Linear Growth in Children With Growth Hormone Deficiency.

Interaction of Growth Hormone, Somatostatin and Amino Acids.

by

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DEDICATION

This thesis is dedicated

to

my mother and father

and to my wife and our children

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Author Preface

It has been said that the difference between a person who has written a Ph.D., thesis and one who has not is that one has written a Ph.D., thesis and one has not . Those who have not written such a thesis will never know the joy of starting such a work , the toil and sweat of continuing , the endless hours of painstaking collection of data characterized by accuracy, specificity, reliability and reproducibility and the boredom, exhaustion, and thoughts of self-slaughter as night merges into day and day into night.

Unfortunately about half way through this work my mother passed away and I was compelled to return to Libya not as a matter of custom but as a matter of duty. The loss touched me very deeply and I will never forget her as long as I am alive.

A very strong stimulus was required to counteract the faintness as the last Chapter of the thesis was nearing completion. Then came the checking of references but that was mechanical for the end was near. In all these trials Dr William Hamilton, my supervisor has been an unparalleled inspiration. Long hours of patient discussion, an education in itself, the harmony and the cadence of the English language woven into scientific work freed thereby from jargon has been a pleasure to write. Thus the work has been completed.

I am indebted to the Amana of Nuclear Research in Libya for supporting me financially to undertake this work and especially to Mr Abdulmajeed Elgwod (Engineer) Secretary of the Amana initially.

My grateful thanks go also to my chief supervisor, Professor Forrester Cockburn, Head of the Department of Child Health in whose laboratories the scientific work was done.

And what shall I more say , for time would fail to tell of the unfailing, devotional support received from my wife and my children, who almost became as involved in this work as myself and to whom jointly the thesis is dedicated.

DECLARATION

None of the work presented in this thesis has been submitted in support of an application for another degree of this or another university or institution of learning.

Summary

Thirty two children (twenty one boys and eleven girls) whose ages ranged from 2.17 to 17.94 years were identified as having growth hormone deficiency. Of these, nineteen (fourteen boys and five girls) had idiopathic growth hormone deficiency, while in thirteen (seven boys and six girls) the growth hormone deficiency was due to cerebral tumours either sui generis as in craniopharyngioma or idiopathic following operation and postoperative cranial irradiation.

Assays of plasma somatostatin and amino acid concentrations were made on each patient three times, firstly at the time of the withdrawal of cadaveric growth hormone, again at the reintroduction of recombinant growth hormone nine months later and finally after one year's treatment with the recombinant growth hormone.

The patients in the idiopathic group fell into two categories, mainly those whose plasma somatostatin concentrations were either abnormaly high or at a high normal concentration, and those in whom the plasma somatostatin concentrations were normal. Those with normal plasma somatostatin concentrations had a better catch-up growth than those whose plasma somatostatin concentrations were high.

In those children with cerebral tumours the plasma somatostatin concentrations were uniformly within normal limits irrespective of their growth response to the recombinant growth hormone treatment.

All the children during the off-growth hormone period had reduced rate of linear growth and plasma amino acid concentrations compared with both the cadaveric and recombinant growth hormone periods. On the

other hand the recovery in both linear growth velocity and plasma amino acid concentrations was greatest in those children whose plasma somatostatin was normal.

Thus normal growth hormone and low plasma concentration of somatostatin are associated with a normal or high plasma amino acid concentrations, a situation advantageous to the linear growth.

Since the interrelationship between growth hormone, somatostatin, insulin like growth factors and linear growth are now clearly linked, immunization against somatostatin (in those children with high somatostatin concentration) or the concurrent administration of IGF-1 might be explored in the treatment of growth hormone deficiency.

It is concluded that firstly, the high plasma concentrations of somatostatin militate against an optimum response to administered growth hormone and the concurrent fall in plasma amino acid concentrations may be contributary to a reduced osseous and muscle growth.

Secondly, that children who do not respond to growth hormone therapy as expected should have their plasma somatostatin concentration assayed before increasing the dosage of the expensive recombinant growth hormone.

Thirdly, it may be that even in children with short stature whose growth hormone status is normal, a high plasma somatostatin concentration is contributing to the short stature.

It is proposed that assay of plasma somatostatin is mandatory in the investigation of children with short stature.

LIST OF ABBREVIATION

ACTH	Adrenocorticotrophic hormone
AGV	Annual growth velocity
BSE	Bovine serum encephalopathy
DAA	Dispensable amino acid
FSH	Follicle stimulating hormone
GAR	Goat anti-rabbit
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone releasing hormone
GHRIH	Growth hormone release inhibiting hormone
IDAA	Indispensable amino acid
IGF-I&II	Insulin like growth factors one and two
IGHD	Idiopathic growth hormone deficiency
LH	Luteinizing hormone
REM	Rapid eye movement
RIA	Radioimmunoassay
SDS	Standard deviation score
SRIF	Somatotrophin release inhibiting factor
T_3	Triiodothyronine
T_4	Tetraiodothyronine
TRH	Thyrotrophin releasing releasing hormone
TSH	Thyroid stimulating hormone
UMOL	Micro-mole

CHAPTER ONE

Introduction

Growth and especially its control is a complex and controversial phenomenon. It represents a profound interaction between genetic, hormonal and nutritional influences, and is the result of a multitude of processes which are controlled by neural and humoral mechanisms. Growth in effect is due to increase in total cell number (hyperplasia), in cell size (hypertrophy) and in the amount of intracellular material.

In this introduction I will review in general terms the present understanding of these mechanisms before embarking on a detailed analysis of the finer aspects of the subject relative to the work presented here.

Historical review

The earliest thoughts on there being a substance related to growth and its assignment to the pituitary gland as its origin has a historical interest but the real association of linear growth and the pituitary gland came as a result of Crow and his colleagues (1) who removed the pituitary glands from dogs and observed thereafter the failure of linear growth. The second phase was the observation that pituitary extracts when injected intraperitoneally into rats resulted in acceleration of their body growth (2). It was a short step to the purification of the earlier crude extracts and credit goes to Li and Evans (3) who isolated from bovine anterior pituitaries in a highly purified form a growth promoting substance which was named growth hormone. Unfortunately a human hypopituitary dwarf was given this bovine growth hormone with disappointing results in terms of linear growth and nitrogen retention (4).The explanation for this failure was later found in the fact that human and bovine growth hormones differ from

each other in physicochemical nature (5, 6). Extracts from human pituitary glands given to humans was found to stimulate linear growth(5). Thus species specificity was established as far as growth hormone was concerned (7,8)*

The molecular structure of growth hormone has now been determined (9) and its exact amino acid sequence shown to be 191 amino acid residues containing two disulphide bridges between residues 53 and 165 and the other between residues 182 and 189 (10). It is a globular protein with a molecular weight of 22,000 (22K) (Figure 1.I). A smaller 20K variant is also present in the circulation but is less predominant, and is identical to the major form of hGH except for a deletion of a 15 amino acid segment, residues 32-46 of hGH. Other components with molecular weights of 45K, 30K, 16K, 12K (11,12,13) are also present in the circulation. It may be that some forms of short stature are due to the 20K variant or other less bioactive forms of growth hormone.

In February 1985 a patient who had been receiving human growth hormone died with a clinical illness of progressive dementia and other neurological features of Creutzfeld-Jacob syndrome (14). A second patient was reported in April 1985, followed by yet a third report of the disease in a 24 year old man who had received growth hormone for life-long hypopituitarism (15).

Since the introduction of human growth hormone as a treatment approximately 2000 children in the United Kingdom and 10,000 children in the United States of America have received this product. Therefore four cases of Creutzfeldt-Jacob disease in 12,000 patients represents an immensely elevated incidence over that in the general population. When

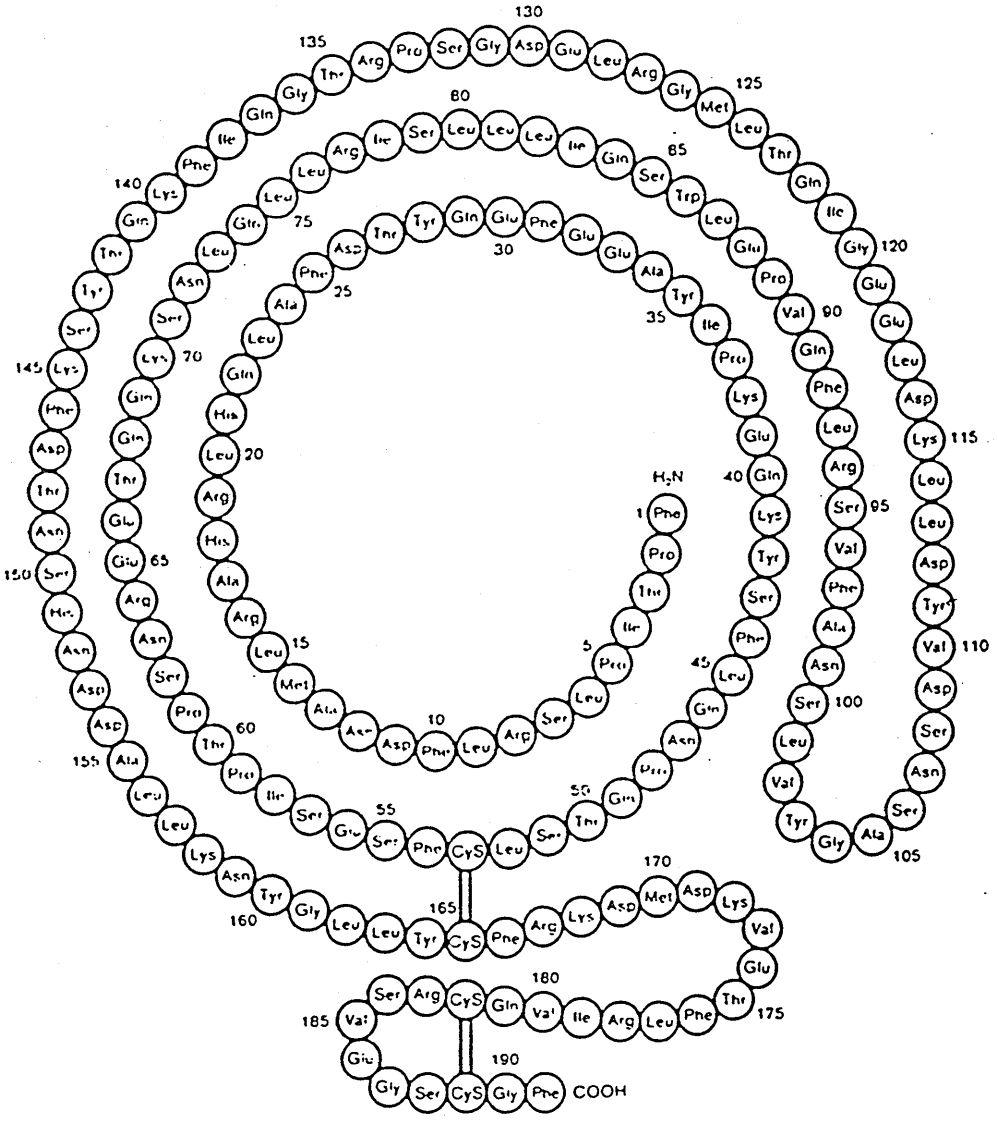


Figure 1.1 Growth hormone structure

the age of the victims all under 35 years is also considered, it is impossible not to suggest a causal link between growth hormone and Creutzfeldt-Jacob disease. This condition is world wide and has a reported incidence of one per million of the population per year. It affects the sexes equally, with the peak incidence of about 55 years of age, and is quite uncommon before the age of 30 years. Although its exact mode of transmission is unknown, it is thought to be transmitted via proteinaceous infectious particles or prions (16). The transmitting agents have also been labelled "slow viruses"but this could be a misnomer, for they contain no nucleic acids and unlike conventional viruses prions are extraordinarily difficult to inactivate, being resistant to formalin treatment, to 70 percent ethyl alcohol, to radiation, heat and ultraviolet light. They are however destroyed by 2N sodium hydroxide (15). Iatrogenic transmission of the disease has followed corneal transplantation and has been acquired from contaminated instruments used in neurosurgical procedures (17,18). Interestingly the scare came at a time when the demand for growth hormone was steadily exceeding supply and commercial (pharmaceutical) companies such as Eli Lilly, Kabi Vitrum and Serono were pressing ahead with research directed to producing a synthetic growth hormone by recombinant DNA techniques. Human growth hormone was immediately withdrawn as a treatment in Britain by May 1985, and patients were therefore without treatment until the new product became available in January 1986. This was methionyl growth hormone.

