is fluorometric and calculation assumes equal amounts of cortisol and corticosterone in dogs.

†Results expressed as mean ± mean deviation from the mean.

CHAPTER IV

DISCUSSION

1. Appropriate Control Group for Anti-SRIF Experiments

Injection of foreign sera into an animal are known to cause immunogenic stress, even on primary exposure (Elias et al., 1962; Wilson and Miles, 1975); though more profound reactions, which may even be fatal (Smith and Hamlin, 1977), occur in "sensitized" animals where a period of some days is allowed to elapse before repetition of the antigenic challenge (Anaphylaxis) (Boyd, 1966). Such reactions are not surprising since in the dog, even first exposure to homologous plasma can produce allergic and hypotensive responses with release of histamine (Baker and Remington, 1958; Bliss et al., 1959; Bliss and Walker, 1959; Gadboys et al., 1963). Thus, even homologous plasma may not be "pharmacologically inactive" (Elias et al., 1962) and handling of blood may activate the mediators that cause a response (Smith et al., 1965). Reaction to the injection of goat sera in both naive and sensitized dogs used in this study are, therefore, not unexpected.

Although one would expect the experimental design with naive dogs receiving control goat sera to serve as an adequate control for the experimental group treated with an antiserum to somatostatin, total glucocorticoid data (see Results) and clinical observations (see Appendix) indicate that reaction to AS-SRIF had been somewhat more severe than those following the various control goat sera in naive

animals, but less severe than the anaphylactic reactions observed in the sensitized animals. In light of these findings, the appropriate control response for the anti-SRIF experiments would be somewhat larger than for the control injections in other naive animals, but would nonetheless be substantially below the extreme responses of the sensitized (anaphylactic) group. However, since the peak GH secretion and extra GH released in the AS-SRIF stimulated spikes are even larger than similar parameters for GH spikes in sensitized animals, it is likely that much of the GH response in AS-SRIF animals occurs because of the antibodies to SRIF contained in this serum.

2. Post-manipulation GH Surges

In the resting control experiments in this study, 6 spontaneous GH surges were observed during 5 six-hour experiments, so that GH spikes occured with a mean frequency of one per 5 hours. This is somewhat less frequent than seen in a larger series of similar experiments (Cowan et al., 1984) that yielded a mean frequency of one such secretory event per 3.9 hours in dogs. Even if one were to use this greater frequency, i.e. to choose a position of disadvantage to prove the case, the occurance of GH spikes obtained with the manipulations made in different experimental designs have very significantly different probabilities from what would be predicted on the basis of randomly occuring control spikes.

Following the injection of a bolus of sheep serum containing antibodies to SRIF, GH burst occured reliably within one min. Since these experiments used short sampling intervals (1 min) while the

other study cited above used samples spaced 5 min apart, even if one were again to choose a position of disadvantage by evaluating a 5 min window following manipulation, the probability of a GH spike occuring in that window in 5 out of 5 trials would still approach zero. Similarly, using a 20 min window for the anaphylaxis experiments (all of the 4 animals responding*, with time lags of upto 14 min), the probability of these results being spontaneous would be 0.0005, indicating beyond any doubt that there is a real response.

In the 5 experiments in which goat serum was injected into naive animals, the response does not separate as readily from the random expectation. Only 2 of 5 gave GH spikes, having lags of 5 and 6 min. Using a 10 min window, for conservation, even this low frequency of response still represents about 10 times the random rate of occurance of GH spikes. Using the binomial theorem, the probability of these results occuring randomly is $P = .0154$.

Examination of time lags between injection of serum and response in these 3 groups is also interesting. While AS-SRIF

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*The fifth experiment of this type (in dog #5) could not be completed as the anaphylaxis was so severe that the animal died about 30 min following goat serum injection. Presumably because of marked hypotension, only a few blood samples could be taken in the post-manipulation period, and plasma from these when assayed showed concentrations of GH that were higher than those obtained in any of the other experiments. It can be concluded, therefore, that this dog did exhibit a high amplitude excursion in GH due to anaphylaxis, although for paucity of samples and death of the animal prior to the termination of the secretory event, the response could not be completely characterized.

stimulated spikes are separable from those obtained in the anaphylactic group ($p < 0.05$) and the naive dogs (lags of 5 and 6 min lie outside the 95% confidence interval of the lag value of 0.2 ± 0.2 min from AS-SRIF experiments), separation between the naive and sensitized group is not achieved for this parameter.

The difference in the GH responses in these 3 groups is also evident in the peak GH secretion rate during spikes. Values obtained in naive dogs are smaller than similar values for anaphylactic animals and lie outside the 95% confidence interval of this parameter for GH spikes in anti-SRIF experiments. Also, since the Ns are relatively small, statistical significance is not apparent in this respect between the AS-SRIF induced GH secretory episodes and those seen in the anaphylaxis experiments, but peak GH secretion rates, nevertheless, are roughly double in the AS-SRIF experiments.

It is clear that the AS-SRIF experiments produced GH spike responses significantly more promptly and considerably larger than any other stimulus. Examining them against their appropriate controls (see section 1 of Discussion) it is clear that their differences in GH response from resting control or naive goat serum controls are huge. Even conceding some tendancy towards a mild anaphylaxis in this group, based upon the stresses associated with the AS-SRIF injection, the differences from expected control response point to a powerful extra ability of this material to set off GH spikes almost instantly.

While there is no proof that this extra action results from the administration of anti-SRIF antibodies, it is true that a major known difference between this test material and ordinary serum was the presence of antibodies to SRIF. It seems likely that the extra ability to evoke GH spikes is due to an antibody effect, driving SRIF rapidly below some critical threshold. This reinforces the evidence from indirect studies done previously in this lab (Cowan et al., 1981; Cowan et al., 1984) which had suggested a role for decline in SRIFin initiating GH bursts, and these will be discussed further in section 5 of this discussion.

GH secretory episodes are reliably seen to occur in the hypovolemia experiments (consecutively in 4 out of 4 trials) with a time lag of up to 40 min between onset of blood withdrawal and initiation of a GH spike. The probability of spontaneous occurance of this result is 0.0008. The values for peak GH secretion rates and amount of GH released in excess of basal secretion during spikes, however, are in the same range as observed in the spontaneous GH secretory bursts occuring during the resting control experiments.

The effect of ether inhalation upon episodic GH secretion is less clear. Certainly, in 2 of the 4 experiments with this design, an augmented release of GH is observed due to the manipulation. However, the duration for which extra GH is released in these cases is short (12 and 15 min) compared to spikes of GH that occur spontaneously or following any of the other manipulations used in this study. When considered in conjunction with the observations that this augmented GH secretion begins during the administration

of ether and that ether has a hypertensive effect in dogs (so that any disruption in hypothalamo-hypophyseal portal flow would seem unlikely), it would appear that these "atypical spikes" involve some mechanism other than that by which GH secretory bursts are elicited in the other experiments of this study.

Many of the dogs used in the series were subjected to 3 different kinds of experiments, and dogs #4 and 6 were studied with 4 and 5 of the experimental designs, respectively (see Table III). Some intra-dog comparisons are therefore possible. Dog #5 had GH spikes with the longest durations in both the resting control and anti-SRIF experiments tending to bias the values for the mean durations of the episodes in these two groups. Dog #2 consistently gave the lowest amounts of extra GH released in spikes with all 3 designs in which it was used (resting control, AS-SRIF, and anaphylaxis experiments).

3. Comparison of Post-Manipulation Secretory Episodes to Spontaneous GH Bursts

Large GH spikes (larger than those typical of spontaneous GH bursts), resulting from the different manipulations utilized, leads to the examination of the possible attendant mechanisms which may evoke them.

The narrow range of the duration of GH secretory bursts *in vivo* suggests the release of a store of GH which has been accumulated in the somatotrophs during a period of SRIF influence. This hypothesis first advanced by Stachura (1977) was based upon

experiments on pieces of rat partes distales in a perifusion system when he found that most of the reduced GH release (below equilibration levels) during short pulses of relatively high concentrations of SRIF was recovered following these pulses. It would seem therefore, that SRIF blocks GH release but not its accumulation in the somatotrophs in a releasable form. However, as pointed out by Cowan et al. (1983), progressively less "lost" GH is recovered with increasing periods (10 and 60 min) of inhibition with SRIF, suggesting that accumulation of releasable GH slows down with time, presumably by some intracellular feedback process. The high peak GH secretion and amount of extra GH released in stimulated spikes in this study may then occur by virtue of recruitment of a larger number of somatotrophs than participate in the spontaneous episodes, or, an increased conversion of the GH stored in the granules in somatotrophs* into a readily releasable form, perhaps under increased GRF influence.

While the first of these two suggestions is not impossible (a transient decline in concentration of SRIF below some critical value only in the milieu of some cells in a portion of the gland occuring in spontaneous secretory bursts) the latter possibility

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*Somatotrophs contain very large stores of GH and only a small fraction of this is released in a normal secretory episode. This conclusion stands on its own from the fact that somatotrophs continue to release measurable amounts of GH in the course of in vitro experiments lasting a few hours and large amounts of GH can be measured in these cells even after they have been used in such studies as is often done following these experiments.

would seem more likely. If one were to hypothesize that stress (a feature in all experiments with the larger than spontaneous spikes) in dogs increases GRF release, the large amounts of GH released and high amplitude of the spikes may be explained. While the stressful manipulations studied presumably lower somatostatin concentration in the dog in some way (since high concentrations of somatostatin prevents GH spikes) allowing a secretory episode to be initiated, GRF may act on GH stored in somatotrophs and render more GH available in a readily releasable form. Hence, these two factors may act in conjunction producing the effects that are observed.

A feature of stimulated spikes that bears commenting upon is the duration of these episodes. Spontaneous GH surges are often preceded by a period of slowly rising GH secretion before a steep sloped burst occurs. This may represent a period of declining SRIF concentration impinging on the somatotrophs, allowing secretion to increase somewhat above the irreducible basal level. At some point, however, a critically low SRIF concentration is seen, triggering the massive release of accumulated GH. In interpreting the effect of traversing such a threshold, it is useful to note that the full expression of the effect of removal of an SRIF signal from somatotrophs <u>in vitro</u> is felt within one minute (Cowan et al., 1983), so even a very short period of dearth of SRIF could trigger such an event. With a sharp decline in portal somatostatin concentration which may occur due to stress in the dog,

or interruption in the supply of SRIF to the anterior pituitary due to changes in portal flow (see 4 below), even if for a very short period of time, a GH secretory episode may be initiated rapidly, without a significant period of gradually rising secretion preceding it.

The remarkably narrow range for steep duration of GH spikes occuring with the different experimental designs (indeed, even when compared to in vitro data from Cowan et al. (1983), and in consideration of the bias introduced by varying sampling intervals between designs, and within some of them), would suggest that the secretory event, once initiated would proceed inexorably to completion. (This conclusion would again suggest a different mechanism involved in the spikes that occur following exposure to ether). This is also supported by evidence from a pilot study that I designed to test this hypothesis in which the dog was primed with an SRIF infusion that was interrupted for 10 min before being restarted. Since there is an increased likelihood for a GH spike to occur within a few minutes of stopping a prolonged SRIF infusion (Cowan et al., 1984) renewal of infusion would again raise SRIF concentration above the critical threshold even while the spike was in progress. In one of two dogs so treated, a post inhibitory spike occured; it was completely unaffected by restarting of the SRIF infusion, suggesting that the renewed high SRIF levels did not prematurely terminate the secretory episode.

Of interest is the high amplitude surge in GH secretion in the AS-SRIF experiment in dog #5, where the injection of the antiserum to somatostatin coincided with the downslope of a spontaneous secretory episode. Although the pilot study mentioned above suggests that the secretory event, once initiated, is not interrupted by concentrations of SRIF greater than the critical threshold, lowering SRIF again may reactivate release of more GH as observed in this experiment (though concurrent increase of GRF due to stress in dogs, which is a hypothesis in this study, may play a role in producing the high amplitude of the response). This result, although interesting, is not unique, and biphasic GH spikes have been observed in previous studies (Martin, 1976; Martin et al., 1978).