The principle of this technique is that the plasmid of the bacterial cell is opened and the DNA-containing strand to be cloned is inserted into it. This strand is derived from the human pituitary cell. The recombinant plasmid is inserted into the bacteria (E-coli) which is now transformed to

synthesize human growth hormone. Recent research has resulted in an amino acid sequence identical to human growth hormone (19,20). Its use in clinical practice shows that it has all the attributes of the former cadaveric material but with remarkably few of the disadvantages of that product.

Biological actions of growth hormone

The actions of growth hormone are many and not all of them are known. Some of the recognised actions are on fat, carbohydrate, and protein metabolism. On fat it causes lipolysis and this was once thought to be due to a lipid mobilizing peptide, a moiety of the growth hormone molecule. The net effect is to raise the plasma lipid level and so facilitate the uptake of free fatty acids by muscle as a source of energy (21).

On carbohydrate metabolism long term effects of growth hormone are to raise plasma glucose concentrations through a combination of glucose production and decreased utilization by limiting glucose transport within the cells. This hyperglycaemic effect takes place after several hours leading to a relatively insulin resistant state which produces hyperinsulinaemia if the growth hormone concentration remains high. The short-term administration of growth hormone exerts an insulin-like effect which results in a modest and transient degree of hypoglycaemia (22).

On protein metabolism the effects of growth hormone are better understood by giving growth hormone to growth hormone deficient patients. There is an induced nitrogen retention for protein synthesis. This action is c-AMP-dependent. Intracellular transport of amino acids is increased as well as mRNA (23) which leads to an increase in the organ size and differentiation as well as increasing functional capacity.

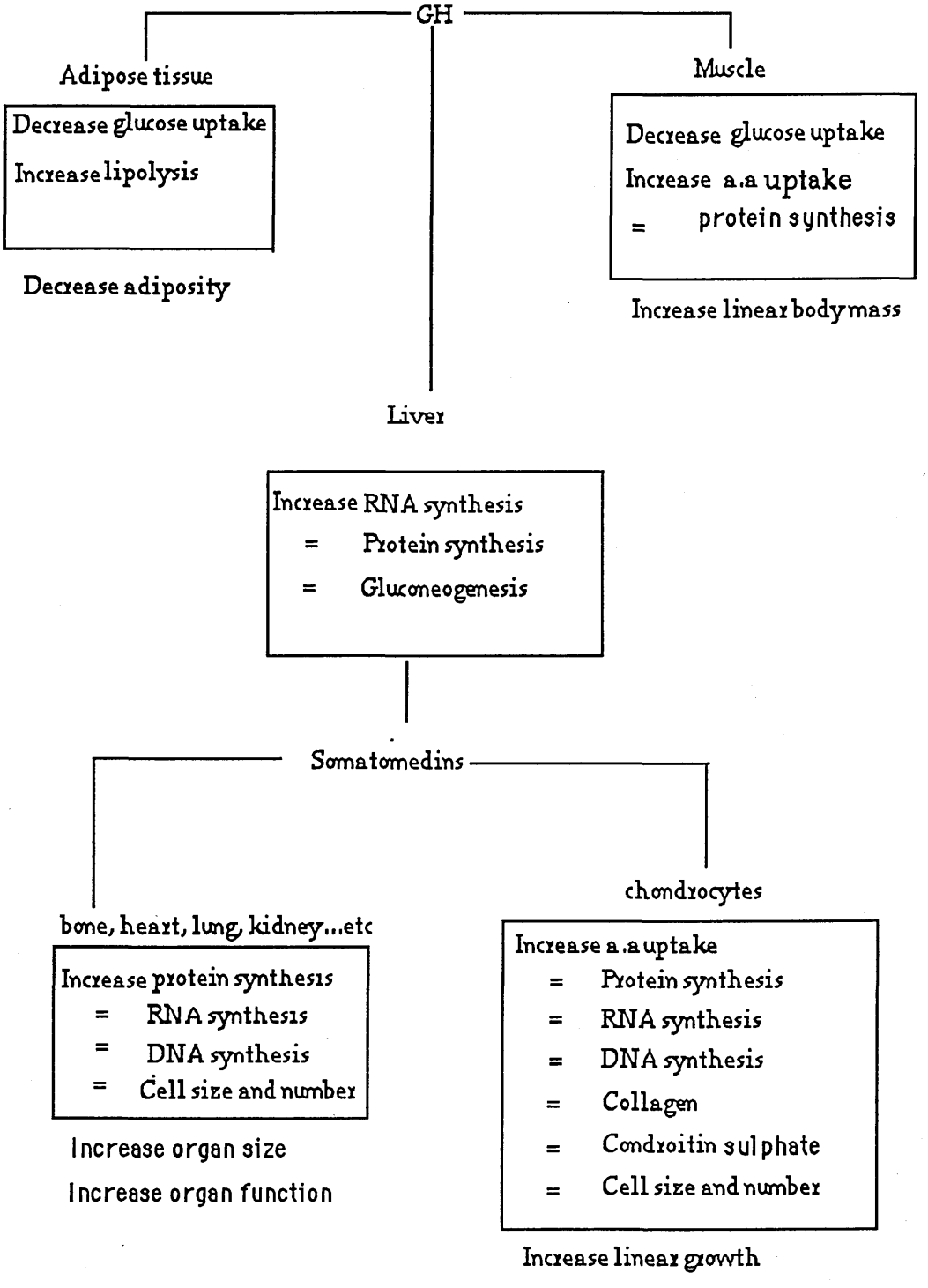
On mineral metabolism growth hormone leads to a large retention of potassium and phosphate intracellularly. In growth hormone deficiency states, growth hormone administration leads to increased concentrations of phosphate. Retention of sodium is less than that of potassium. Serum calcium concentrations are not increased, although there is an increased urinary excretion of calcium, related to an increase in calcium absorption (24). Sometimes during treatment a negative calcium balance may occur, the aetiology of which is unknown. Table 1.1 summarises some of the biological actions of growth hormone.

Important to this thesis is the action of growth hormone on skeletal growth. In association with locally produced IGF-I (25) growth hormone acts directly on the chondrocytes of the epiphyseal plate to cause proliferation with the formation of palisades (26,27). These cells ossify to be incorporated into the metaphysis of the bone, so lengthening it.

Pertinent to this work is also the role of growth hormone and IGF-I in nutrition. For example, in kwashiorkor there are elevated concentrations of growth hormone and depressed concentrations of IGF-I (28). The increase in circulating growth hormone is due to increased production rather than a decrease in the rate of clearance.

In patients with diabetic hyperglycaemia there is increased growth hormone secretion, probably due to relative intracellular starvation (glucopenia) due to the lack of insulin. Badly controlled diabetic children are frequently short statured especially in association with hyper as well as hypoglycaemia and clearly the interactions between cellular nutrition, circulating blood sugar concentrations and growth hormone secretion account for this observation.

Table 1.1 Biological actions of growth hormone



Growth hormone receptors

In its action growth hormone becomes attached to a specific membrane receptor. This receptor consists of three components: an extracellular portion which presumably binds growth hormone, a transmembrane portion and a cytoplasmic portion. The receptor has a value of 130,000 Daltons and is heavily glycosylated. It is a unique protein without similarity, and is highly specific for human growth hormone (29). Undoubtedly this explains the earlier mention of lack of response when bovine growth hormone was administered to a child with growth hormone deficiency. Cells secrete growth hormone binding proteins into the circulation. This circulating binding protein is similar to the receptor protein and may also be the extruded extracellular portion of the membrane receptor. In patients with Laron dwarfism there is a lack of this circulating binding protein and this has led to the conclusion that they may have a lack of the receptor for growth hormone (30,31).

Insulin-like growth factors

Two such are identified, namely IGF-I and IGF-II. It was formerly thought that the liver was the main source of supply to the circulating plasma pool as far as IGF-I is concerned. This is still correct but it is now recognized that IGF-I is elaborated at the site of action of growth hormone where it mediates growth hormone action (25) and at least locally produced IGF-I has its local action before entering the circulating pool.

These IGF's are secreted in response to the circulating levels of growth hormone and circulate bound to high affinity proteins. IGF-I is more growth hormone dependent than IGF-II. There are two receptors for the IGF's – one for IGF-I which binds it preferentially and is similar to the

insulin receptor but binds also IGF-II and insulin although with less affinity.

These IGF-I receptors are associated with the growth promoting actions of growth hormone. The IGF-II receptors are structurally different; they bind IGF-II preferentially but also IGF-I with less affinity (32). IGF-II receptors are associated with other somatic actions of growth hormone.

Interestingly, while growth hormone stimulates increased production of IGF-I, the IGF-I inhibits the secretion of growth hormone directly acting on the pituitary and also indirectly through the stimulation of somatostatin secretion in the hypothalamus forming a part in the regulation of growth hormone secretion as will be seen later.

Growth hormone secretion throughout life

In fetal life growth hormone is detectable in the pituitary at the 5th to the 7th week of gestation (33), but its presence and accumulation is not thought to be responsible for fetal growth. Fetal growth has been related to nutritional circumstances mediated through insulin and the IGFs. At birth the plasma concentration is greater than the corresponding maternal level but it declines after the first 48 hours of life (34).

In childhood, the fasting growth hormone level is lower than that of the newborn, but higher than the adult value. During the 24 hours of the day there are spontaneous peaks of plasma growth hormone higher and more frequent than in adults (35). Also tall children secrete more growth hormone than small children; the rates of growth of the tall child and the child with pituitary gigantism being similar but in pituitary giants associated with massive amounts of growth hormone beyond that of the normal tall child. The manner in which the growth hormone is secreted

in the two instances is different. The tall child has short bursts of high amplitude, while the child with pituitary gigantism has longer bursts of lower amplitude (36).

In pubertal children the basal plasma growth hormone concentration is not significantly different from that of adults but more peaks may occur during the day with greater amplitude during the night than occur in adults. The diurnal pattern of growth hormone secretion shows peaks these being associated with food ingestion, exercise and REM sleep. This fact is made use of when testing children for neurosecretory growth hormone deficiency.

Control of Growth Hormone Secretion

Preformed growth hormone in the pituitary is released under the influence of growth hormone releasing hormone. This substance is a small peptide elaborated by nuclear aggregates in the hypothalamus. The substance is delivered to the infundibular stalk via the long axons of these cells. From the infundibulum the releasing hormone is taken up by the long pituitary portal vessels and delivered to the eosinophil cells in the anterior hypophysis, thus stimulating growth hormone release. Another small peptide also elaborated in hypothalamic aggregates of cells and reaching the pituitary by the same means is somatotrophin release–inhibiting factor (SRIF). At the pituitary cell level SRIF damps down the action of the releasing hormone, so smoothing the continuous growth hormone secretion. SRIF is now termed somatostatin (37).

Growth hormone releasing hormone has been characterized and produced by recombinant DNA techniques. It is now available for clinical use – diagnostically at present and to a lesser extent therapeutically.

Somatostatin not only inhibits growth hormone secretion stimulated by GRF but under different temporal conditions, somatostatin may act in a paradoxical positive manner to sensitize the pituitary growth hormone response to GRF (38). This co-operative interaction may well be necessary to optimize pulsatile growth hormone release. Linear growth in children is recognized to be associated intimately with the pulsatile nature of growth hormone release as already mentioned.

Growth hormone deficiency

The identification of children with growth hormone deficiency is now according to nationally accepted tests. Children to be tested are identified either because they are in stature less than the third centile (usually < 2.5 SDs) or because over a twelve month period they have a height velocity below the 25th centile (46). Other systemic, genetic, chromosomal, osseous and endocrine diseases are excluded as a cause of the short stature before specifically testing for growth hormone reserve.

Causes of growth hormone deficiency

When considering this subject it is patently obvious from the foregoing discussion that a failure to synthesize growth hormone will result in growth hormone deficient short stature. Failure to synthesize the hormone could be inherited as an autosomal dominant or recessive trait (39), with pituitary aplasia or hypoplasia or destruction of the pituitary gland during operative procedures such as removal of a prolactinoma.

Secondly the hypothalamic stimulus of growth hormone releasing hormone may be absent and although the pituitary may contain adequate growth hormone, none of it can be released. Herein is an interesting observation. Induced hypoglycaemia acting at the hypothalamic level

through gluco-privation may fail to stimulate the release of growth hormone releasing hormone. Additionally hypoglycaemia also stimulates the release of corticotrophin releasing hormone with a resultant rise in ACTH and plasma cortisol. Thus during hypoglycaemia a rise in plasma cortisol without a significant rise in growth hormone would indicate deficiency of growth hormone releasing hormone. Other factors which might destroy the nuclear aggregates producing growth hormone releasing hormone are, irradiation (the hypothalamus being more sensitive to the effects of radiation than the pituitary) (40), tumours, infiltrations (reticuloendotheliosis) and other causes. Table I.2 shows the common causes of growth hormone deficiency.

Somatostatin is well recognized as an inhibitor of growth hormone secretion. Were somatostatinomata common in children, short stature would be a likely accompaniment. But less gross increases in somatostatin can inhibit growth hormone release and the degree of inhibition may reduce plasma growth hormone concentrations. A secondary effect of somatostatin is to reduce the concentrations of plasma amino acids and it could be that a combination of these two facts could restrict growth.

A psychosocial aetiology for growth hormone deficiency is also recognized. In these children depressed levels of stimulated growth hormone normalize when the child is placed in an improved social circumstance. The mechanism here is unclear, and there is no response to GH treatment; the children restart growth by changing their environment (41).

Finally here it must be mentioned that other endocrine substances such as glucocorticosteroids inhibit release of growth hormone; this is seen in Cushing's disease and Cushing's syndrome (42). In terms of glucocorticoid

Table 1.2 Common causes of growth hormone deficiency

1. Congenital.		
	Hereditary .	autosomal recessive
		autosomal dominant
		idiopathic GHRHD
	Developmental defects	
		pituitary aplasia.
		pituitary hypoplasia.
		midline anomalies
2. Acquired.	**Tumours.**	hypothalamic
		pituitary.
		other intracranial
	Irradiation	
	Infection.	meningitis
		encephalitis
	Infiltration.	histiocytosis X
		haemochromocytosis.
	Injury .	perinatal insult.
		head injury.
3.Transient.		peripubertal.
		psychosocial.
		primary hypothyroidism.

therapy it is stated that replacement therapy should ideally not exceed 10-15 mg hydrocortisone per m^2 per day. However it has been postulated that glucocorticoids inhibit growth hormone secretion above the level of the pituitary (43). In panhypopituitary multiple replacement therapy is required with growth hormone, ACTH or hydrocortisone, thyroxine and possibly the gonadotropins.

It has been quoted that in a general population the incidence of endogenous growth hormone deficiency may range from 1 in 3700 children to 1 in 30,000 (44-45). This disparity may reflect the variations in diagnostic criteria used by different investigators.

Provocative tests for diagnosis of growth hormone deficiency

(a) Most simple is the post-prandial (3 to 4 hours after glucose ingestion: 1.4 g/kg) plasma growth hormone concentration. Values above 15 mu/L are normal.

(b) The post exercise test requires the patient to undertake vigorous exercise for a period of 25-30 minutes. Concentrations of plasma growth hormone reach upwards of 20 mu/L in more than 50 to 90 per cent of normal children tested

(c) The sleep test. It is known that during REM sleep growth hormone is secreted in high concentration. In 60 to 70 per cent of normal children REM sleep concentrations exceed 20 mu/L (47-48).

(d) Arginine infusion test (0.5 g/kg; maximum dose 40 gm). The arginine hydrochloride (10 per cent solution) is given intravenously at a constant rate over 30 minutes. Blood is taken at 30 minute intervals over 2.5 hours. In some 85 per cent of normal children, concentrations of plasma growth hormone greater than 15 mu/L are achieved. (In growth hormone

deficient children there is a risk of hypoglycaemia and blood glucose concentrations should be monitored.) In a sense this is an important test to this thesis, although it was not used to identify any of the children, the subjects of this work. But certain amino acids may facilitate the release of growth hormone by suppressing somatostatin; this subject will be dealt with later.

(e) Glucagon test. Glucagon (30-100 micrograms per Kg up to a maximum of 1 mg) normally gives rise to a plasma concentrations of growth hormone exceeding 15 mu/L after 120 minutes in 75 to 80 per cent of healthy children. Concentrations less than 15 mu/L indicate deficiency of growth hormone reserve (49).

(f) Clonidine Test. This alpha adrenergic receptor stimulant facilitates growth hormone release. When given orally (0.15 mg/m^2) peak concentrations of plasma growth hormone are noted between 90 and 120 minutes after ingestion (50).

(g) The Insulin Tolerance Test (ITT). This test is by far the most reliable and it was the first test to be used. Historically it was referred to as the insulin unresponsiveness test before growth hormone could be measured in the plasma. Delayed recovery from hypoglycaemia till 2.5 hours post injection of insulin (I.V. 0.1 units per kg) indicated lack of growth hormone reserve. Nowadays peak plasma concentrations of growth hormone 30 minutes after the nadir of blood glucose are expected. Absolute growth hormone deficiency is indicated by peak concentrations of plasma growth hormone less than 7.0 mU/l, concentrations from 7.0 to 15 mU/L are regarded as indicating partial growth hormone deficiency, while normal concentrations are greater than 15.0 mU/l and they may reach 40-50 mU/l. This test has become the backbone of investigation of growth hormone deficiency although somewhat modified in immediate

prepubertal and pubertal children by the administration of testosterone (im) or oestrogen (orally) to the respective sexes. This practice is referred to as the priming procedure. The sex hormone is thought to make the hypothalamus more sensitive to glucoprivation and the basis for the practice is the well recognized fact that during normal puberty the growth spurt is associated with both an increased plasma concentration of growth hormone and of sex hormones.

Factors stimulating and inhibiting growth hormone secretion

It is well recognized that preformed growth hormone in the pituitary is released under certain physiological, pharmacological and pathological states. These have been well summarized in Table 1.3.

Additionally there are factors of similar type which inhibit the release of growth hormone. These are classified as under Table 1.4

Table 1.3 Factors stimulating growth hormone secretion.

Physiological	Pharmacological	Pathological
1. Spontaneous	1. Hypoglycaemia	1. Acromegaly
2. Sleep	2. Amino acids	2. Starvation
3. Stress - physical	3.Hormones: glucagon	3. Protein deprivation
- psychosocial	oestrogen	4. Anorexia nervosa
4. Exercise	ACTH	5. Renal failure
5. Postprandial hypoglycaemia	vasopressin	
6. Fasting	4. Monoamines: L-dopa	
	propranolol	
	epinephrine	
	serotonin	

Table 1.4 Factors inhibiting growth hormone secretion.

Physiological	Pharmacological	Pathological
1.Postprandial hyperglycaemia.	1.Phentolamine	1.Acromegaly;
2.Elevated fatty acids.	2.Chlorpromazine	L-dopa
3.Elevated GH levels.	3.Theophylline.	phentolamine
4.Somatostatin.	4.Morphine.	apomorphine
	5.Depot-ACTH.	somatostatin
	6.Glucocorticoids.	2.Hyperthyroidism
		3.Hypothyroidism.

Somatostatin

Historical Aspects

While searching for a hypothalamic factor capable of releasing growth hormone from the anterior pituitary a substance which inhibited growth hormone release from rat anterior pituitary was unexpectedly detected (51). This finding led these workers to suggest that the secretion of growth hormone from the pituitary was regulated by two different interacting neurohumoral factors, one stimulatory and the other inhibitory and both under the control of the nervous system. Later on this substance was isolated and characterized from ovine hypothalami (52), it was found to be a tetradecapeptide with 14 amino acid residues, cyclic in structure and joined by two intramolecular disulphide bonds between the two cysteine residues at position 3 and 14 (Figure 1.2).

Subsequently, the tetradecapeptide was isolated from porcine hypothalami and characterized (53). It was isolated from the pancreas of anglerfish (54) and it was found also in rat pancreas(55). Indeed it has been isolated from human adrenal medulla and pheochromocytoma, this being the first occasion when non-physiological tissue was analysed (56). Following these very basic discoveries and the synthesis of somatostatin in quantity, many researchers have administered the peptide to both animals and humans to study its biological activities and its precise mode of action.

Somatostatin was at first referred to as growth hormone release-inhibiting factor (GH-RIF) or somatotrophin release inhibiting factor (SRIF). Now all these names seem inappropriate since somatostatin has been isolated from cells unrelated to growth hormone regulation as we shall shortly consider.

However its early isolation from animal hypothalami and more precisely

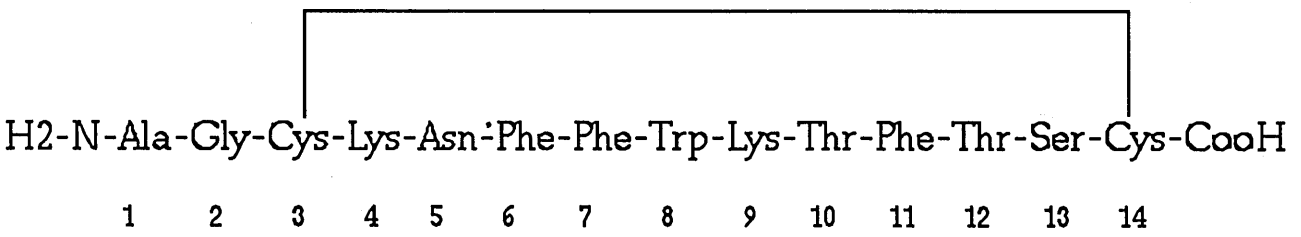


FIG. 1.2 Amino acid sequence of somatostatin-14

from cell aggregates located mainly in the median eminence, periventricular and medial preoptic areas of the anterior hypothalamus justifies the original name (57,58,59).

Distribution of somatostatin

Reliable methods for detection and quantitation of somatostatin are now widely in use. By immunocytochemistry somatostatin can be localized in tissues, but only if the somatostatin is immunoreactive. Radioligands specific for somatostatin receptors (using SS-14 analogues, SS-28 analogues or analogues of the stable octapeptide SMS 201-995) has facilitated somatostatin detection in post-mortem material either by binding techniques or by visual detection with autoradiography. Somatostatin in biological fluids and extracts may not only be detected but quantified by radioimmunosassay. Somatostatin immunoreactivity has now been detected in the central nervous system, in the gastrointestinal tract, in the genitourinary system, heart, eye, thyroid, thymus, skin and in some neuroendocrine tumours (60,61,62,63). More detailed comment will be made on some of these findings as seems appropriate to this work. But before doing so it is of interest to note that somatostatin cells display characteristic morphological features such as cytoplasmic elongations suggesting a local or paracrine role. The cells are round in shape, have a flocculent matrix and closely opposing limiting membranes.