4. Effect of Stress on GH Secretion in Dogs

The finding that different stress situations used in this study do not decrease basal GH secretion or prevent GH secretory surges affirms the observations that stress, in general, does not depress GH secretion in dogs as it does in the rat (Schalch and Reichlin, 1968; Takahashi et al., 1971; Rice and Critchlow, 1976; Takahashi, 1978; Lenox et al., 1980). In fact, the reliability with which GH spikes occur with anaphylaxis and blood withdrawal stress indicate that the dog more resembles the primate (Glick et al., 1965; Meyer and Knobil, 1967; Schalch, 1967) in this respect.

In the present study, a significant rise in plasma glucocorticoids has been used to confirm the presence of stress. From this standpoint, a rise in concentration of circulating glucocorticoids can occur in the dog without elevations in GH so that the rise in GH is not an invariable accompaniment of stress as is observed in some of the ether experiments (dogs #4 and 7) and in some cases of primary injection of control goat serum in naive animals (dogs #8 and 9) as well as the inverse being possible (high GH response with relatively small glucocorticoid response - injection of goat serum in dog #6, previously sensitized). In stress studies done on human subjects, increase in both GH and cortisol are less frequent than elevations in cortisol alone, and dissociations between GH and cortisol response suggest a separate mechanism controlling the secretion of these two stress-labile hormones (Rose and Sachar, 1981).

Since GH secretory episodes occur with increased frequency in the (hypotension-producing) anaphylaxis and hypovolemia experiments, and such clear cut responses are not reliably seen with ether, which has a hypertensive effect, speculation can be made regarding the role of disturbances in hypothalamo-hypophyseal portal circulation in precipitating some GH spikes. If reasonable assumptions are to be made regarding pressures directing flow in the portal circulation to the pituitary gland (or in any portal circulation, for that matter), it seems likely that large declines in mean anterial pressure will have a greater impact on

the small gradients in pressure available for portal flow than on the larger gradients available for systemic flow. This is made even more likely by the absence of a typical range of arteriolar adjustment for regulation of portal flows. Moreover, should portal flow to the pituitary be interrupted and circulating GH continue to increase (as experiments in this study show), a strong case is made for an alternative collateral systemic circulation to the adenohypophysis in the dog as has been demonstrated by dye studies in the rat (Ambach and Palkovits, 1979).

However, this apparent effect of hypotension on GH secretory bursts does not always occur; in an additional experiment on dog #4 which was exposed to a bolus of foreign serum for the 3rd time, with a 4-fold rise in plasma glucocorticoids within 20 min and a precipituous decline in mean arterial pressure to about 20 mmHg which was sustained for about an hour, no post-manipulation GH spike was observed. Besides, it is beyond the scope of this study to evaluate whether hypotension, even if it did contribute to offsetting some GH secretory episodes, had a direct effect through causing changes in hypothalamo-hypophyseal portal flows or through some intermediary mechanism as yet unknown.

The observation made here regarding stressful effects of injection of foreign sera into an animal bear interestingly upon preceding studies where administration of antisera to somatostatin produced elevated basal GH secretion in rats but did not evoke pulsatile secretion (Ferland et al., 1976; Terry and Martin, 1981; Eikelboom and Tannebaum, 1983). Stress in the rat increases SRIF

secretion and inhibits GH secretion, including suppression of basal secretion. If the sera themselves caused stress, endogenous SRIF production would increase, whereas antibodies to SRIF would act in the opposite direction by neutralizing circulating somatostatin. These 2 effects must generate ambiguous results, and operating as they would in opposite directions, can invalidate some of the conclusions made without consideration of the stressful effects of the sera themselves. Even the use of control sera, without recognizing the presence of a variable stress component (this study) may not resolve these questions. Indeed, it is clear that the rat is not a suitable experimental animal for any stressful manipulation in which a positive result relies upon stimulation of GH secretion.

5. Examination of Models for Episodic GH Release

The secretion of GH has been shown to be episodic in several mammalian species including the dog (Takahashi et al., 1981; Cowan et al., 1981; Cowan et al., 1984).

The role of the hypothalamic inhibitor for growth hormone, somatostatin (SRIF), in the short-term regulation of such episodic secretion has been unclear. Certainly stress-induced SRIF secretion lowers GH secretion in the rat and this effect can be abolished by antiserum to SRIF (Arimura et al., 1976; Terry et al., 1976; Tannenbaum et al., 1978). An antiserum to SRIF increases trough (basal) GH concentrations in the unstressed rat (Eikelboom and Tannenbaum, 1983), but has little or no effect on GH secretory bursts (Terry and Martin, 1981). These confirm similar, though less clear-

cut earlier findings also in the rat (Ferland et al., 1976), and have led many workers to reject changes in SRIF as an important factor in initiating episodic secretion. Recent efforts have concentrated on identifying the role of GRF in control of pulsatile secretion. The availability of GRF preparations (Brazeau et al., 1981), and consequently monoclonal antibodies to GRF (Luben et al., 1981; Luben et al., 1982) has led to efforts to reduce circulating GRF in rats with such antibodies (Wehrenberg et al., 1982). While the reduction in circulating GRF so accomplished did reduce and ultimately eliminate GH secretory bursts, the interpretation by the authors that GRF excursions initiate the bursts is not an exclusive model. Hence, while GRF is clearly important to any substantial GH secretion it is as yet not clear whether its excursions initiate secretory bursts, or whether it has a steadier tonic effect without which other superimposed factors cannot act fully.

Thus the way in which SRIF and GRF interact to produce GH secretory bursts in vivo remains poorly defined.

Conclusions from the present series regarding the stressful effects of injection of foreign sera on the secretion of GH point to the inappropriateness of the use of the rat as the experimental animal in studies which employ antisera. For experimental designs with serial sampling, the blood volume of rats is too small to additionally assess the status of glucocorticoids as an indicator of the stress that manipulations may produce. When the study involves the GH system, this can be further emphasized since the rat differs from

most larger mammals in that the basal GH secretion is reducible and stress itself has a negative influence on both basal and episodic GH release. Although useful information has been obtained from experiments using this species, clearer characterization of the mechanism by which GH secretion is controlled, would require other animal models.

A modest constant infusion of SRIF abolishes the spontaneous GH secretory surges normally seen in dogs (Cowan et al., 1981; Cowan et al., 1984) but does not lower basal secretion rate. These results imply that during basal GH secretion, a surfeit of SRIF impinges on the somatotrophs, as extra SRIF does not further lower secretion. However, as exogenous SRIF blocks the secretory bursts, very little SRIF must be present in order to allow them to occur. The injection of the antiserum to somatostatin used in this study reliably evokes GH surges with almost no delay between injection of the antiserum and initiation of the secretory event, strongly suggesting that a decline in concentration of SRIF must be implicated in the causation of these episodes.

No doubt the supra-hypophyseal influences on the release of GH must have some facilitatory effect, probably mediated by GRF, as in it's absence there is a decline in GH secreted by the pars distalis, both in basal secretion and episodic release. This is evidenced by experiments where the integrity of the "hypothalamo-hypophyseal unit" is interrupted by pituitary stalk section (Wehrenberg et al., 1980) and extrasellar transplant of the gland (Schalch and Reichlin, 1966). <u>In vitro</u> work

on transplanted homologous partes distales (Zanini et al., 1979) and the gradual decline in equilibration levels of GH secreted by somatotrophs in a perifusion system (Cowan et al., 1983) also suggest this conclusion.

The initiation of episodic bursts of GH, then, must occur because of decline in circulating SRIF below some critical concentration with GRF exerting a tonic effect, possibly influencing the amplitude of the secretory surge, or conceivably when a decline in SRIF and an elevation in GRF occur concurrently (Terry and Martin, 1981). Since the effect of GRF alone on in vivo GH secretion has not yet been elucidated, further work is necessary before this problem comes to be resolved and the mechanism by which GH secretory bursts are offset is understood with more clarity. Some in vitro work utilizing perifused somatotrophs which are subjected to SRIF and GRF as isolated pulses or used together, is currently being done by Dr. J. Kraicer at Queen's University and this could progress on to further experiments to test their results in vivo. Also, with refinement in the techniques of preparing antibodies in cell cultures, availability of pure antibodies would eliminate the effects of the serum in which they have been raised, and may permit experiments with more clear cut results.

However, the admittedly multifactorial data already existing, along with the speculations in this discussion (in sections 3 & 4), provide a possible model which may integrate all of the results obtained with these six designs of experiments.

This model, though somewhat fanciful, would be based upon the following facts or speculative hypotheses:

i) Typical secretory bursts need a decline in SRIF to occur.

ii) Extra GRF can increase the size of bursts when they occur, but can also increase basal GH release (hypothesis).

iii) Stress in the dog releases extra GRF (hypothesis).

iv) Stress in the rat releases extra SRIF (Arimura et al., 1976; Terry et al., 1976).

v) Certain manipulations in the dog which are stressful (and release GRF as a result) also can diminish SRIF impinging on the somatotrophs. Perhaps all hypotensive stresses do this, but certainly our AS-SRIF serum does this, as it is both a stress, and binds some circulating SRIF.

vi) Extra SRIF in the rat lowers basal GH, but it does not do so in the dog (Cowan et al., 1984) or man (Mortimer et al., 1974).

vii) Progressive removal of GRF lowers basal GH in the rat (Wehrenberg et al., 1982) but the converse has not been tested in vivo.

Examining the different designs used and applying these seven elements:

a) Resting controls: the interaction of a declining SRIF effect, superimposed on a steady or rising GRF has already been reviewed.

b) AS-SRIF: the decline in SRIF due to the antibodies (and possibly due in part - later - to circulatory changes (see section 4 of this chapter) may trigger a GH spike. Extra GRF due to stress increases the amplitude greatly.

c) Experiments using goat sera in naive and sensitized dogs, and hypovolemia experiments: varying degrees of stress from low (naive goat sera or hypovolemia) to high (anaphylaxis) may adjust GRF release and hence, account for near-control spike amplitudes for the naive exposure and hypovolemia experiments, and for the larger ones in the anaphylactic group. Hypotensive changes may diminish SRIF delivery to the somatotrophs to below a critical threshold; this is not reliably prompt in any of these designs, but clearly reflects a hierarchy - from infrequent spike effects in the naive group, to reliable but slow in the hypovolemia group, to reliable and moderately prompt in the anaphylactic group. Nonetheless, none of these speculatively indirect influences on SRIF is nearly as prompt as the AS-SRIF effect noted in (b) above.

d) Ether: If ether is a stress (doubtless), but, being hypertensive has no effect on SRIF supply to the somatotrophs, the increase in GRF might predict a rising baseline, and possible short upward excursions not restrained by the ambient SRIF levels, but still not reflecting the expected 20 min + spike resulting from SRIF withdrawal.

6. Sources of Error in this Study

a) Assay-Related Errors. The coefficient of intra-assay variation for the GH-RIA was always less than 10%, and would be expected to contribute little to errors in calculated values for continuous GH secretion rates. Discontinuities are unlikely, as individual experiments were never split, but were always assayed in their entirety within one assay. From time to time, the re-assay of

of experiments was done, and suggested very small inter-assay variation as well, though it was not numerically evaluated.

The use of hypophysectomized dog plasma and a known pool sample in each assay helped to reassure us that (i) low values were not artifacts and (ii) inter-assay variation was small.

b) Model-Related Errors. The use of the single compartment model for GH as a first approximation has certain faults. It is unlikely to represent real physiology, since the multiexponential disappearance curves for labelled GH (Glick et al., 1964; Peake et al., 1968; Kramer et al., 1973) suggest more compartments. Nonetheless, for steady states, any model will do (as they all degenerate to Ra = MCRxC), and for relatively rapid departures from steady state, the input of "slower" compartments is negligible.

However, the single compartment model is likely to introduce some artifacts:

i) The composite "V" is unlikely to be a true constant. It probably should vary slightly with concentration, and is probably overestimated at higher concentrations of GH, yielding brief negative values for secretion rates on some rapid downslopes after very high GH peaks.

ii) Other slow compartments, each with their own "V's", must absorb small amounts of GH during periods of high GH concentration, only to return it later, occasionally raising baselines somewhat longer after major secretory events. These are very much second order effects, but are also seen in MCR and V validation studies in an earlier series

in this lab (Cowan et al., 1984).

Errors associated with the use of a constant MCR are likely to be small (Cowan et al., 1984), except during periods of cardiovascular perturbation (see subsection (c) below).

c) The Impact of Stress and Cardiovascular Changes on Calculated GH Secretion Rates. (i) Changes which reduce hepatic perfusion would be expected to lower the MCR for any peptide hormone. Such changes undoubtedly occur briefly during all experiments except resting controls and ether exposure groups. This would result in a brief period during which GH secretion rates would be overestimated using the constant MCR calculation approach. However, during most of a secretory episode evoked by a manipulation, plasma GH concentration is rising rather steeply (the gradual fall in concentration is mostly after the actual secretory episode). Hence the $V\frac{dC}{dt}$ term is usually very important during secretory bursts and the input of the MCRxC term (and its possible over estimation) is diminished.

Should a slightly reduced MCR persist, it might also help to account for the few cases where return to basal GH secretion rates appeared incomplete.

(ii) Reductions in circulating volume, as an anaphylaxis, might have an impact on V, the apparent distribution volume for GH. Such an effect would not likely be great, however, since not all of V is in plasma (V exceeds plasma volume in any event) and a reduction of the component of V in plasma, would probably be matched by an increase in the position of apparent V in ECF, due to increased capillary

permeability.

V may be somewhat overestimated in the hypovolemia experiments (perhaps by 10-20%) and this may artifactually increase the amplitude of spikes seen in these experiments by a proportion less than this.

(iii) "Stress" may alter MCR (but not V) by alterations in hepatic perfusion. However, while stress-induced increases in cardiac output do occur, they usually reflect primarily changes in flow to areas other than the viscera. Hence, stress effects not covered in (i) above, as in ether experiments, would be expected to have little effect on rate calculations.

d) The Effect of "N". The small "N" in the groups in this study make the statistical separation even of markedly different responses difficult. Purer anitbody preparations (expected in 1984), and more animals, will improve this aspect.

7. Summary of Conclusions

The indirect evidence suggesting that decline in circulating somatostatin concentration below some critical threshold is implicated in the initiation of episodic bursts of GH has already been reviewed. More direct evidence for the role of somatostatin in pulsatile GH secretion is obtained from the finding in this study that decrease in endogenous SRIF produced by injection of an antiserum to somatostatin promptly and reliably evokes GH secretory surges.

Stresses reduce GH secretion in rats and increase the GH levels in man. Results from this series of experiments show that stress does not lower GH in the dog, and may increase GH secretion, so that the dog more resembles primates than it does rodents in this respect.

In the stresses examined herein, the manipulations which produced hypotension reliably resulted in the occurance of GH secretory episodes while ether, a hypertensive one, did not.

More work needs to be done to more definitely explain some of the effects on release of growth hormone observed in this study. Through the use of pure antibodies to SRIF raised in cell cultures, and experiments using GRF alone or in conjunction with SRIF, the physiological mechanisms involved in the control of GH secretion may be further elucidated and more clearly characterized.

APPENDIX

Summary of Clinical Observations

Nothing unusual was noticed throughout the resting control experiments, all dogs being quiet and responsive. Dog #1 passed urine 315 min after the start of the experiment, but this event occured 35 min after the termination of the sole spontaneous GH secretory episode in this experiment and did not produce any change in the resting plasma glucocorticoid values.

The reaction of the animals to sheep serum containing antibodies to SRIF was evident within 2 or 3 min of it's i.v. administration as all dogs exhibited some restlessness and had to be petted and reassured. Occurance of some degree of stress is also suggested by the following observations within 10 min of the bolus injection: foul smell from opening up of the animals' anal glands (dogs #3 and 4); short lived episode of retching (dog #4); bowel movement (dogs #1 and 5). Darkening of blood withdrawn during sampling was observed 3-10 min following the manipulation in 3 of the 5 dogs (#2, 3 and 4), although a transient increase in hematocrit could be surmised only in dog #5 from decrease in the volume of plasma recovered from blood samples that had been centrifuged.

The reaction to control goat sera in naive dogs was somewhat milder than the preceding group with slight restlessness and darkening of blood seen in 3 of 5 dogs (#7, 9 and 10). Dog #10 had two episodes of vomiting and passed stool between 30 and 45 min after

injection of serum. A decrease in blood pressure is suggested in dog #6 from a difficulty in drawing blood samples from the indwelling vena caval catheter, similar to that observed with dog #10 (relevant portion of mean arterial pressure tracing from the latter experiment shown in Fig. 9(b) of this thesis). In the other 3 dogs (#7, 8 and 9), injection of the bolus of goat serum had no effect on mean arterial pressure (see Fig. 9(a)).

Injection of control goat sera in previously sensitized dogs showed reactions similar to, but clinically more severe, than the preceding two groups. In addition to the features noted above, there was shivering, retching or vomiting, and severe prostration with diminished responsiveness to external stimuli (all the animals), jerky muscular movements (dogs #2 and 6) rhinorrhoea (dogs #2 and 4), and dyspnea (dogs #4 and 6) with laryngeal stridor (dog #6). These reactions were more severe in the dogs (#3, 4 and 5) that had received the anti SRIF containing serum only a week earlier (1 of these 3 animals, dog #5, died during the experiment) and were milder when the intervals to subsequent exposure were greater (2 to 4 months). Blood pressure tracings from experiments of this type, whenever recorded, showed rapid development of severe hypotension (see Fig. 10) following injection of the foreign serum, as is characteristic of anaphylaxis (Smith and Hamlin, 1977).

In the hypovolemia experiments, the induced declines in arterial pressure were recorded (see Fig. 11). Only one dog (#4) appeared to become drowsy from the blood withdrawal and became

alert again part way through the reinfusion.

During the ether inhalation experiments, dog #7 passed urine and flatus soon after onset of the manipulation and dog #8 showed a short lived darkening of blood for about 2 min during the administration of ether. Blood pressure tracings from these experiments showed immediate rise of M.A.P. from resting values of about 100 mmHg to about double that value within seconds of the face mask with ether being put on the animal, and some degree of hypertension persisted throughout the manipulation, with gradual return to resting values over several minutes following removal of the face mask (see Fig. 12).

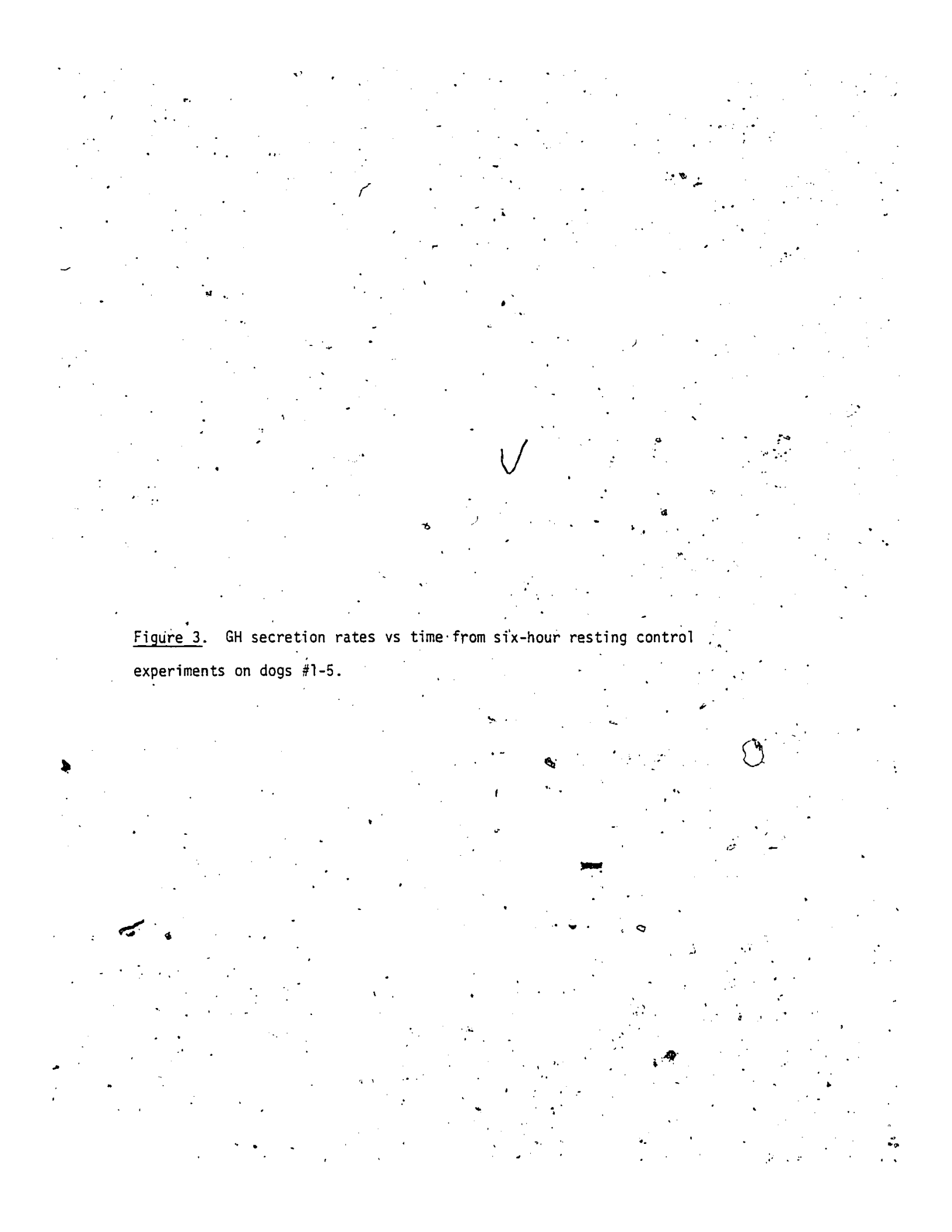


Figure 3. GH secretion rates vs time from six-hour resting control experiments on dogs #1-5.