In Brain Tissue

Somatostatin is present in the hypothalamus, the cerebral cortex, and the hippocampus. Earlier reference has been made to such specific sites as the median eminence, periventricular and preoptic areas. It is present in cells which are part of intrinsic local circuits but also in long axons of other cells

which suggest a projection activity to other areas from where the cells are located. Recently it has been shown that somatostatin is consistently and selectively depleted in the cerebral cortex of patients with Alzheimer's disease but localized in the characteristic birefringent plaques and neurofibrillary tangles so typical of this disease (64&65). This is of extreme interest for Alzheimer's disease is a degenerative disease with the clinical picture of deteriorating memory, reason, judgement and will, and is seen most often in the elderly. Presumably post-mortem examination of the cases of Creutzfeldt-Jacob disease 'due' to growth hormone administration did not reveal plaque formation in the brain. Also the ataxic features of the Creutzfeld-Jacob disease are not features of Alzheimer's disease. Nonetheless while the long tract pathologies of the two conditions may be different, the dominating features are similar and these are related to cortical function particularly of the anterior lobes. It is in the cortex that most somatostatin immunoreactivity has been detected. The question now is whether or not the early preparations of human cadaveric growth hormone recognized as being significantly contaminated with other hormones like TSH, LH and FSH also contained pituitary somatostatin. This somatostatin could have acted antigenically in the recipients to form antibodies to the cortical cells elaborating somatostatin. It is known that there are somatostatin receptors on the thyrotrophs and lactotrophs of the pituitary (66,67). There are some ampoules of the early UK human growth hormone still in the laboratories of the Department of Child Health and it would be of extreme interest to re-analyse that material with somatostatin content in mind for it may be a more plausible theory than the prions of Creutzfeld-Jacob could be. In Huntington's Chorea there is an increase of somatostatin in the caudate nucleus which is not of local production but

rather delivered by long fibres from elsewhere to the nucleus. The significance of this is as yet uncertain (68).

In the human fetus, somatostatin has been found in the hypothalamus as early as 16 weeks gestation (69). This is not surprising for until then is the period of cell differentiation and organogenesis. But noxa which inhibit organogenesis could readily inhibit the cerebral cortical and hypothalamic acquisition of somatostatin synthesis and thus may give rise to as yet undetermined cerebral malfunctioning. Somatostatin immunostaining cells and fibres are found throughout the spinal cord. This system appears prenatally. Activity is greatest in the posterior horns and columns and is related to cells rather than fibres (70).

In Gastrointestinal Tissue

Immunoreactive somatostatin can be extracted from all layers of the gastrointestinal tract. Mucosal somatostatin is somatostatin-28, while somatostatin-14 is of nonmucosal neural origin (71). ie. the somatostatin from mucosal endocrine cells and from enteric innervation. In the pancreas it is of D-cell origin. Somatostatin containing cells are found along the entire length of the gut and in particular at the gastric fundus and antrum and in the colon. The somatostatin endocrine cells have an apical pole which reaches the lumen and a basal cytoplasmic elongation to which a paracrine role is attributed. This elongation lies near to non-endocrine cells such as the parietal cells of the fundus or to other endocrine cells such as gastrin-containing cells. Somatostatin is known to suppress the release of gastrin and gastric acid secretion (72).

Somatostatin-containing cells are found by the eighth fetal week in the small intestines and stomach (69). In the fetal and neonatal pancreas the

D-cells constitute up to 25 per cent of the total islet population. In neonates with nesidioblastosis(73) the population shows marked depletion even lower than in the developed pancreas when it is reduced to around 10 per cent.

In the genitourinary system

Somatostatin has been detected both by radioimmunoassay and by immunocytochemistry in animals and man. In the human male genital tract a few somatostatin immunoreactive nerve fibres have been found in the interstitial tissue and amongst the smooth muscles of the prostate gland and in the muscular coat of the vas deferens. Interestingly, the highest concentrations of somatostatin have been found in the younger subjects (rats) of 1.5 and 3 months of age and this concurs with findings in humans (60, 74)

In thyroid and thymus

Somatostatin co-exists with calcitonin in the parafollicular C-cells of the thyroid and as scattered cells in the thymus. It has been found that the number of somatostatin cells decreases with age (75-76). Is this a possible factor in the higher occurrence of thyrotoxicosis in adults than in children? Somatostatin is known to (a) reduce the synthesis and release of T3 and T4 stimulated by TSH (77); (b) to inhibit the nocturnal spike of TSH (78); and (c) to block the TRH-stimulated TSH secretion (79). Relatively high juvenile and adolescent thyroid levels of somatostatin may therefore act as a protective mechanism against over-stimulation with TSH. It would be extremely interesting to study cases of paediatric thyrotoxicosis at least some benefit may be obtained from the administration of synthetic somatostatin. Its role in the thymus is less clear.

In other tissues

There is a vast literature on the total body occurrence of somatostatin, much of it irrelevant to this present work. Suffice it to say that it is found in heart, eye, placenta, and skin. Its presence in the latter suggests a sensory role and this fits well with the already mentioned presence of somatostatin in the dorsal spinal ganglia. Its presence in some tumour tissue is of interest. In pituitary tumours causing acromegaly some contain high density somatostatin receptors while others have only low amounts of non-homogeneously distributed somatostatin receptors (80-81). This correlates well with clinical experience in that some cases of acromegaly respond to administered somatostatin while others do not. While acromegaly is not a paediatric illness, pituitary gigantism is and this information opens up a possible line of treatment for these children. However such an exercise would require somatostatin monitoring before and during treatment to determine the effective plasma concentration of somatostatin in relation to growth restriction.

A further point of developing interest is the fact that when the somatostatin analogue (sandostatin) is administered to acromegalic patients, the IGF-I plasma concentration is reduced as is also the plasma level of growth hormone (82,83).

Mechanism of Action

The action of somatostatin at the various sites of occurrence is uniformly that of inhibition. The detail of its action on pituitary derived cells has been most studied and it is likely that a similar mechanism may apply in all tissues. Somatostatin binds to a specific membrane receptor and inhibits adenyl cyclase activity. This it does through coupling with an N_i

unit which has an opposing effect to growth hormone releasing hormone which itself stimulates adenyl cyclase by coupling to an N_S unit.

Somatostatin also has effects independent of its action on cAMP. These effects cause a fall in intracellular calcium. This fall reduces growth hormone secretion probably via increasing permeability to potassium, resulting in membrane hyperpolarization (84).

Physiological action of somatostatin on various tissues

Some of the tissues affected have been mentioned above but a fuller list of tissues involved and the net effects of somatostatin is given in Table 1.5 At this point it is pertinent to say something on the structure of the various somatostatins. Most work has been done on somatostatin-14 and hence more is known of the action of this compound. Chemically it is a sequence of fourteen amino acids as has been seen in Figure 1.2.

Somatostatin-28 is somatostatin-14 N-terminally extended (Fig 1.3) (85). There are two other species of 25 and 20 amino acids each containing the same COOH terminal 14-amino acid sequence (86). These derive from a 116 amino acid precursor (87). Most if not all the physiological activity of all the somatostatins resides in this COOH terminal 14-amino acid sequence i.e. somatostatin-14. Within the molecule it has been found that only the amino acid sequence at positions 7, 8 and 9 are absolutely necessary for physiological activity, while modifications at positions 6 and 11 markedly decrease the biological activity. Changes at position 1-5 and 12-14 do not affect physiological activity (88). Interestingly, substitution of the D: isomer at position 8 for the L-trp increases the potency of the somatostatin. This increased potency could be due to increased resistance to proteolytics. Also, substitution of Phe instead of Lys in the non-essential

Table 1.5 Inhibitory actions of somatostatin

Tissue	Response
1..Anterior pituitary	Secretion of GH, TSH and occasionally PRL
	and ACTH.
2. Pancreas.(islets)	Secretion of insulin, glucagon and pancreatic polypeptide
3. Pancreas exocrine (exocrine)	Secretion of water, bicarbonate and enzymes.
4. Stomach.	Acid and pepsin secretion and gastric emptying.
5. Intestine.	Secretion of fluid and bicarbonate, absorption of calcium,glucose, fructose, glycerol, xylose, lactose , triglycerides, amino acids and mesenteric blood flow.
6. Gall bladder.	Bile flow.
7. Kidney.	Renin secretion, aldosterone response to angiotensin II.
8. Parathyroid.	Parathyroid hormone secretion.
9. Salivary gland.	Salivary flow.
9. Platelets.	Aggregation
10. Thyroid	Calcitonin secretion.
11. Miscellaneous	Splanchnic blood flow,

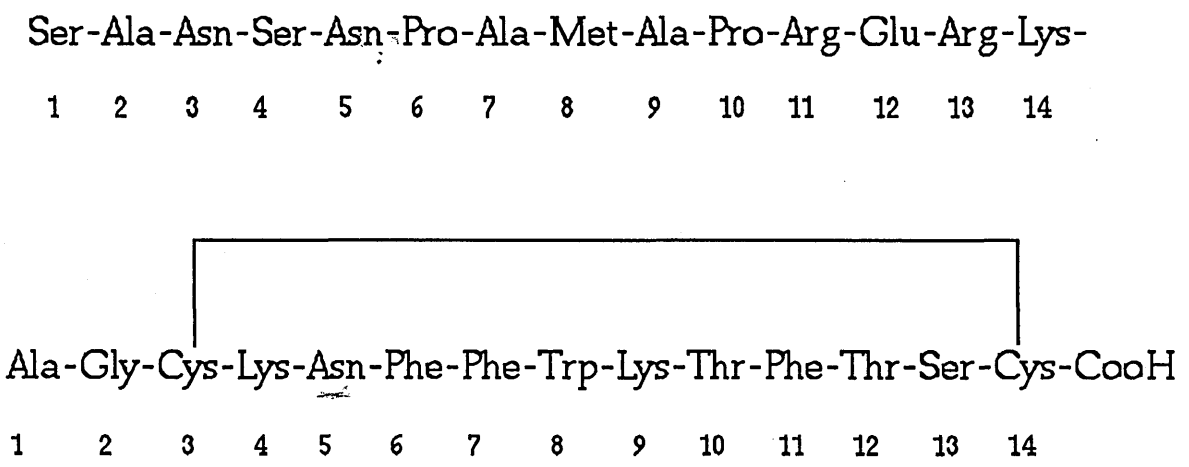


FIG. 1.3 Amino acid sequence of somatostatin-28

position 4 of somatostatin increases its selectivity for inhibiting growth hormone release (89). Deletion of Asn at position 5 increases the relative insulin selectivity (90).

The configuration of active somatostatin-14 is like a hairpin, with the position held by the disulphide bridge between the cysteine at positions 3 and 14, with a twin occuring around the tryptophan and lysine at position 8 and 9. If by reduction the bridge is disrupted, the molecule assumes a linear position and is physiologically inactive. Many attempts have been made to find a synthetic analogue which would combine increased potency with specificity; workers in the Sandoz Swiss Laboratories in Basle by trial and error have synthesized an octapeptide (91):

D-Phe–Cys–Phe–D–Trp–Lys–Thr–Cys–Thr–ol

This compound has been found to be twice or thrice as active in inhibiting growth hormone secretion by GRF-stimulated pituitary (rat) cells in culture. Its increased potency is due to its biological half-life of about 2 hours compared with the natural hormone of 2 minutes (92). This product, known as Sandostatin, is in clinical use for the management of acromegaly, thyrotoxicosis and other conditions, as will be seen later. Interestingly, several other somatostatin analogues have been shown during their experimental trials to have different potency and selectivity (93) as shown in Table 1.