RESTING CONTROL EXPERIMENTS

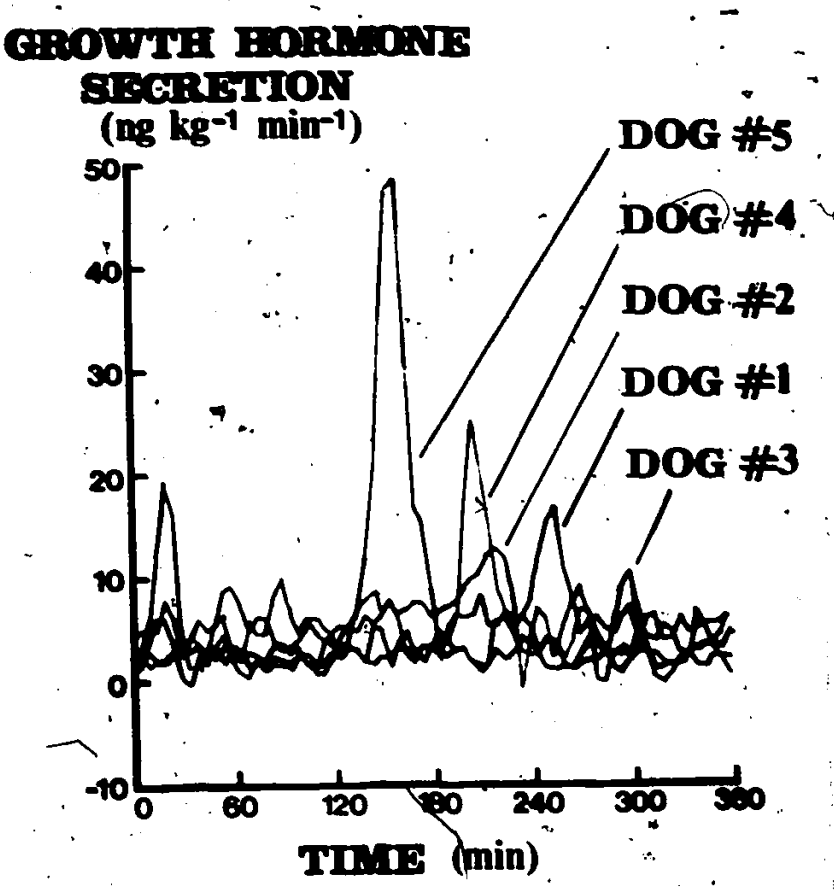
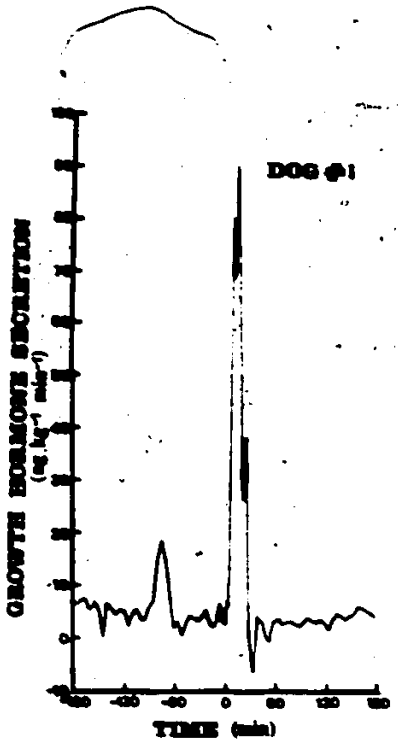
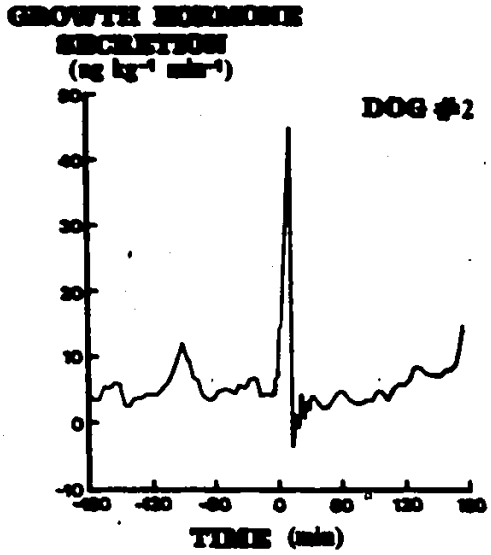


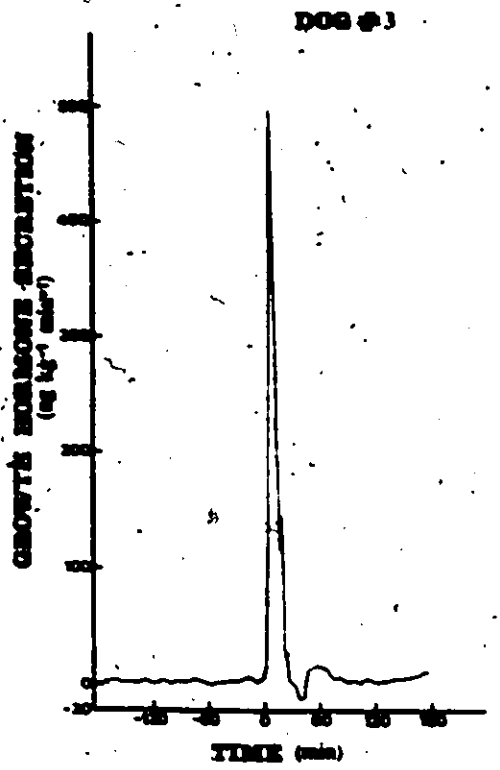
Figure 4 (a-e). GH secretion rates vs time from AS-SRIF experiments on dogs # 1-5. At time = 0, each dog received a bolus injection of 8.5 mL of an antiserum to somatostatin through an indwelling venous catheter. Note the near absence of lags following injection to onset of a GH secretory surge.


DOG #1
GROWTH HORMONE SECRETION
TIME (min)

(a)

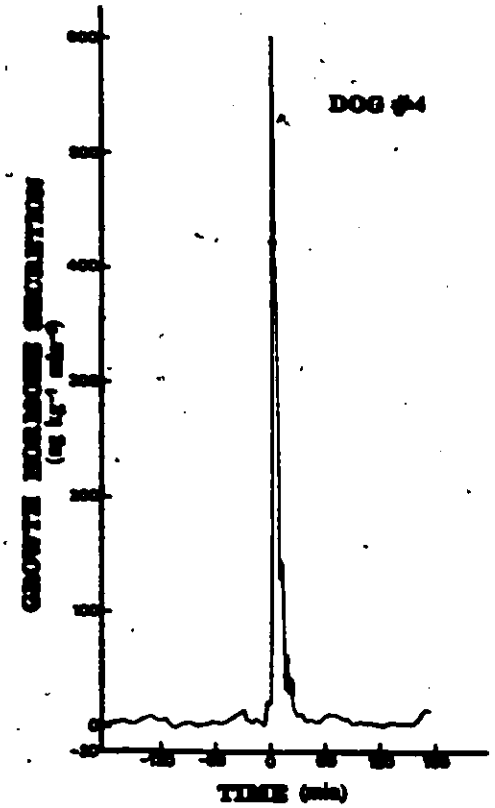

GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #2
TIME (min)

(b)



(c)

Fig. 4



(d)

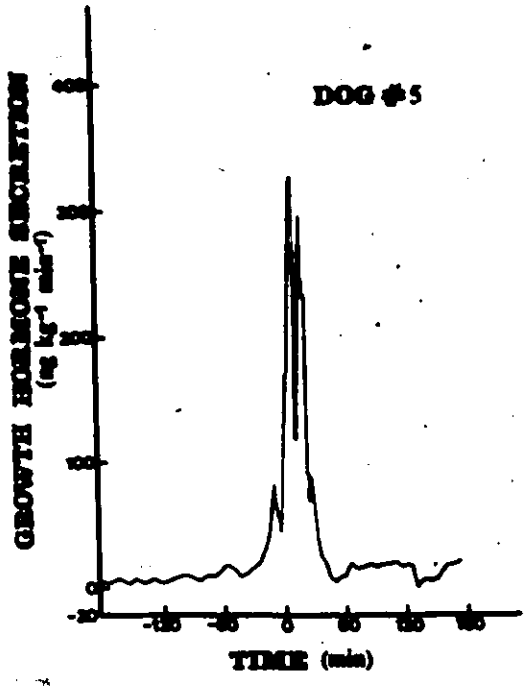
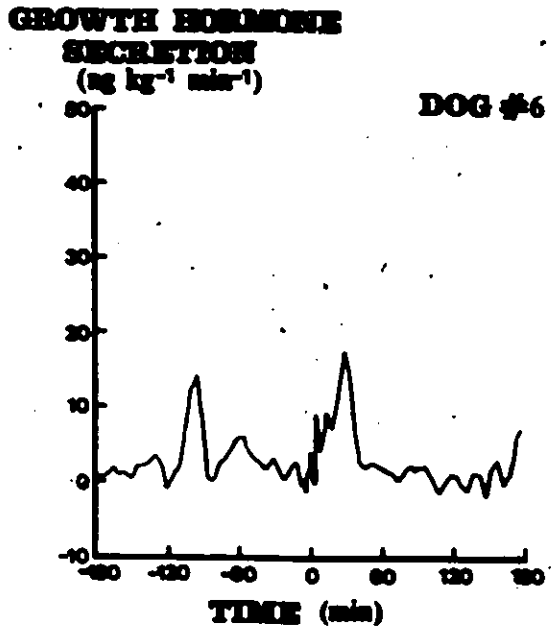
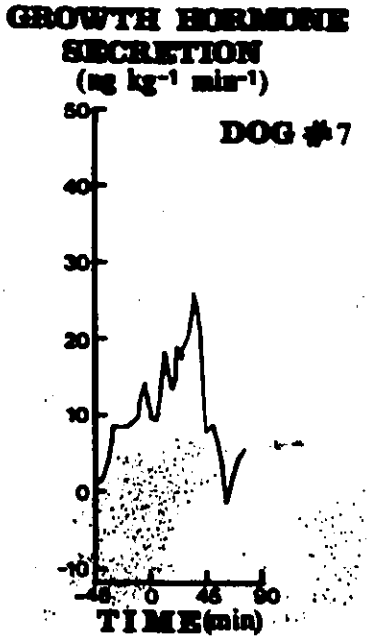


Fig. 4

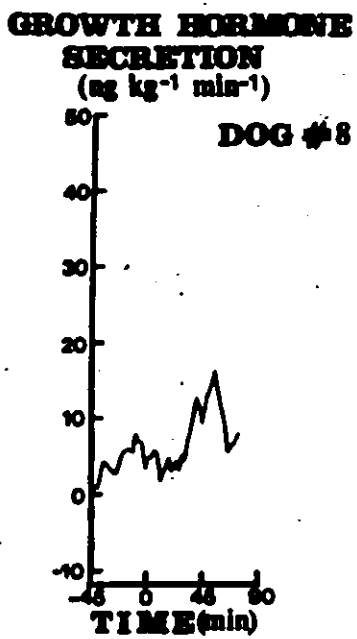
(e)


GROWTH HORMONE
SECRETION
(ng kg⁻¹ min⁻¹)
DOG #6
50
40
30
20
10
0
-10
-180
-120
-60
0
60
120
180
TIME (min)

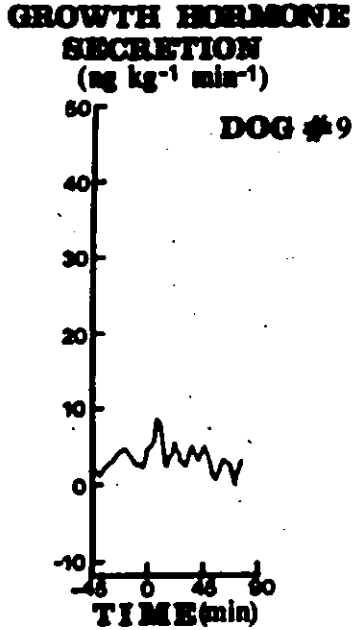
(a)


GROWTH HORMONE
SECRETION
(ng kg⁻¹ min⁻¹)
DOG #7
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

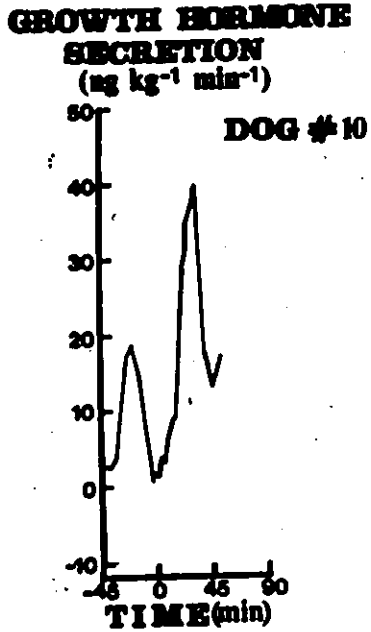
(b)


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #8
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

(c)


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #9
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

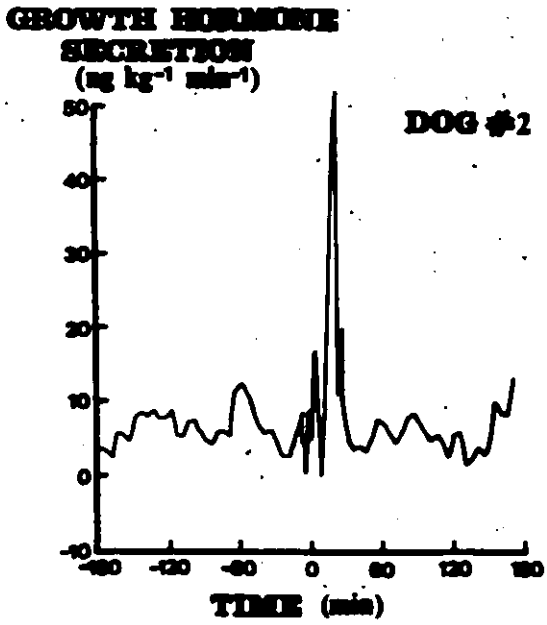
(d)


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #10
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

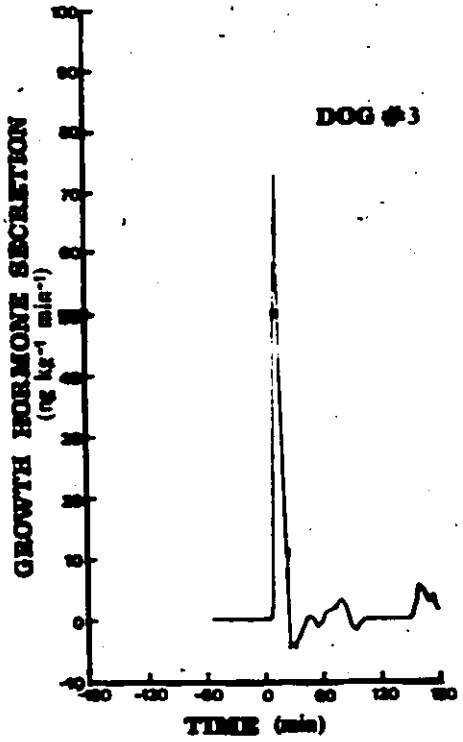
(e)

Fig. 5

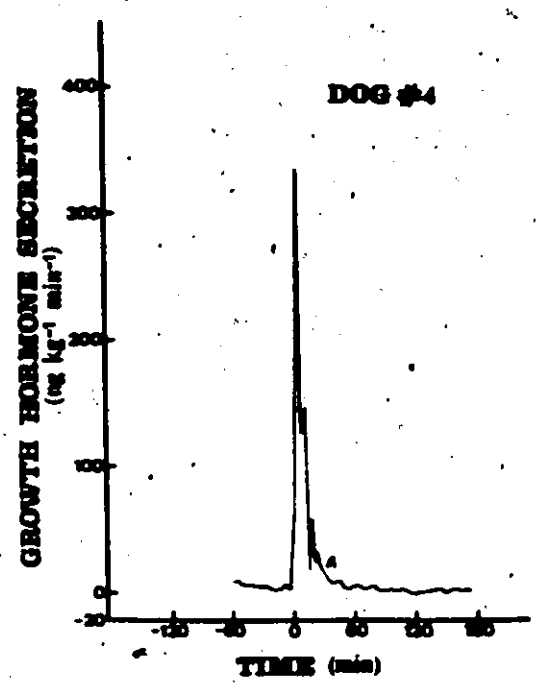
Figure 5 (a-e). GH secretion rates vs time from experiments with injection of control goat sera in naive dogs (#6-10). At time = 0, each dog received a bolus injection of control goat serum through an indwelling venous catheter. Note that when the manipulation is followed by a GH secretory episode, it occurs following lag of 5 or 6 min after injection.


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #2
50
40
30
20
10
0
-10
-180
-120
-60
0
60
120
180
TIME (min)

(a)

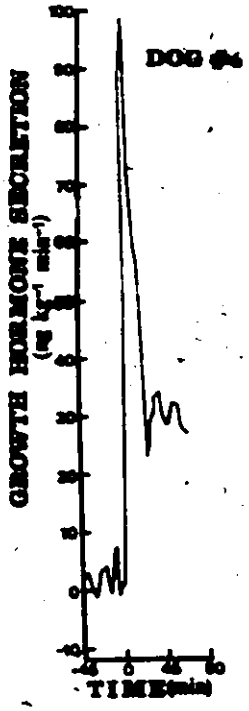

GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #3
0
-10
-180
-120
-60
0
60
120
180
TIME (min)

(b)


DOG #4
GROWTH HORMONE SECRETION
TIME (min)

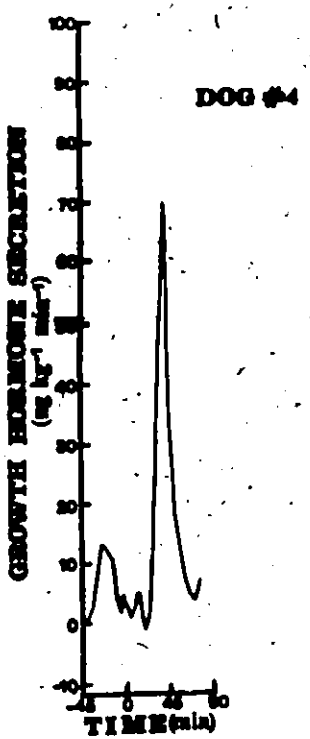
(c)

Fig. 6

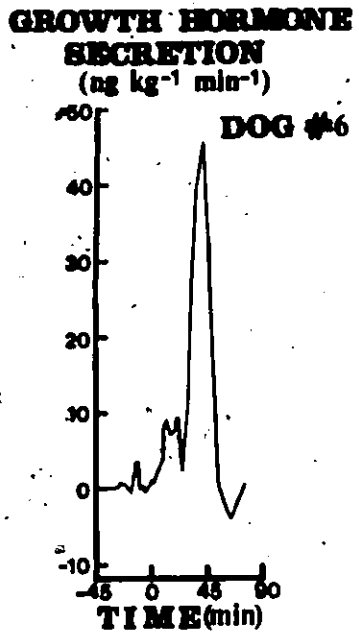

DOG #6
GROWTH HORMONE SECRETION

(d)

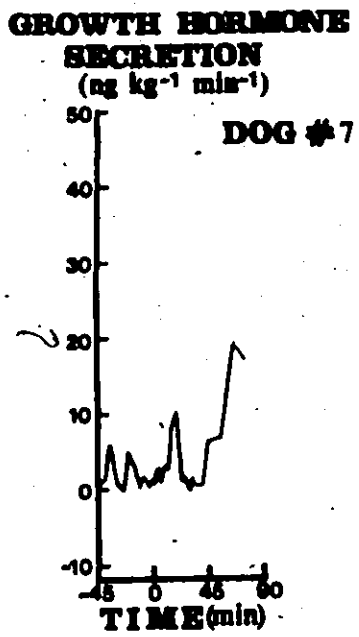
Figure 6 (a-d). GH secretion rates vs time from experiments with injection of control goat sera in dogs previously sensitized to it. At time = 0, each dog received a bolus injection of control goat serum through an indwelling venous catheter. Note the presence of appreciable lags between manipulation and response.


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #4
TIME(min)

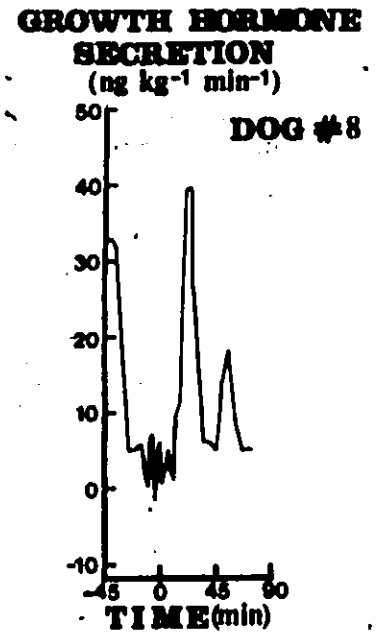
(a)


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #6
TIME(min)

(b)

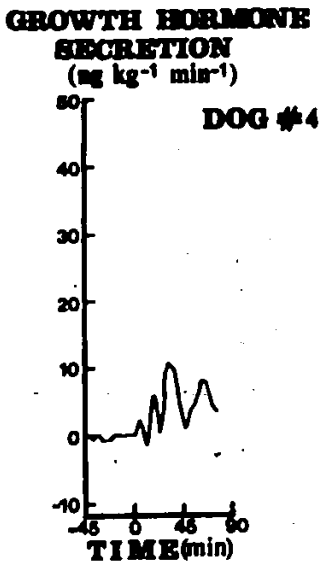

GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #7
TIME(min)

(c)

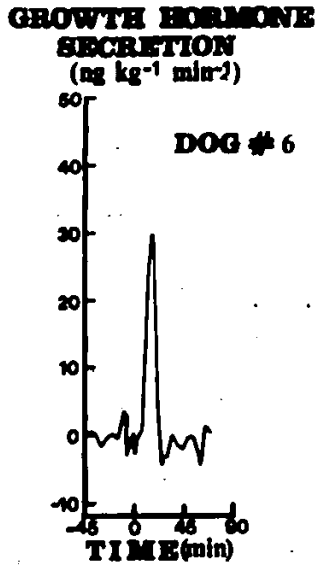

GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #8
TIME(min)

(d)

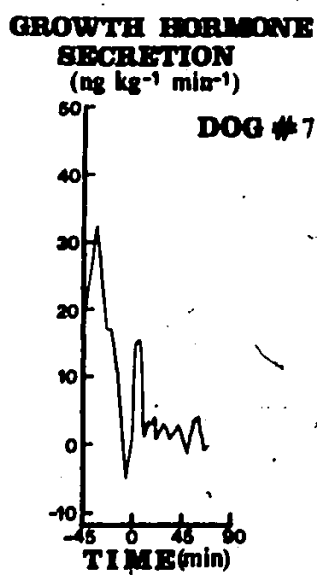
Figure 7 (a-d). GH secretion rates vs time from hypovolemia experiments Blood withdrawal was started in each of the experiments at time = 0 and reinfused about 30 min later. Note the presence of substantial time lags from start of blood withdrawal to onset of GH spike.


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #4
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

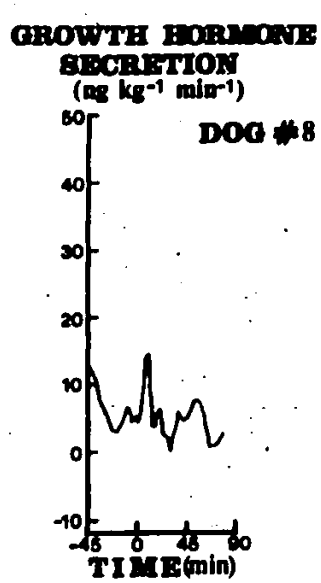
(a)


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG # 6
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

(b)


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #7
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

(c)


GROWTH HORMONE SECRETION
(ng kg⁻¹ min⁻¹)
DOG #8
50
40
30
20
10
0
-10
-45
0
45
90
TIME(min)

(d)

Figure 8 (a-d). GH secretion rates vs time from ether inhalation experiments. Face mask with ether was placed over the animal's snout at time = 0 and removed at time = 10 min. Note that the GH surges when they occur following manipulation, are of short duration.