On gastrointestinal system

The greatest contribution of somatostatin to the plasma pool is from the gut and related tissues. Somatostatin immunoreactivity is present from the oesophagus to the colon. In the stomach, the antral area (D-cells) has

Table 1.6 Relative potencies of specific somatostatin analogues.

Peptide	GH (in vitro)	Insulin (in vivo)	Glucagon (in vivo)
SRIF	100	100	100
DesAsn^{5}-(D-Trp^{8}, D-Ser^{13})-	13	1,750	<1
(D-Trp^{8}, D-Cys^{14})-	650	130	1,000
(D-Lys^{4})-	22	<1	<1
DesAsn^{19}(D-Trp^{22} , D-Ser^{27})-SS28	<5	14,500	<100
SMS 201-995	300	300	2,300

high somatostatin-like immunoreactivity, followed by the upper small intestines and the D-cells of the pancreatic islets. It inhibits endocrine and exocrine secretion, gut mobility, blood flow, absorption, and the growth of the gastrointestinal mucosa(Table 1.5). My interest here firstly is concerned with its relationship to protein and the absorption of amino acids.

It is recognized that the ingestion of food stimulates the secretion of somatostatin into the blood. Fats and protein are potent stimuli of somatostatin release, while carbohydrate is rather a poor stimulus (94). Nonetheless, despite these differences, somatostatin inhibits the absorption of glucose, xylose and amino acids(95). Thus, in patients with high plasma somatostatin concentrations, caution must be exercised when interpreting the significance of the results of glucose and xylose absorption tests during the investigation of short statured patients. In these same patients some of whom may have low plasma growth hormone concentrations either because of an absolute growth hormone deficiency state, or because of neurosecretory growth hormone failure, low plasma amino acid levels are likely, for one of the actions of growth hormone is to increase the nitrogen retention. It follows then that in some children with growth hormone deficiency and high concentrations of plasma somatostatin, the combined effect will be undoubted low plasma amino acid concentrations. This will be explored in this work.

My second interest is in the action of somatostatin in inhibiting gastric emptying, gall bladder contraction and slowing of small intestinal transit time. It is the experience of many clinical endocrinologists that short statured children with and without growth hormone deficiency have extremely poor appetite. Could therefore high somatostatin plasma concentrations by this acting slow down the total process of eating,

digestion, absorption and defecation to such a degree that appetite is significantly impaired?

Pancreatic tryptic activity is induced by food ingestion. This effect can be reduced by the administration of somatostatin. Indeed, it has been shown (96) that somatostatin administered reduces the cholecystokinin-pancreozymin induced trypsin, chymotrypsin and amylase. But also cholecystokinin-pancreozymin secretion itself is inhibited by somatostatin. Hence with reduced secretion of trypsin and chymotrypsin it is likely that protein digestion and free amino acid absorption are reduced by high concentrations of plasma somatostatin. This induced incomplete digestion of protein may however lead to an absorption of peptides rather than free amino acids.

Finally here, the well recognized high plasma levels of somatostatin found in younger children (74) may well be a built-in protective measure against overfeeding and gastrointestinal shock from excessive bolus in the upper small bowel.

Action on TSH secretion

Interestingly growth hormone and TSH secretion are both stimulated by separate releasing hormones, but the secretion of both is inhibited by somatostatin(97). Detailed studies of this phenomenon indicate that the primary inhibitory effect of somatostatin in vivo is on growth hormone secretion, while in vitro its action on the secretion of both growth hormone and TSH is equal. Thus an in vivo suppression of TSH is only seen when pituitary concentrations of somatostatin are unphysiologically high. At thyroid level its presence has already been noted and its action on the thyroid to reduce the synthesis and release of T3 and T4. Peripherally

somatostatin reduces the deiodination of T4 to T3, and increases the production of rT3 (77).

On Brain

Most of the research work on somatostatin has utilized somatostatin-14 as has been mentioned but interestingly somatostatin-28 exhibits potent inhibitory activity on spontaneous GH release which persists for a significantly longer period of time than that of somatostatin-14. Somatostatin-28 may not therefore be merely a prohormone but a regulatory hormone in its own right (98). The biological half-life of each respectively is 2.4 minutes and 6.1 minutes.

However, while it is recognized that the pulsatile nature of growth hormone secretion as reflected by circadian rhythm is influenced by a unique balance between hypothalamic GRF and somatostatin, the concentration of the systemic circulating somatostatin also influences the basal concentrations of plasma growth hormone. This has been demonstrated by infusing rats with somatostatin so as to achieve ultra-physiological concentrations, plasma growth hormone diminished sharply to almost below detectable levels immediately (99). The interplay of these and other growth promoting agents with the hypothalamopituitary axis may be summarized by the following schematic drawing (Fig 1.4 shows Schematic representation of events leading to control of GH secretion).

Growth hormone is released from the anterior pituitary somatotrophs under the influence of GRF. The extent of the release is controlled by hypothalamic somatostatin. These hormones have separate receptors (i.e acting in a non-competitive fashion) somatostatin binding to N_i receptors and GRF binding to N_s receptors on the somatotrophs. The circulating

growth hormone by passage through the liver and kidneys increases the circulating concentration of somatomedin (IGF-I). This in turn acts firstly on the somatotrophs to inhibit further growth hormone secretion, but also secondarily on the hypothalamus to increase the release of somatostatin which in turn suppresses growth hormone release. The growth hormone however has a short retrograde loop back to the hypothalamus where it inhibits the cells producing GRF. This mechanism integrates physiologically the plasma peaks of growth hormone in relation to exercise, food and REM sleep. Thus it is now accepted that while diurnal inhibition of growth hormone release is likely to be partially under the control of systemic somatostatin the necessary plasma peaks are neuroregulated by the harmonious integration of growth hormone itself, GRF, somatostatin and IGF-I.

Finally to complete this consideration of somatostatin relevant to the present work a short reference to insulin and hypoglycaemia is offered. Insulin per se stimulates hypothalamic somatostatin release (100), while glucose is an inhibitor of hypothalamic somatostatin release. It is therefore of interest that peripherally insulin increases plasma somatostatin indirectly through its hypoglycaemic action, for in hypoglycaemic states somatostatin is increased. The relevance of this to our subject is that patients with growth hormone deficiency lack GH-anatagonism to insulin and are therefore likely to experience plasma glucose concentrations in the lower normal range. It could be that thereby there is an added tendency to increased plasma somatostatin concentrations. In persistently low plasma glucose states, glucagon secretion increases as part of the glycaemic restorative mechanism. Again glucagon stimulates D-cell somatostatin secretion. Thus several mechanisms may be at work to raise the plasma

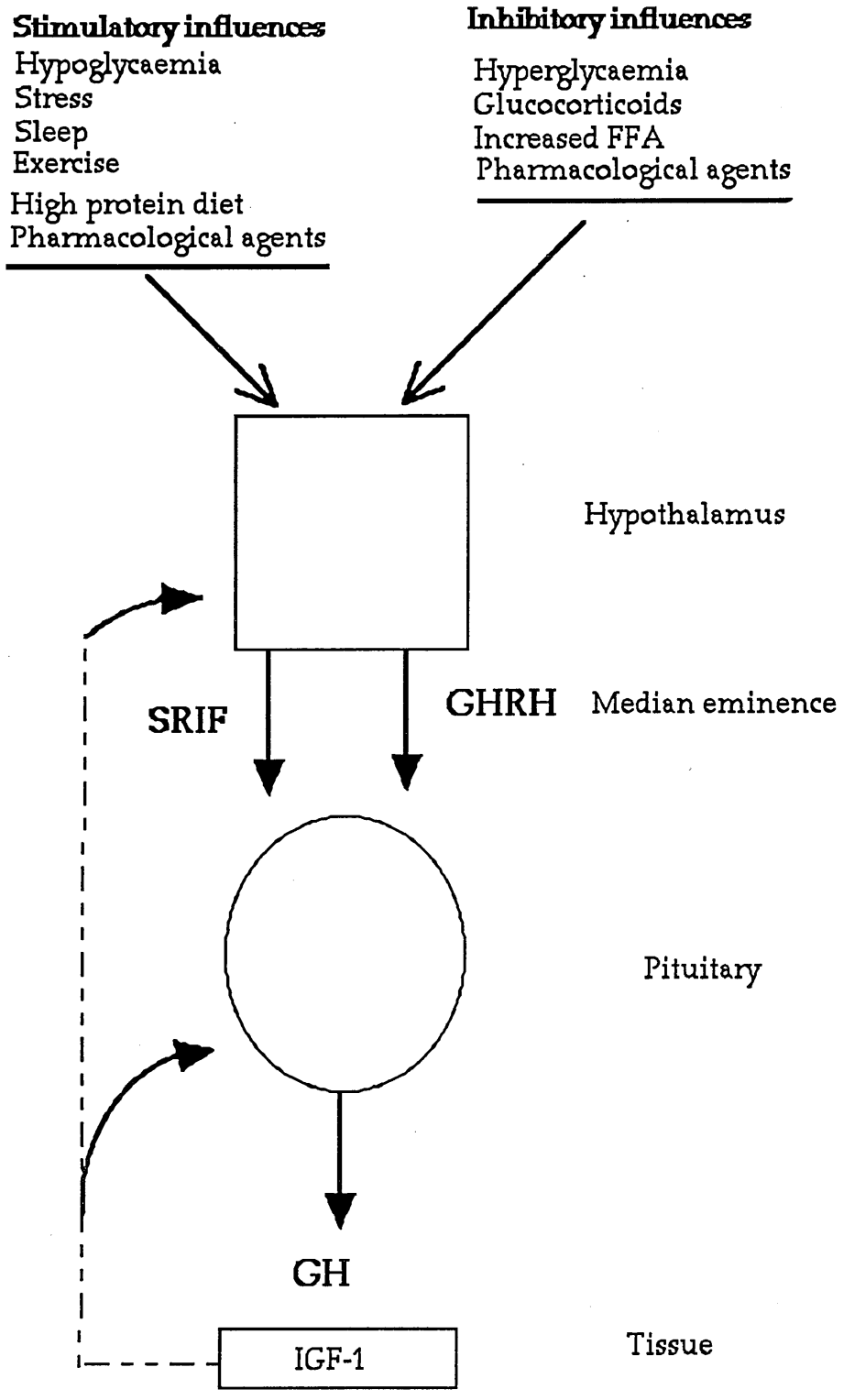


Figure 1.4 Main influences in the control of growth hormone secretion.

somatostatin concentrations in the GH-deficient patients which I shall present in this work.

Clinical Uses of Somatostatin

Because of the various modes and sites of action of somatostatin, it is not surprising that it has been experimentally used in many conditions. The formulation of the more potent octapeptides with somatostatin activity has emboldened clinicians to therapeutic endeavour. Table 1.7 gives a list of conditions in which somatostatin has had clinical and therapeutic trials.

At our level of interest, somatostatin may have a role investigatively and therapeutically. Firstly, the interesting work (99), in which infusion of somatostatin leads to an immediate fall in plasma growth hormone concentrations which were followed by rebound peaks of growth hormone on sudden withdrawal of the infused somatostatin, suggests that such a principle could form the basis for a research tool to determine the status of GRF in short statured patients. The procedure could readily be combined with a 1h GRF test and so only two samples of blood, one for each test, would be necessary to determine the pituitary synthetic capacity for growth hormone and the hypothalamic status of GRF.

Secondly, because of its action in suppressing insulin secretion it may have a place in the seldom occurring cases of nesidioblastosis. But its therapeutic actions would require close monitoring of the plasma somatostatin concentrations achieved, for in the final analysis its role can only be symptomatic and not curative. In Type I diabetes somatostatin also has a role – here because of its suppressing action on growth hormone and glucagon release to reduce nocturnal hyperglycaemia. While insulin

replacement will always be required, also in Type I diabetic patients, the insulin dosage requirement may be reduced and the course of the brittle diabetic smoothed if somatostatin can be found to be a suitable adjuvant to treatment. Additional support for the concept involving growth hormone suppression comes from the link between growth hormone and microvascular complications in diabetic patients since it has been found that growth hormone-deficient dwarfs with diabetes usually lack microvascular complications (101) and, more interestingly(102) was the diabetic retinopathy produced in dogs as a result of growth hormone injection.

Table 1.7 Clinical uses of somatostatin.

1. Central neuroendocrine disorders.

Acromegaly.

Excessive TSH secretion.

Defective ACTH feedback (Addisson, Nelson.)

2. GIT tumours and related conditions.

Insulinoma.

Nesidioblastosis

Vipoma.

Glucagonoma

Gastrinoma.

Carcinoid.

3. Non-tumoural GI conditions.

Malignant diarrhoea

Pancreatic fistula and pancreatitis.

GI bleeding.

4. Diabetes. Type I (insulinopenic)

Type II (peripheral resistance)

5. Blood pressure regulation.

Hyperreninaemia.

Aldosteronism.

6. Tissue growth inhibition.

Skin (EGF ?)

Malignant tissue.

7. Cytoprotective.

8. Miscellaneous.

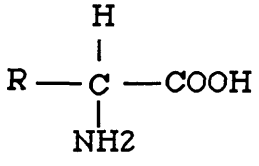
Burns, trauma, sepsis.

Amino Acids

Protein (Gr; proteois) was early recognized as necessary for life in a more fundamental way than carbohydrates or fats. Protein destruction in the body was observed to result in increased urinary nitrogen so that the estimation of urinary nitrogen became a measure of protein turnover. The smallest unit of the protein is the amino acid. Glycine was the first amino acid to be isolated; gelatine was the source material.

Tryptophan was found to be required preformed in the diet of rodents (103) and it was confirmed later that lysine and sulphur-containing amino acids were nutritionally essential(104). The last nutritionally essential amino acid to be recognized was threonine (105).

All amino acids contain at least one amino group (–NH2) in the α-position and a carboxyl group. The simplest structure of an amino acid then is glycine as under.

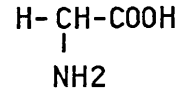
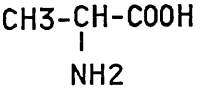
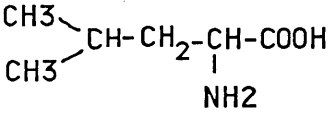
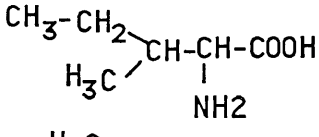
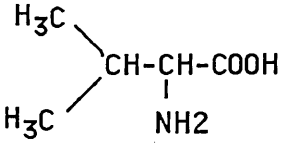
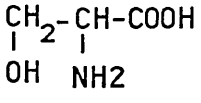
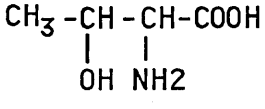
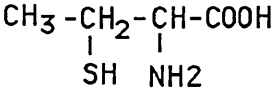
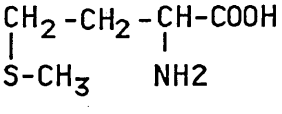


All amino acids contain an asymmetric carbon atom, except glycine .

Most amino acids contained in protein are in the laevo form, in which they are important in human metabolism. D-forms occur mainly in micro-organisms. D-amino acids are poorly absorbed and rapidly excreted. The common amino acids are listed according to their chemical structure and property in Table 1. 8.

The terms glucogenic and ketogenic amino acids are now obsolete, but they

Table 1.8 Proteinderived L-amino acid structure and their chemical characteristics.

Amino acid group	Trivial name	Symbol	Chemical characteristics	Structural formula
Neutral amino acids	Glycine	Gly	Straight chain	
	Alanine	Ala	= =	
	Leucine	Leu	Branched 'R'	
	Isoleucine	Ile	= =	
	Valine	Val	= =	
	Serine	Ser	OH-containing "R'	
	Threonine	Thr	= =	
	Cysteine	Cys	SH-containing "R'	
	Methionine	Met	= =	

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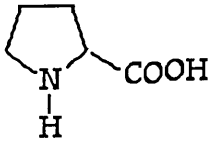
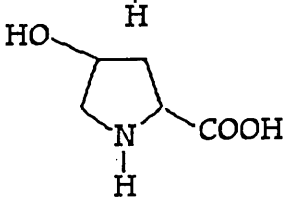
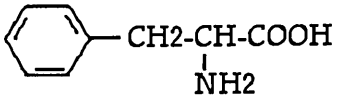
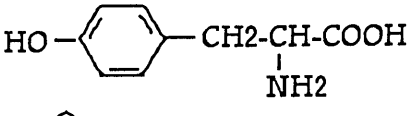
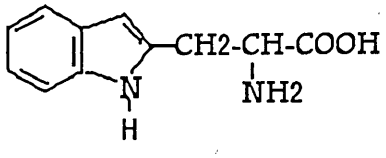
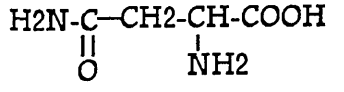
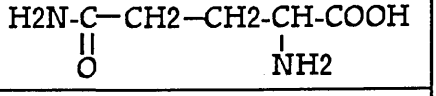
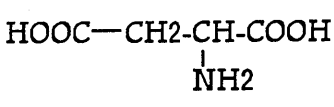
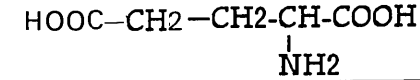
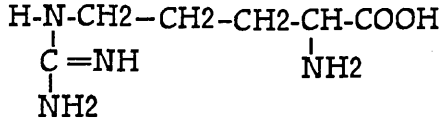
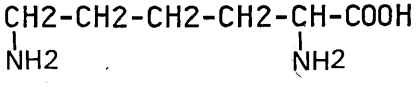
Amino acid groups	Trivial name	Symbol	Chemical characteristics	Structural formula
Neutral amino acids	Proline	Pro	Imino acid	
	OH-proline	Hyp	= =	
	Phenylalanine	Phe	Aromatic"R"	
	Tyrosine	Tyr	= =	
	Tryptophan	Trp	= =	
	Aspargine	Asn	Amides cotai-ning'R"	
	Glutamine	Gln	= =	
Acidic amino acids	Aspartic acid	Asp	(COOH)-Contai-ning"R"	
	Glutamic acid	Glu	= =	
Basic amino acids	Arginine	Arg	NH2-contai-ning "R"	
	Lysine	Lys	= =	

serve to indicate their behaviour in vivo. For instance, the glucogenic amino acids are capable of stimulating insulin secretion, presumably to deal with the increase in blood sugar derived from their utilization. Phenylalanine, tyrosine, leucine and isoleucine are degraded in part to acetoacetate, whereas other amino acids are degraded chiefly to pyruvate, oxaloacetate, α-ketoglutarate, succinate and fumarate. The metabolic fate of the amino acids indicates whether they are glucogenic or ketogenic .

The former nomenclature of essential and nonessential amino acids has given place to two new terms, namely indispensable amino acids (IDAA's) and dispensable amino acids (DAA's) (106). In terms of the nutritional value of amino acids, it is important to note that a few amino acids do not fit into either category. Some of these amino acids such as arginine, which although synthesized by the body are not synthesized in sufficient amounts required for the physiological needs of the young rapidly growing subject. Cysteine and tyrosine synthesized only from methionine and phenylalanine respectively are indispensable amino acids if their precursors are not present in a diet in adequate amounts or if there is a defect in the pathway by which they are synthesized. Such a situation has been found in the absence of cystathionase activity (the enzyme which cleaves cystathionine to cysteine) in fetal liver and brain. Cystathionase in low activity has also been found in premature infants and in full term infants who died immediately after birth (107). This observation has been confirmed by the fact that cystathionase activity is significantly correlated with gestational age and increases rapidly after birth (108), Such amino acids as cysteine are termed semi-indispensable or even more suitable conditionally indispensable. A classification of amino acids according to their nutritional function and metabolic fate is shown in Table. 1.9

Table 1.9 classification of amino acids according to their functional and metabolic fate.

Nutritional function			Metabolic fate		
Indispensable	Dispensable	*Cond.indisp	Glucogenic	Ketogenic	Gluco-Keto
Valine	Alanine	Cysteine	Alanine	Leucine	Isoleucine
Leucine	Glycine	Tyrosine	Glycine		Lysine
Isoleucine	Serine	Arginine	Serine		Phenylala-nine
Threonine	Cysteine	Histidine	Histidine		Tyrosine
Methionine	Proline		Proline		Tryptophan
Phenylalanine	OH-proline		OH-proline		
Tryptophan	Asparagine		Asparagine		
Lysine	Aspartic acid		Aspartate		
	Glutamine		Cysteine		
	Glutamic acid		Glutamate		
			Methionine		
			Threonine		
			Valine		

* conditionally indispensable.

When young animals are fed a diet containing a high proportion of indispensable amino acids, the dispensable amino acids synthesized from these are slow to reach optimum levels necessary for maximum growth. Thus the dispensable amino nitrogen can become a limiting factor for growth in the human infant who is on a low intake of high quality protein or who, from this thesis's point of view, may lack a stimulus for the utilization of nitrogen from DAA's or IDAA's. An important point is that the IDAA's other than lysine and threonine can be replaced in diets by their α-keto analogues. Lysine and threonine therefore may be the only amino acids which are truly nutritionally indispensable, since they do not pass through an α-keto stage in their resynthesis.

The IDAA's must be ingested. On the other hand in normal man during a 24-hour period almost about 16 g protein from the digestive enzymes are found in the lumen of the gut, 50 g from the shed cells from the intestinal mucosa and 6 g from the serum albumen, these latter are actively secreted into the intestinal lumen. From this protein pool some indispensable amino acids must result so contributing to that ingested in the diet. Dietary protein will therefore cause a fluctuation of the plasma concentrations of amino acids, but the endogenous supply as above will maintain an evenness of plasma amino acids throughout the 24-hour period. (109)

Valine, leucine and isoleucine (branched chain amino acids)

These amino acids are of particular interest in that they are the only amino acids which are only partially degraded in the liver. After a meal rich in protein the effusion of blood to the systemic circulation contains a higher percentage of these branched chain amino acids than was contained in the

protein of the meal. The excess of the other amino acids (both indispensable and dispensable) are selectively degraded by the liver so that more than 70 per cent of the increase in free amino nitrogen leaving the liver after a meal is accounted for by the branched chain amino acids while only some 20 per cent of these amino acids are contained in the meal (110). The circulating branched chain amino acids are transaminated and degraded peripherally in muscle, kidney, adipose tissue and brain. Apart from their role in protein synthesis, it is this peripheral catabolic process which makes them energy-yielding substrates. They are deaminated in muscles especially during fasting to their oxo-acids which in turn are utilized in the liver as glucogenic or ketogenic substrates. Following a 24-h starvation there is a considerable increase in these amino acids while the concentration of some other amino acids falls. Leucine also acts as a regulator of protein turnover. Low concentrations of leucine stimulate protein synthesis while high physiological levels inhibit catabolism of tissue protein (111).

There is one aminotransferase acting on all these branched chain amino acids converting them to their corresponding oxo-acids. A very specific leucine aminotransferase does exist in some tissues. The catabolic aminotransferase is found mainly in skeletal muscle and after cleavage of the amino group alanine is formed which is exported to the liver as a glucogenic substrate (112).

The importance of these facts to the present work is that growth hormone in its protein synthesizing role will facilitate and perhaps accelerate the incorporation of these branched chain amino acids into muscle to increase the mass of muscle protein and probably also facilitate the increase in muscle length (commensurate with increase in bone length). Also leucine

reduces the rate of protein catabolism. The high concentration of leucine in maple syrup disease inhibits muscle protein catabolism and so reduces the availability of alanine as a glucogenic amino acid. Hence the frequent attacks of hypoglycaemia in this disease of branched chain amino acid metabolism (109).