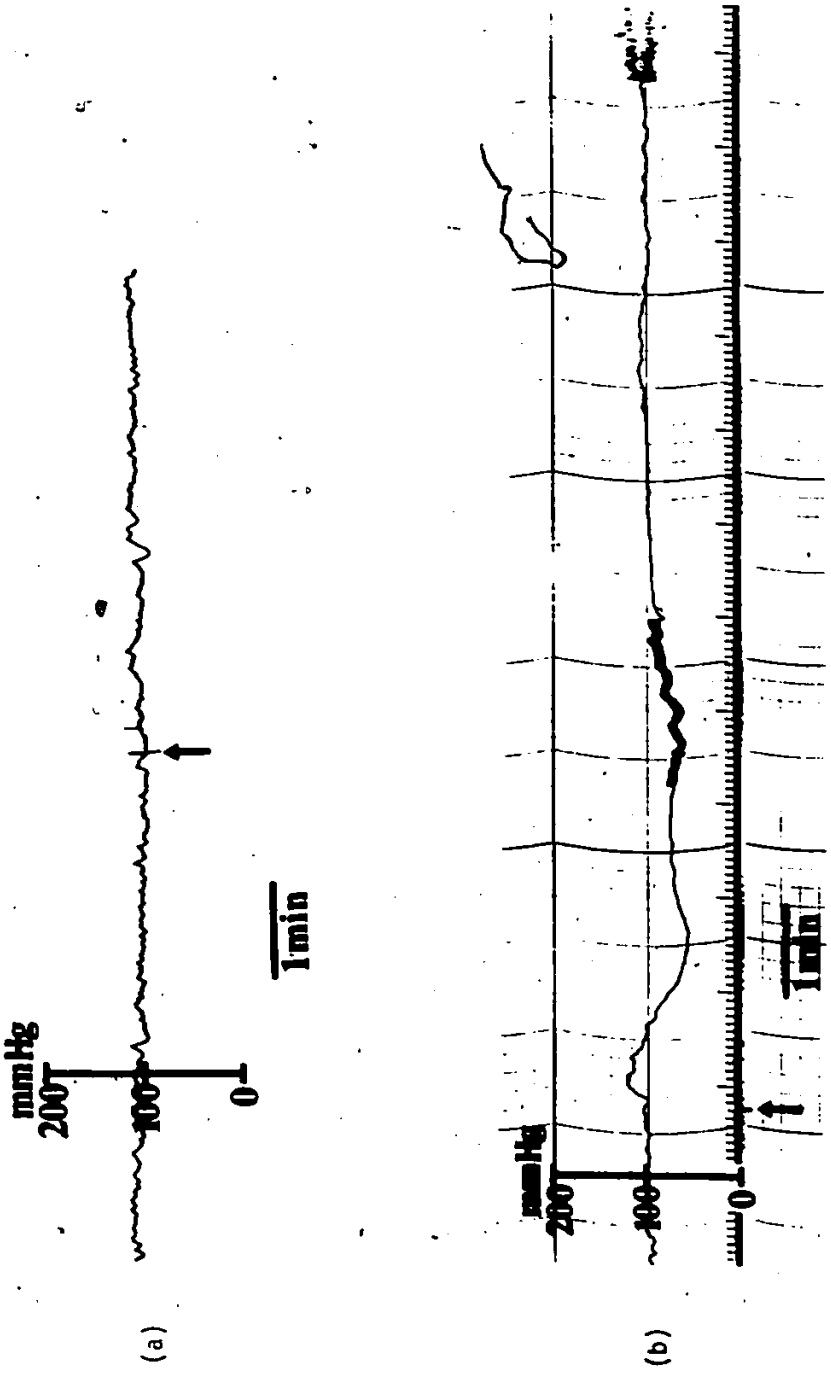

mmHg
200
100
0
1min
(a)
mmHg
200
100
0
1min
(b)

Figure 9 (a). A portion of the mean arterial pressure trace from experiment with first injection of control goat serum in dog #7. The arrow indicates the time at which serum was injected as a bolus into the indwelling venous catheter. Note that M.A.P. is unaffected by administration of the serum.

Figure 9 (b). A portion of the mean arterial pressure trace from experiment with first injection of control goat serum in dog #10. The arrow indicates the time at which the serum was injected as a bolus into the indwelling venous catheter. Note decline in M.A.P. within 1 min of administration of serum and recovery to resting levels 5 min later. Broader segments of tracing are recordings of the pulse pressure, which seems reduced during the transient hypotension and shows a return to normal when normotension was restored.

Fig. 10

Fig. 11

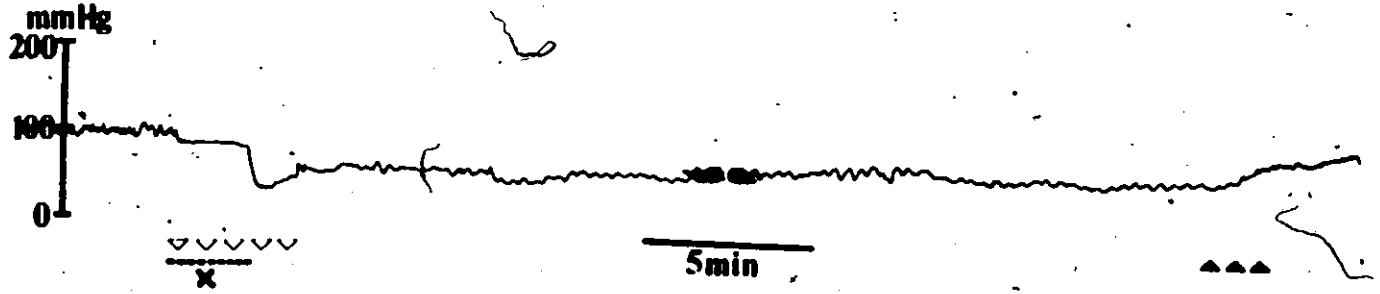


Fig. 12

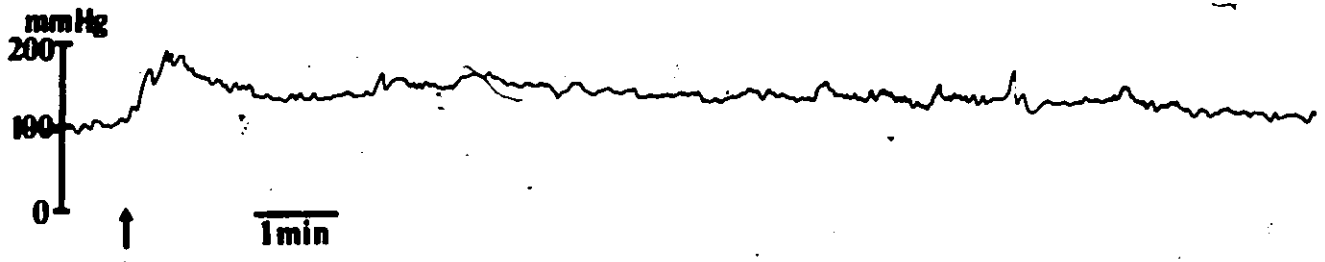


Figure 10. A portion of the mean arterial pressure trace from experiment with injection of control goat serum in dog #6 when this animal had already been sensitized to goat serum in a previous experiment. The arrow indicates the time of bolus injection of the serum through the indwelling venous catheter. Note the immediate rise in M.A.P. for a few seconds before a precipitous fall to about 40 mmHg where it stabilized for several minutes. Broader segments of the tracing are recordings of the pulse pressure, which seems to be gradually decreasing as hypotension becomes more profound.

Figure 11. A portion of the mean arterial pressure trace from the hypovolemia experiment on dog #6. Hollow triangles indicate the period when blood was being withdrawn and solid triangles indicate the period when blood was being reinfused. The broken line marked with a cross indicates the period where the blood pressure was not being recorded as the arterial cannula was being used for the withdrawal of blood, in addition to the indwelling venous catheter. Note that M.A.P. is reduced to ≈ 60 mmHg by the hypovolemia produced and stays at about that level till normotension is rapidly restored by reinfusion of the withdrawn blood about 30 min later.

Figure 12. A portion of the mean arterial pressure trace from the ether inhalation experiment on dog #4. The arrow indicates the time at which the face mask with ether was placed over the animal's snout and this procedure was continued for 10 min. Note the immediate rise in M.A.P. from resting values of about 100 mmHg to about 200 mmHG with the hypertensive effect sustained to an extent throughout the manipulation, gradually declining towards resting values thereafter.

References

Abrams, R.L., M.L. Parker, S. Blanco, S. Reichlin and W.H. Daughaday (1966) Hypothalamic regulation of growth hormone secretion. Endocrinology 78: 605-613.

Althen, T.G. and R.J. Gerrits (1976) Metabolic clearance and secretion rates of porcine growth hormone in genetically lean and obese swine. Endocrinology 99: 511-515.

Ambach, G. and M. Palkovits (1979) The blood supply of the hypothalamus in the rat. In: Handbook of the Hypothalamus, Vol. 1: Anatomy of the Hypothalamus, by P.J. Morgane and J. Panksepp (Editors), p. 274. Marcel Dekker, New York.

Anderson, E. and W. Haymaker (1974) Breakthroughs in hypothalamic and pituitary research. Progress in Brain Research 41: 1-60.

Arimura, A. and J.B. Fishback (1981) Somatostatin: regulation of secretion. Neuroendocrinology 32: 246-256.

Arimura, A., W.D. Smith and A.V. Schally (1976) Blockade of the stress-induced decrease in blood GH by anti-somatostatin serum in rats. Endocrinology 98: 540-543.

Arimura, A., H. Sato, D.H. Coy and A.V. Schally (1975) Radioimmunoassay for GH-releasing inhibiting hormone. Proceedings of The Society of Experimental Biology and Medicine 148: 784-789.

Arnold, M.A. and J.D. Fernstrom (1980) Administration of anti-somatostatin serum to rats reverses the inhibition of pulsatile growth hormone secretion produced by injection of metergoline but not yohimbine. Neuroendocrinology 31: 194-199.

Baker, C.H. and J.W. Remington (1958) Acute circulatory collapse of the adrenalectomized dog following plasma infusion. Circulation Research 6: 294-300.

Benker, G., K. Hackenberg, B. Hamburger and D. Reinwein (1976) Effects of growth hormone release inhibiting hormone and bromocryptine (CB154) in states of abnormal pituitary-adrenal function. Clinical Endocrinology 5: 187.

Berelowitz, M., S.L. Firestone and L.A. Frohman (1981) Effects of growth hormone excess and deficiency on hypothalamic somatostatin content and release on tissue somatostatin distribution. Endocrinology 109: 714-719.

Besser, G.M., C.H. Mortimer, D. Carr, A.V. Schally, D.H. Coy, D.C. Evered, A.J. Kastin, W.M.G. Tunbridge, M.O. Thorner and R. Hall (1974) Growth hormone release inhibiting hormone in acromegaly. British Medical Journal 1: 352-355.

Birge, C.A., G.T. Peake, I.K. Mariz and W.H. Daughaday (1967) Radioimmunoassayable growth hormone in the rat pituitary gland: effects of age, sex and hormonal state. Endocrinology 81: 195-204.

Bliss, J.Q., D.G. Johns and A.S.V. Burgen (1959) Transfusion reactions due to plasma incompatibility in dogs. Circulation Research 7: 79-85.

Bliss, J.Q. and J.D. Walker (1959) Histamine release by homologous plasma in the dog. Canadian Journal of Biochemistry and Physiology 37: 371-375.

Borgeat, P., F. Labrie, J. Drouin, A. Belanger, H. Immer, K. Sestanj, V. Nelson, M. Gotz, A.V. Schally, D.H. Coy and E.J. Coy (1974) Inhibition of adenosine 3', 5'-monophosphate accumulation in the anterior pituitary gland in vitro by growth hormone release in inhibiting hormone. Biochemical and Biophysical Research Communication 56: 1052.

Boyd, W.C. (1966) Immediate hypersensitivity, anaphylaxis, production of anaphylactic sensitivity. Fundamental of Immunology, IV Ed., pp. 417-418. Interscience Publishers, New York.

Brazeau, P., P. Bohlen, N. Ling, F. Esch, R. Benoit and R. Guillemin (1981) Purification and partial characterization of the hypothalamic growth hormone releasing factor. Endocrinology 108: A837.

Brazeau, P., J. Rivier, W. Vale and R. Guillemin (1974) Inhibition of growth hormone secretion in the rat by synthetic somatostatin. Endocrinology 94: 184-187.

Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, R. Guillemin (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive growth hormone. Science 179: 77-79.

Brown, G.M., D.S. Schalch and S. Reichlin (1971) Hypothalamic mediation of growth hormone and adrenal stress response in the squirrel monkey. Endocrinology 89: 694-703.