Lysine and Threonine

It has already been stated that these are the two truly indispensable amino acids because they cannot be generated from their α-keto-forms. They are required for muscle protein synthesis and hence their prime importance as dietary constituents and subsequent absorption.

Threonine has an hydroxyl side chain and because of this contributes to the hydrophilicity of proteins. This hydroxyl group is readily available for phosphorylation catalysed by protein kinases. Thus such catabolism is a means of exporting phosphate e.g. casein which is heavily phosphorylated and provides most of the phosphate required for bone mineralization in the growing child (113). Thus in the absence of an adequate intake of casein, threonine in a small way may compensate as a phosphate donor. Adequate plasma concentrations of this amino acid are therefore desirable. However on an average dietary protein intake there is a relatively narrow range of threonine content and high threonine foods such as eggs and meat are required to provide recommended daily requirements of threonine i.e.40 mg /kg /day. On the other hand L-lysine is catabolised into α-aminoadipic acid and to piperidine carboxylate. Failure of this pathway results in hyperlysaemia with an accumulation of saccharopine. While threonine is catabolised into an α-ketobutyrate which in turn is oxidatively decarboxylated to yield proponyl CoA. This latter will then enters the Krebs cycle through certain chemical reactions .

Alanine, Glycine, Serine and Tyrosine

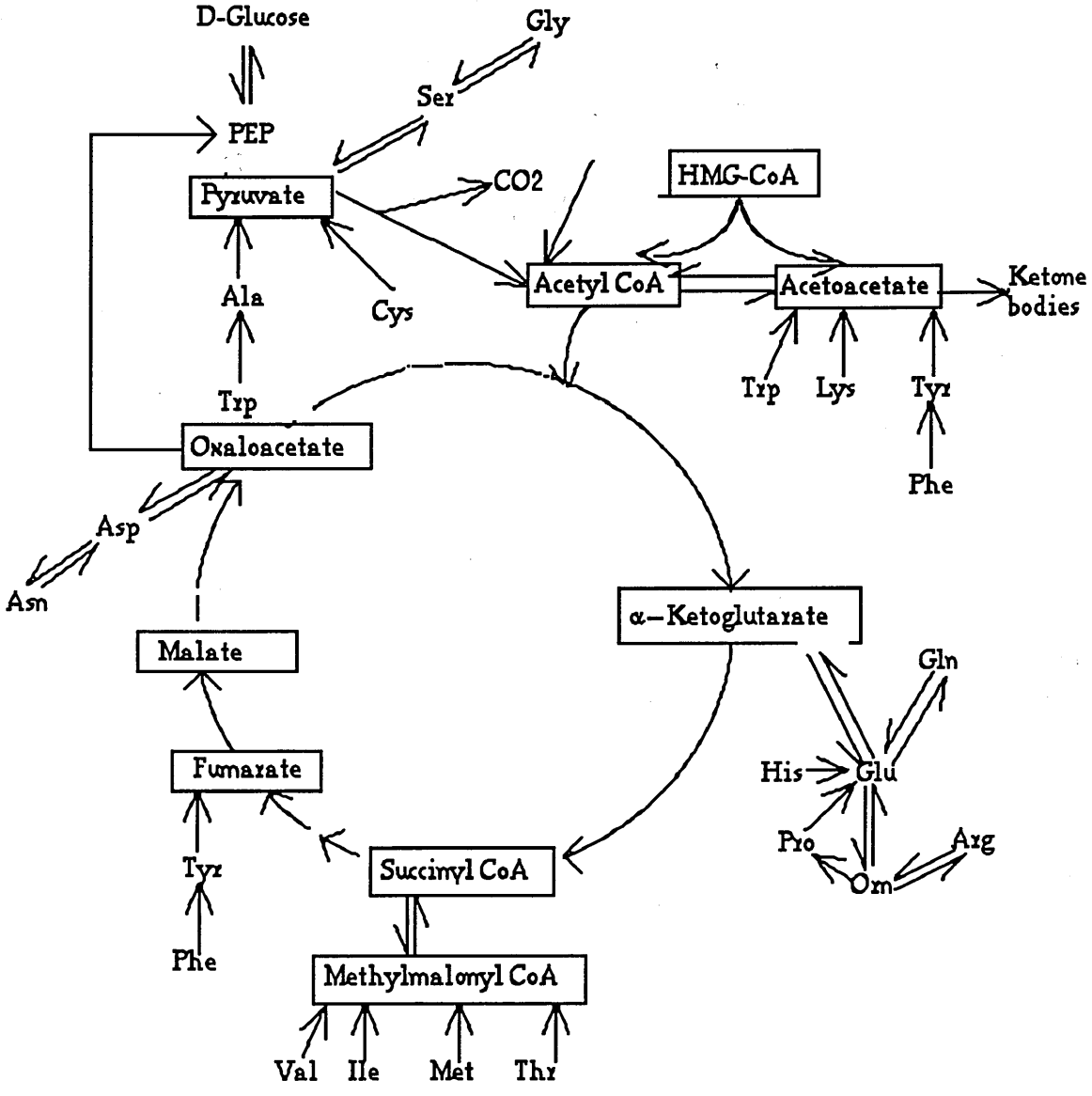
We have already noted that alanine is derived from the branched chain amino acid catabolism in muscles and is transported to the liver as a glucogenic amino acid. Additionally alanine is formed in the mucosal cells of the gut from glutamic acid and glutamine and transported via the portal vein to the liver for gluconeogenesis (114).

Glycine has serine as its precursor. It can also be derived from glyoxalate derived from any other source. In catabolism it may also condense with another molecule of glycine to form serine. Serine synthesis occurs through the pentose phosphate pathway, from glycine cleavage. Metabolic fate of serine is to pyruvate.

Tyrosine which is considered to be conditionally indispensable amino acid is derived from phenylalanine. In catabolism it is deaminated through homogentisic acid to acetoacetate and fumarate.

In Figure 1.5 are shown the various pathways by which the carbon containing chains and the amino groups are separated. The mechanisms of this separation are transaminations or oxidative deaminations. The disposition of the carbon chains is the factor determining the formation of either ketone bodies or glycogen. The portions of the amino acids which form Krebs cycle intermediates are glucogenic, i.e. forming glucose through oxaloacetate and phosphoenolpyruvate. The ketogenic portions give rise either to acetoacetate or acetyl CoA. Whether glucogenic or ketogenic, the importance is that either substance can be quickly mobilized for energy production.

Figure 1.5 Metabolic fates of carbon skeleton of amino acids.



Protein as source of amino acids

Dietary protein is the principal source of amino acids although we have seen that there is a component from proteinous secretion into the gut. Proteolytic enzymes break down the proteins to amino acids and peptides in the gastrointestinal tract. But some small proteins and peptides are absorbed from the intestines. Amino acids are the main and smallest fractions of protein digestion. They circulate in the blood and may be measured as plasma amino nitrogen or as individual amino acids.

Digestion commences in the stomach where pepsinogen from the chief cells in the mucosa is converted to pepsin which then proceeds to break down the protein to polypeptides (115). The peptide gastrin from the distal end of the stomach actually triggers the chief cells to produce pepsinogen. Similarly the pancreas releases its digestive juice stimulated by secretin a hormone of the duodenum stimulated by the presence of acid gastric chyme in the duodenum. The acid gastric chyme is then alkalinized by bile from the liver and bicarbonate from the pancreas. Also from the pancreas comes chymotrypsinogen, trypsinogen, proelastase and procarboxy-peptidase. Trypsin, chymotrypsin and elastase are endo-peptidases, they cleave proteins and polypeptides at internal sites, usually at specific amino acid residues (116). Carboxypeptidases (A and B) are exopeptidases cleaving amino acids from the carboxyl ends of polypeptide chains. The resulting proteolytic enzymes have remarkable specificities for cleaving protein chains at certain amino acid residues. The products of these enzymes are free amino acids, dipeptides and small peptides. The residual peptides are hydrolyzed in the intestinal mucosal cells by aminopeptidases and dipeptidases.

Absorption of Amino Acids

A substantial amount of small peptides as well as amino acids are absorbed by stereospecific systems. Mainly amino acids are found in the portal blood, peptides being hydrolyzed by peptidases in the absorptive cells.

Amino Acid Transport System

The absorption of neutral and basic amino acids is mediated by separate carrier systems. In Hartnup disease neutral amino acids are not absorbed, although the basic amino acids are adequately absorbed (117). The reverse is true in patients with cystinuria.

The intestinal absorption of L-amino acids is much faster than for the corresponding D-isomers. Indeed D-amino acids may not be actively transported, although at a much reduced affinity D-amino acids share in the L-amino acid carrier system (118).

Amino acids with a long side chain have a greater affinity for the transport system than do those with a short side chain (119). The importance of this is the selective rate of absorption of amino acids containing equivalent amounts of either eight indispensable or the eighteen common dietary amino acids.

Absorption of amino acids is not affected within a wide range of changes in the intraluminal concentration of sodium, nor by the pH (120) . Neutral amino acids are more rapidly absorbed from the distal jejunum than from the proximal jejunum, and certainly more rapidly than from the distal ileum. Recently active transport of neutral amino acids was demonstrated in the duodenum and is described as being an active sodium dependent mechanism (121). Amino acid absorption seems to be reduced by

starvation, although starvation does not alter the selectivity for absorption(122).

Although these facts have been well established during the past two decades, there is a current volume of literature on this subject using more modern techniques involving human intestinal brush-border membrane vesicles experiments. These works confirm the presence of different systems for amino acid transport and they even have shown that for the transport of the acidic amino acids there is an apparently electroneutral manner which utilizes an inward sodium gradient and an outward potassium gradient(123, 124).

Peptide transport system

Dipeptides and tripeptides are also absorbed by the human intestine. Neutral, basic and acidic dipeptides share a common mediated mechanism for transport (125) which is a non-concentrative, Na^+ independent, carrier mediated system There is also here a preference for L-peptides with short chain N-terminal amino acids in the absorption system and lowering of the intraluminal pH results in a less restricted absorption of peptides than of amino acids. Absorption of peptides in man is maximal in the jejunum, minimal in the duodenum and intermediate in the ileal region, where hydrolysis of peptides exceeds that of the jejunum (126).

Interestingly, in coeliac disease free amino acid absorption is more impaired than is that for peptides and this even in the face of villous atrophy and hence loss of brush border. It may well be that in this disease ileal absorption, with its greater hydrolytic capacity, compensates for loss due to the jejunal pathology.

Amino acid imbalance and antagonism

Body mechanisms in health are geared to handle a balanced intake of amino acids as they are represented in dietary protein. A relatively large excess of any one essential amino acid administered along with a protein reduces the biological value of the protein and especially when the excess of the amino acid is gross and the protein intake marginally adequate. This situation is said to depress growth (127). Differences in the concentration of the essential amino acid versus the adequacy of the protein diet determines the degree of growth restriction.

When the total intake of protein is increased to slightly above the minimum requirement, the addition of the excess amino acid has no effect on growth (128). Thus in humans, eating more protein than the minimum amount of protein required imbalances of any single or combined indispensable amino acids are unlikely to have any detrimental effect. From these data it is clear that when treating protein malnourished children, dietary protein increase is much to be preferred to an increased intake of isolated indispensable amino acids.

Interestingly, there is an antagonism between some amino acids when one is present in relative excess. For example, the addition of an excess of arginine to a diet reduces the biological value of proteins that are not limited by lysine, yet only the addition of lysine will restore nitrogen balance. Similarly the branched chain amino acids are mutually antagonistic – excess of leucine impairs utilization of dietary protein and only the addition of isoleucine and valine will overcome the effect. In infants after an overnight fast, the concentration of threonine in the plasma shows an inverse relationship with dietary lysine (129),whereas in young adults such a reciprocal relationship does not obtain (130).