Chihara, K., N. Minamitani, H. Kaji, A. Arimura and T. Fujita (1981) Intraventricularly injected growth hormone stimulates somatostatin release into rat hypophyseal portal blood. Endocrinology 109:2279-2281.

Chihara, K., A. Arimura and A.V. Schally (1979) Immunoreactive somatostatin in rat hypophyseal portal blood: effect of anesthetics. Endocrinology 104: 1434-1441.

Christensen, S.E., Aa.P. Hansen, J. Weeke and K. Lundback (1978) 24-hour studies of the effects of somatostatin on the levels of plasma growth hormone, glucagon, and glucose in normal subjects and juvenile diabetics. Diabetes 27 (3): 300-306.

Cowan, J.S., P. Gaul, B.C. Moor and J. Kraicer (1984) Secretory bursts of growth hormone secretion in the dog may be initiated by somatostatin withdrawal. In press, Canadian Journal of Physiology and Pharmacology.

Cowan, J.S., B. Moor, A. Chow and J. Kraicer (1983) Characteristics of the post-somatostatin rebound in growth hormone secretion from perifused somatotrophs. Endocrinology 113: 1056-1061.

Cowan, J.S., P. Gaul and J. Kraicer (1981) Does somatostatin (SRIF) cause pulsatile growth hormone secretion? Proceedings of the Canadian Federation of Biological Societies, Vol. 24, 249 (Abstract).

Cowan, J.S. (1978) Validation of a single-compartment approach to the calculation of secretion rates of ACTH. Canadian Journal of Physiology and Pharmacology 56: 410-415.

Coy, D.H., E.J. Coy, A. Arimuro and A.V. Schally (1973) Solid phase synthesis of growth hormone release inhibiting factor. Biochemical and Biophysical Research Communication 54: 1267-1273.

DeGroot, L.J., G.F. Cahill, Jr., W.D. Odell, L. Martini, J.T. Potts, Jr., D.H. Nelson, E. Steinberger and A.I. Winegrad (Editors) (1979) Neuroendocrinology. In: Endocrinology, Volume 1, pp. 3-302. Grune and Stratton, New York.

Deuben, R.R. and J. Meites (1964) Stimulation of pituitary growth hormone release by a hypothalamic extract in vitro. Endocrinology 74: 408-414.

Dorsa, D.M. and M.H. Connors (1979) Canine growth hormone responsiveness during pentobarbital anesthesia: a method for evaluating serotoninergic stimulatory action. Endocrinology 104: 101-104.

Efendic, S. and R. Luft (1980) Somatostatin: A classical hormone, a locally active polypeptide and a neurotransmitter. Annals of Clinical Research 12: 87-24.

Efendic, S., P.E. Lins, K. Uvnas-Wallensten (1978) Extrahypophyseal effects of somatostatin. Annals of Clinical Research 10: 151-156.

Eikelboom, R. and G.S. Tannenbaum (1983) Effects of obesity inducing ventromedial hypothalamic lesions on pulsatile growth hormone and insulin secretion: evidence for the existence of a growth hormone-releasing factor. Endocrinology 112: 212-219.

Elias, G.L., F.N. Gurd, L.G. Hampson and A.S.V. Burgen (1962) Comparison of effectiveness of autologous and nonautologous plasma in oligemic shock in dogs. Circulation Research 11: 857-861.

Ferland, L., F. Labrie, M. Jobin, A. Arimura and A.V. Schally (1976) Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion. Biochemical and Biophysical Research Communication 68: 149-156.

Finklestein, J.W., H.P. Roffwarg, R.M. Boyar, J. Kream and L. Hellman (1972) Age related changes in the twenty four hour spontaneous secretion of growth hormone. Journal of Clinical Endocrinology and Metabolism 35: 665-670.

Frohman, L.A. and L.L. Bernardis (1970) Growth hormone secretion in the rat: metabolic clearance and secretion rates. Endocrinology 86: 305-312.

Frohman, L.A. and L.L. Bernardis (1968) Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. Endocrinology 82: 1125-1132.

Frohman, L.A., L.L. Bernardis and K. Kant (1968) Hypothalamic stimulation of growth hormone secretion. Science 162: 580-582.

Gadboys, H.L., A.R. Jones, R. Slonin, B.G. Wisoff and R.S. Litwak (1963) The homologous blood syndrome III: Influence of plasma, buffy coat and red cells in provoking it's manifestations. American Journal of Cardiology 12: 194-202.

Ganong, W.F. (1981) Review of Medical Physiology, Xth Edition, p. 290. Lange Medical Publications, Los Altos, California.

Garcia, J.F. and I.I. Geschwind (1968) Investigation of growth hormone secretion in selected mammalian species. In: Growth Hormone, by A. Pecile and E.E. Muller (Editors), pp. 267-291. Excerpta Medica Foundation, Amsterdam.

Gillioz, P., P. Girand, B. Conte-Devolx, P. Jaquet, J.L. Codaccioni and C. Oliver (1979) Immunoreactive somatostatin in rat hypophysial portal blood. Endocrinology 104: 1407-1410.

Glick, S.M., J. Roth, R.S. Yalow and S.A. Berson (1965) The regulation of growth hormone secretion. Recent Progress in Hormone Research 21: 241-283.

Glick, S.M., J. Roth and E.T. Lonergan (1964) Survival of endogenous human growth hormone in plasma. Journal of Clinical Endocrinology 24: 501-505.

Gomez-Pan, A., W.M.G. Turnbridge, A. Duns, R. Hall, G.M. Besser, D.H. Coy, A.V. Schally and A.J. Kastin (1975) Hypothalamic hormone interactions in acromegaly. Clinical Endocrinology 4: 455-460.

Guillemin, R., P. Brazeau, P. Bohlen, F. Esch, N. Ling and W.B. Wehrenberg (1982) Growth hormone releasing factor from a human pancreatic tumor that caused acromegaly. Science 218: 585-587.

Guillemin, R. (1978) Biochemical and physiological correlates of hypothalamic peptides. The new endocrinology of the neuron. In: The Hypothalamus, by S. Reichlin, R.J. Baldessarini and J.B. Martin (Editors), pp. 155-194. Raven Press, New York.

Hall, R., G.M. Besser, A.V. Schally, D.H. Coy, D.C. Evered, D.J. Goldie, A.J. Kastin, A.S. McNeilly, C.H. Mortimer, C. Phenekos, W.M.G. Turnbridge and D.R. Weightman (1973) Action of growth hormone release inhibitory hormone in healthy men and in acromegaly. Lancet 2: 581.

Hampshire, J., N. Altszuler, R. Steele and L.J. Greene (1975) Radioimmunoassay of canine growth hormone: enzymatic radioiodination. Endocrinology 96: 822-828.

Jacoby, J.H., J.F. Sassin, M. Greenstein and E.D. Weitzman (1974) Patterns of spontaneous cortisol and growth hormone secretion in Rhesus monkeys during the sleep waking cycle. Neuroendocrinology 14: 165-173.

Kasting, N.W., J.B. Martin and M.A. Arnold (1981) Pulsatile somatostatin release from the median eminence of the unanesthetized rat and its relationship to plasma growth hormone levels. Endocrinology 109: 1739-1745.

Kato, Y., K. Chihara, S. Ohgo and H. Imura (1974) Inhibiting effect of somatostatin on growth hormone release induced by isoprenaline or chlorpromazine in rats. Journal of Endocrinology 62: 687-688.

Kramer, M.W., S.E. Pearson and S.M. Michaelson (1973) Plasma disappearance of radioiodinated dog-growth hormone in the dog. Hormone and Metabolic Research 5: 470-471.

Krulich, L., A.P.S. Dhariwal and S.M. McCann (1968) Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology 83: 783-790.

Lenox, R.H., G.J. Kant, G.R. Sessions, L.L. Pennington, E.H. Mougey and J.L. Meyerhoff (1980) Specific hormonal and neurochemical responses to different stressors. Neuroendocrinology 30: 300-308.

Lovinger, R., A.T. Boryczka, R. Shakelford, S.L. Kaplan, W.F. Ganong and M.M. Grumbach (1974) Effect of synthetic somatotropin release inhibiting factor on the increase in plasma growth hormone elicited by L-dopa in the dog. Endocrinology 95: 943-946.

Lovinger, R.D., M.H. Connors, S.L. Kaplan, W.F. Ganong and M.M. Grumbach (1974) Effect of L-dihydroxyphenylalamine (L-Dopa), anesthesia and surgical stress on the secretion of growth hormone in the dog. Endocrinology 95: 1317-1321.

Luben, R.A., P. Brazeau, P. Bohlen and R. Guillemin (1982) Monoclonal antibodies to hypothalamic growth hormone releasing factor with picomoles of antigen. Science 218: 887-889.

Luben, R.A., P. Brazeau and R. Guillemin (1982) Production of monoclonal antibodies to hypothalamic growth hormone releasing factor by exclusive use of in vitro immunization. Endocrinology 110: A539.

Lucke, C., B. Hoffken and A. von zur Muhlen (1976) Studies on the postponed growth hormone secretion following the infusion of somatostatin. Acta Endocrinologia 82: 460-466.

Lumpkin, M.D., A. Negro-Vilar and S.M. McCann (1980) Paradoxical elevation of growth hormone by intraventricular somatostatin: possible ultrashort-loop feedback. Science 211: 1072-1074.

Malven, P.V. (1974) Altered release of GH, LH and prolactin (PRL) induced by electrical stimulation of the median eminence (ME) in unanesthetized sheep. Endocrinology 94: A127.

Martin, J.B. (1979) Brain mechanism for integration of growth hormone secretion. 23rd Bowditch Lecture. Physiologist 22: 23-29.

Martin, J.B., P. Brazeau, G.S. Tannenbaum, J.O. Willoughby, J. Epelbaum, L.C. Terry and D. Durand (1978) Neuroendocrine organization of growth hormone regulation. In: The Hypothalamus, by S. Reichlin, R.J. Baldessarini and J.B. Martin (Editors), pp. 329-357. Raven Press, New York.

Martin, J.B. (1976) Brain regulation of growth hormone secretion. In: Frontiers in Neuroendocrinology, Vol. 4, by L. Martini and W.F. Ganong (Editors), pp. 129-168. Raven Press, New York.

Martin, J.B., G.S. Tannenbaum, J.O. Willoughby, L.P. Renaud and P. Brazeau (1975) Functions of the central nervous system in regulation of pituitary GH secretion. In: Hypothalamic Hormones: Chemistry, Physiology, Pharmacology and Clinical Uses, by M. Motta, P.G. Crosignani and L. Martini (Editors), pp. 217-236. Academic Press, New York.

Martin, J.B., L.P. Renaud and P. Brazeau (1974) Pulsatile growth hormone secretion: suppression by hypothalamic ventromedial lesions and by long acting somatostatin. Science 186: 538-540.

Martin, J.B. (1974) Inhibitory effect of somatostatin (SRIF) on the release of growth hormone (GH) induced in the rat by electrical stimulation. Endocrinology 94: 497-502.

Mason, J.W. (1971) A re-evaluation of the concept of 'non-specificity' in stress theory. Journal of Psychiatric Research 8: 323-333.

McCann, S., (Section Editor) (1979) Endocrinology and Metabolism. Annual Review of Physiology 41: 553-631.

McIntyre, H.B. and W.D. Odell (1974) Physiological control of growth hormone in the rabbit. Neuroendocrinology 16: 8-21.

Meites, J., B.T. Donovan and S.M. McCann (Editors) (1975) Pioneers in Neuroendocrinology. Plenum Press, New York.

Meyer, V., and E. Knobil (1967) Growth hormone secretion in the unanesthetized rhesus monkey in response to noxious stimuli. Endocrinology 80: 163-171.

Molitch, M.E. and L.E. Hlivyak (1980) Growth hormone short-loop feedback: anatomic specificity of growth hormone stimulation of hypothalamic somatostatin concentration. Hormone and Metabolic Research 12: 559-560.

Mortimer, C.H., D. Carr, T. Lind, S.R. Bloom, C.N. Mallinson, A.V. Schally, W.M.G. Turnbridge, L. Yeomans, D.H. Coy, A.J. Kastin, G.M. Besser and R. Hall (1974) Effects of growth hormone release inhibiting hormone on circulating glucagon, insulin and growth hormone in normal, diabetic, acromegalic and hypopituitary patients. Lancet 1: 697-701.

Muller, E.E., A. Pecile, M. Felici and D. Cocchi (1970) Norepinephrine and dopamine injection into lateral brain ventricle of the rat and growth hormone-releasing activity in the hypothalamus and plasma. Endocrinology 86: 1376-1382.

Norstedt, G., A. Mode, T. Hokfelt, P. Eneroth, R. Elde, L. Ferland, F. Labrie and J.A. Gustafsson (1983) Possible role of somatostatin in the regulation of the sexually differentiated steroid metabolism and prolactin receptor in rat liver. Endocrinology 112: 1076-1090.

Parker, D.C., L.G. Rossman, T.M. Siler, J. Rivier, S.S.C. Yen and R. Guillemin (1974) Inhibition of sleep related peak in physiologic human growth hormone release by somatostatin. Journal of Clinical Endocrinology and Metabolism 38: 406-499.

Patel, Y.C. and S. Reichlin (1978) Somatostatin in hypothalamus, extra-hypothalamic brain, and peripheral tissues of the rat. Endocrinology 102: 523-530.

Peake, G.T., I.K. Mariz and W.H. Daughaday (1968) Radioimmunoassay of growth hormone in rats bearing somatotropin producing tumors. Endocrinology 83: 714-720.

Pelletier, G. (1980) Immunohistochemical localization of somatostatin. Progress in Histochemistry and Cytochemistry 12(3): 1-14

Peracchi, M., E. Reschini, L. Cantalamessa, A. Catania and G. Giustina (1976) Inhibitory effect of somatostatin on dibutyryl cyclic AMP-induced insulin and growth hormone release in human subjects. Metabolism 25(3): 321-328.

Pimstone, B.L., D. LeRoith, S. Epstein and S. Kronheim (1975) Disappearance rates of plasma growth hormone after intravenous somatostatin in renal and liver disease. Journal of Clinical Endocrinology and Metabolism 41: 392-394.

Porter, J.C. (Editor) (1977) Hypothalamic peptide hormones and pituitary regulation. Advances in Experimental Medicine and Biology, Vol. 87. Plenum Press, New York.

Prange-Hansen, A.A., H. Orskov, K. Seyer-Hansen and K. Lundback (1973) Some actions of growth hormone-release inhibiting factor. British Medical Journal 3: 523.

Reichlin, S. (1981) Neuroendocrine aspects of control of specific pituitary tropic hormones. In: Textbook of Endocrinology, VIth Edition, by R.H. Williams (Editor), pp. 610-622. W.B. Saunders, Philadelphia.

Reichlin, S. (1981) Systems for the study of regulation of neuropeptide secretion. Advances in Biochemical Psychopharmacology 28: 571-597.

Reichlin, S. (1974) Regulation of somatotropic hormone secretion. In: Handbook of Physiology, Section 7, Part 2, Volume 4, by E. Knobil and W.H. Sawyer (Editors), p. 405. American Physiological Society, Washington, D.C.

Reynaert, R., S. Marcus, M. DePaepe and G. Peeters (1976) Influences of stress, age and sex on serum growth hormone and free fatty acid levels in cattle. Hormone and Metabolic Research 8: 109-114.

Rice, R.W. and V. Critchlow (1976) Extra-hypothalamic control of stress-induced inhibition of growth hormone secretion in the rat. Endocrinology 99: 970-976.

Rose, R.M. and E. Sachar (1981) Psychoendocrinology: Endocrine responses to stress. In: Textbook of Endocrinology, VIth Edition, Chapter 12, by R.H. Williams (Editor), pp. 654-657. W.B. Saunders Company, Philadelphia.

Sakuma, M. and E. Knobil (1970) Inhibition of endogenous growth hormone secretion by exogenous growth hormone infusion in the Rhesus monkey. Endocrinology 86: 890-894.

Schalch, D.S. and S. Reichlin (1968) Stress and growth hormone release. In: Growth Hormone, by A. Pecile and E.E. Muller (Editors), pp. 211-225. Excerpta Medica Foundation, Amsterdam.

Schalch, D.S. (1967) The influence of physical stress and exercise on growth hormone and insulin secretion in man. Journal of Laboratory and Clinical Medicine 69: 256-269.

Schalch, D.S. and Reichlin (1966) Plasma growth hormone concentration in the rat determined by radioimmunoassay: influence of sex, pregnancy, lactation, anesthesia, hypophysectomy and extrasellar pituitary transplant. Endocrinology 79: 275-280.

Schindler, W.J., M.O. Hutchins and E.J. Septimus (1972) Growth hormone secretion and control in the mouse. Endocrinology 91: 483-490.

Schusdziarra, V., E. Zyznar, D. Rouiller, V. Harris and R.H. Unger (1980) Free somatostatin in the circulation: amounts and molecular sizes of somatostatin-like immunoreactivity in portal, aortic and vena caval plasma of fasting and meal stimulated dogs. Endocrinology 107: 1572-1576.

Silber, R.H., R.D. Busch and R. Oslapas (1958) Practical procedure for estimation of corticosterone and hydrocortisone. Clinical Chemistry 4: 278-285.

Siler, T.M., G. Vandenberg, S.S.C. Yen, P. Brazeau, W. Vale and R. Guillemin (1973) Inhibition of growth hormone release in humans by somatostatin. Journal of Clinical Endocrinology and Metabolism 37: 632-634.

Sinha, Y.N., S.R. Baxter and W.P. Vanderlaan (1979) Metabolic clearance rate of growth hormone in mice during various physiological states. Endocrinology 105: 685-689.

Smith, C.R. and R.L. Hamlin (1977) Circulatory shock. In: Duke's Physiology of Domestic Animals, by M.J. Swenson (Editor), IXth Edition, pp. 150-156. Cornell University Press, Ithaca.

Smith, E.L., S. Deavers and R.A. Huggins (1965) Effects of autologous and homologous blood replacement in hypovolemic dogs. Proceedings of the Society of Experimental Biology and Medicine 120: 199-202.

Stachura, M.E. (1977) Interaction of somatostatin inhibition and dibutyryl cyclic AMP on potassium stimulation of growth hormone release from perifused rat pituitaries. Endocrinology 101: 1044-1053.

Stachura, M.E. (1976) Influence of synthetic somatostatin upon growth hormone release from perifused rat pituitaries. Endocrinology 99: 678-683.

Takahashi, K. (1978) Effect of various traumatic stresses on growth hormone release in pentobarbital anesthetized rats. Neuroendocrinology 26: 1-7.

Takahashi, K., W.H. Daughaday and D.M. Kipnis (1971) Regulation of immunoreactive growth hormone secretion in male rats. Endocrinology 88: 909-917.

Takahashi, Y., S. Ebihara, Y. Nakamura and K. Takahashi (1981) A model of human sleep-related growth hormone secretion in dogs: effects of 3, 6 and 12 hours of forced wakefulness on plasma growth hormone, cortisol and sleep stages. Endocrinology 109: 262-272.

Takahashi, Y., D.M. Kipnis and W.H. Daughaday (1968) Growth hormone secretion during sleep. Journal of Clinical Investigation 47: 2079-2090.

Tannenbaum, G.S. (1980) Evidence for autoregulation of growth hormone secretion via the central nervous system. Endocrinology 107: 2117-2120.

Tannenbaum, G.S., J. Epelbaum, E. Colle, P. Brazeau and J.B. Martin (1978) Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion Endocrinology 102: 1909-1914.

Tannenbaum, G.S. and J.B. Martin (1976) Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology 98: 562-570.

Taylor, A.L., J.L. Finster and D.H Mintz (1969) Metabolic clearance and production rates of human growth hormone. Journal of Clinical Investigation 48: 2349-2358.

Terry, L.C. and J.B. Martin (1981) The effects of lateral hypothalamic-medial forebrain stimulation and somatostatin antiserum on pulsatile growth hormone secretion in freely behaving rats: evidence for a dual regulatory mechanism. Endocrinology 109: 622-627.

Terry, L.C. and W.R. Crowley (1980) The effect of hypophysectomy on somatostatin-like immunoreactivity in discrete hypothalamic and extra-hypothalamic nuclei. Endocrinology 107: 1771-1775.

Terry, L.C., J.O. Willoughby, P. Brazeau and J.B. Martin (1976) Antiserum to somatostatin prevents stress-induced inhibition of growth hormone secretion in the rat. Science 192: 565-567.

Tindal, J.S., G.S. Knaggs, I.C. Hart, L.A. Blake (1978) Release of growth hormone in lactating and non-lactating goats in relation to behaviour, stages of sleep, electro-encephalograms, environmental stimuli and levels of prolactin, insulin, glucose and free fatty acids in the circulation. Journal of Endocrinology 76: 333-346.

Tsushima, T., M. Irie and M. Sakuma (1971) Radioimmunoassay for canine growth hormone. Endocrinology 89: 685-693.

Vale, W., C. Rivier and M. Brown (1980) Physiology and pharmacology of hypothalamic regulatory peptides. In: Handbook of the Hypothalamus - Physiology of the Hypothalamus, by P.J. Morgane and J. Panksepp (Editors), Vol. 2, pp. 165-252. Marcel Dekker, New York.

Vale, W., P. Brazeau, C. Rivier, M. Brown, B. Boss, J. Rivier, R. Burgus, N. Ling and R. Guillemin (1975) Somatostatin Recent Progress in Hormone Research 31: 365-397.

Ward, F.R., H. Leblanc and S.S.C. Yen (1975) The inhibitory effect of somatostatin on growth hormone, insulin and glucagon secretion in diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 41(3): 527-532.

Wehrenberg, W.B., P. Brazeau, R. Luben, P. Bohlen and R. Guillemin (1982) Inhibition of pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). Endocrinology 111: 2147-2148.

Wehrenberg, W.H., D. McNicol, A.G. Frantz and M. Ferin (1980) The effects of serotonin on prolactin and growth hormone concentrations in normal and pituitary stalk sectioned monkeys. Endocrinology 107: 1747-1750.

Willoughby, J.O. and J.B. Martin (1978) Pulsatile growth hormone secretion: inhibitory role of medial preoptic area. Brain Research 148: 240-244.

Willoughby, J.O., J.B. Martin, P. Brazeau and L.P. Renaud (1976) Pulsatile growth hormone release in the rat: failure to demonstrate a correlation with sleep phases. Endocrinology 98: 991-996.

Wilson, G.S., A. Miles (1975) Antibody mediated allergy, serum sickness and related phenomena. Topley and Wilson's Principles of Bacteriology, Virology and Immunity, VIth Edition, Volume 2, Chapter 49, p. 1497. The Williams and Wilkins Company, Baltimore.

Yamashiro, D. and C.H. Li (1973) Synthesis of a peptide with full somatostatin activity. Biochemical and Biophysical Research Communication 54: 882-888.

Yen, S.S.C., T.B. Siler and G.W. DeVane (1974) Effect of somatostatin in patients with acromegaly: suppression of growth hormone, prolactin, insulin and glucose levels. New England Journal of Medicine 290: 935-938.

Zanini, A., G. Giannattasio, P. DeCamilli, A.E. Panerai, E.E. Muller and J. Meldolesi (1979) Studies on rat pituitary homografts. I. In vitro biosynthesis and release of growth hormone and prolactin. Endocrinology 104: 226-236.

Zierden, E., H. Wagner, G. Wust, G. Baumister and W.H. Hauss (1975) HGH, corticoids, insulin and glucose in serum during excessive hyperthermia and effect of somatostatin in male volunteers. Acta Endocrinologia Supplement 193: 73.