Free Amino Acids in Plasma

The level of plasma amino acids is related to their quantity in food proteins, although the true nature of the relationship is one of complexity. Nonetheless the plasma amino acid levels reflect a state of equilibrium – a balance between the entry of amino acids into the circulating pool and their removal by various tissues.

In the pool the amino acids are in the free state. It cannot yet with certainty be stated which portion of the amino acids removed from the pool will be used for protein synthesis and which for combustion, so that the relationship between dietary and plasma amino acids is of very doubtful significance. At most the pattern of resorbed and free plasma amino acids will correspond when dietary proteins have a relatively high biological value. This fact has given rise to the study of plasma amino acid ratios, especially indispensable to dispensable amino acids. Changes in this ratio depend on the nature of the protein ingested, and the subsequent effects on the dispensable amino acids. In other words, restriction in protein ingestion and absorption alter the nutritional ratio by increasing the dispensable amino acids, thus lowering the ratio, while correction of the ratio can be achieved by increasing the quantity of protein ingested.

Clinical importance of amino acids

We have already considered the glucogenic and ketogenic actions of amino acids. As precursors in metabolic pathways they are important. For example, tyrosine from phenylalanine is necessary for thyroxine synthesis. Sulphur containing amino acids give rise to taurine. Glycine contributes to the porphyrin ring of haemoglobin and with serine provides part of the structure of purines and pyrimidines of the nucleic acids. Glycine is also a constituent of glutathione. It also conjugates with cholic acid to form bile,

and with benzoic acid to form hippuric acid which is used in liver function tests. Amino acids are indispensable also for the formation of enzymes and hormone. In screening for inherited disease, specific amino acids are identified. For example, in phenylketonuria, phenylalanine blood concentrations are estimated. This is the basis for the Guthrie test, and facilitates early detection and treatment of that disease.

Therapeutically, amino acids have special value such as in chronic liver disease with encephalopathy. Because the branched chain amino acids are not metabolized by the liver, they may be given by infusion as a source of energy for extrahepatic metabolism. Also a second benefit from an infusion of the branched chain amino acids is that there will be a restriction of the aromatic amino acids which in turn spares the production of serotonin and noradrenalin both of which increase drowsiness in case of hepatic encephalopathy.

The Rationale

The reader might be justified in wondering what is the relationship between growth hormone, amino acids and somatostatin. Throughout this introductory chapter I have here and there hinted that there is or may be indeed a relationship which when applied to children with growth hormone deficiency, would offer an explanation for their response to administered growth hormone.

Basic to the theory to be tested is firstly the fact, generally accepted that growth hormone in one of its actions raises the concentration of plasma amino acids. This of course is logical if perhaps firstly physiological for if a child is to grow then all the growing structures, cartilage, bone, muscles and organs require protein, the building bricks of which are amino acids. That is easily understood and accepted.

Secondly, it is now well accepted that somatostatin is uniformly an inhibitory substance and in the context of this work it inhibits the release of preformed growth hormone from the pituitary and it also inhibits the absorption of amino acids. This really is a nett effect for in so doing it inhibits gastric and gut motility, it inhibits digestive enzymes and it inhibits the transport of intraluminal contents through the gut wall ie. absorption. Thus by preventing adequate plasma concentrations of both growth hormone and amino acids , it is in effect an inhibitor of linear growth.

But it might be argued that it is the hypothalamic somatostatin which inhibits the release of growth hormone, reaching the pituitary via the axons of the cells which synthesize it and along with GRF is taken to the eosinophils of the anterior pituitary by the long and short pituitary portal

vessels. Why then do I study plasma somatostatin concentrations ? For this reason - the long and the short pituitary portal vessels are the channels by which the pituitary receives its blood supply, and these vessels are twigs from the superior and inferior hypophyseal arteries. Thus the anterior pituitary is constantly being bathed with systemic blood containing a measurable concentration of somatostatin. My hypothesis is therefore that hour by hour the pituitary is under the tonic control of plasma somatostatin by way of controlling the release of growth hormone and only in response to the spikes of GRF associated with food, exercise and REM sleep, does the hypothalamic somatostatin buffer the possible gross fluctuations of growth hormone release. Thus high plasma concentrations of somatostatin may therefore not only depress the release of growth hormone but may also lower the concentrations of plasma amino acids, setting the stage for possible short stature.

At the clinical level it is well recognized that some children make more gains in linear growth than others who are treated similarly with growth hormone. The interesting point then is that could it be that those children who do not respond to growth hormone therapy as might be expected, have unduly, or pathologically high plasma concentrations of somatostatin ? Were this to be so, the question arises as to the reason for the increased concentrations of plasma somatostatin, Are we here dealing with a two-way inseparable relationship between plasma somatostatin and amino acids, viz when plasma somatostatin concentrations are high, are the plasma concentrations of amino acids low and vice versa? There is one point which might support such a view namely that infused arginine and other amino acids result in a rise in plasma growth hormone concentrations. Could it be that the growth hormone is released because the inhibiting somatostatin concentration has been lowered thereby? And

reciprocally does administered growth hormone raise the plasma concentrations of amino acids? thereby lowering the plasma concentration of somatostatin - thereby normalizing the high plasma somatostatin values.

While in the patients to be studied here all have growth hormone deficiency, the data to be derived may have some application to children with short stature not due to growth hormone deficiency. In other words, could non-growth hormone deficient short stature in otherwise normal children be due to either high plasma concentrations of somatostatin, or to low plasma concentrations of amino acids or a combination of both?

To what extent the study to be presented here will answer these questions and justify the thesis will unfold as the data are presented in the following chapters.

CHAPTER TWO.

Materials and Methods.

It is seldom that medical catastrophes can be utilized to the advantage of scientific research but such has been the outcome of the scare of Creutzfeldt-Jacob disease as a possible illness following cadaveric growth hormone administration. In the Spring of 1985 following reports of Creutzfeldt-Jacob disease occurring in patients who had received human growth hormone (14. 15), the drug was withdrawn from the 32 patients of this thesis who were then attending the Growth Clinic of the Royal Hospital for Sick Children, Yorkhill, Glasgow. At that time blood was taken from each patient so that the virus or an antiviral titre could be studied in each sample. The plasma samples were stored at -20^{o}C.

About the same time increasing awareness was developing regarding the acquired immune deficiency syndrome and while little was known of the neurotrophic nature of that virus, it seemed reasonable against possible claims to preserve plasma samples for the future.

Fortunately from the date of withdrawal of cadaveric growth hormone as therapy to the becoming available of recombinant growth hormone only nine months elapsed so that most patients recommenced growth hormone therapy within the nine month period.

Almost concurrent with the announcing of the Creutzfeldt-Jacob scare I arrived in the Department of Child Health to commence studies which would be suitable for a Ph.D., degree. My attention was therefore drawn to the study of the effects of growth hormone withdrawal and its recommencement in terms of growth and some plasma biochemical

changes relative to plasma somatostatin and plasma amino acid concentrations.

Clinical Material

The cohort of patients studied consisted of 21 boys and 11 girls whose ages ranged from 2.17-17.94 years. These children had been diagnosed as having growth hormone deficiency and all had been accepted by the Health Services Human Growth Hormone Committee as suitable for growth hormone therapy. Anthropometric data were taken for each child at the time of withdrawal of cadaveric growth hormone, at the occasion of recommencement of recombinant growth hormone and one year later. These data will be presented in Chapter 3. The fluctuations in height and osseous maturation scoring will also be shown in Chapter 3.

Care was made to obtain an accurate standing height estimations during this work by using apparatus supplied by Raven Equipment Ltd., Dunmow, Essex (Figure 2.1 A)

In general to ensure reliable and accurate height measurement, irrespective of the apparatus used, the position of the child is crucial; poor positioning results in inaccurate measurement, thus:-

a. The shoes and the socks should be removed, and the feet should be together and flat on the platform with the heels touching the backboard.

b. The legs should be straight with the buttocks and the scapulae against the backboard.

c. The head should be positioned carefully with the lower orbital margins in the same horizontal plane as the external auditory meati. Sufficient

downward pressure should be exerted to compress the hair. The mean of three readings was obtained for accuracy.

Actual heights were converted to standard deviation scores as generally accepted in paediatric practice (1). Indeed I have expressed plasma concentrations of somatostatin and amino acids also as standard deviation scores. Since standard deviation scores relate an observation to the 50th. centile, the age factor is accounted for in the calculation. To obtain the 50th centile values for the patients in my cohort particularly for plasma somatostatin and amino acid concentrations it was necessary to extend the range of those normal values which were already available in the Laboratories of the Department of Child Health by adding to them data personally derived from an older age group of normal subjects (Appendix Table 1&2). From the combined data I will show in Chapter3 some examples of the regression analyses for both plasma somatostatin and amino acid concentrations in relation to the chronological age.

Biological Sample Collection and Storage

Venous blood (10ml) was collected from a suitable vein following an overnight fast. An aliquot (5ml) for plasma somatostatin assay was placed in a chilled test tube (10ml) containing 7.2 mg. EDTA and Trasylol (Aprotinin) 500 KIU. The remaining blood was heparinized, the plasma separated and stored at -20^{o}C against amino acid assay. The blood for somatostatin assay was immediately extracted and the extract freeze-dried and stored at -20^{o}C against final assay.

Laboratory Material

Chemicals: Acetic acid, acetone, acetyl chloride, ammonia solution, n-butanol, ethyl acetate, chloroform, dichloromethane, isobutylalcohol,

hydrochloric acid, methanol, petroleum ether and sodium hydroxide solution were purchased from British Drug Houses Limited (BDH), Poole, Dorset, U.K. All were of Analar grade .

Toluene (Scintillation Grade) was purchased from BDH Ltd. Both PPO (2:5-diphenyloxazol and POPOP (1,4-di-(2-(5-phenyl-oxozalyl)-benzene)] were purchased from Hopkins and Williams Ltd., Reading Industrial Estate, Falkirk.

Heptafluorobutyric anhydride was purchased from Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire, U.K.

Crystalline amino acids were bought from Sigma, Fancy Road, Poole, Dorset. Also Sigma supplied an amino acid calibration standard solution (2.5 umol per ml in 5ml 0.05 M HCl.) and an ion-exchange resin (Dowex-50 W 8 percent cross link, 200-400 dry mesh).

Ninhydrin spray (0.5 percent in n-butanol) was purchased from BDH Chemicals Ltd.

^{14}C-labelled amino acids valine, leucine, isoleucine and cycloleucine (SA 50 mCi per mmol) were purchased from Amersham International PLC., Buckinghamshire, England, in 2 percent ethanol/water solution(1ml) (50uCi per ml).

Gases. Nitrogen (oxygen-free), oxygen and Argon/Propane (98 percent/2 percent) were obtained from BOC Ltd., Polmadie Avenue, Glasgow.

Equipment.

Gas liquid chromatography apparatus (Figure 2.1 B). A Pye Unicam Series 304 (Pye Unicam, Cambridge, U.K.) equipped with dual column oven,

flame ionizing detectors and a built in temperature programmer was used. A computing integrator (COPI) which gave prints out of retention times and either peak height or peak area was attached. A single pen recorder (Phillips) and standard chart paper (PM 9920/00) was used for trace recording.

Liquid Scintillation Counter. The radioactivity of the standards for initial and recovery experiments was measured by a liquid scintillation spectrophotometer (Pye Unicam LSC, York Street, Cambridge, U.K.). The instrument was calibrated for simultaneous counting of ^{14}C and ^{3}H.

Radiochromatogram Scanner. A Panax thin layer Radiochromatogram Scanner Model RTLS (Panax ESI, Rotheroe and Mitchell, Redhill, Surrey, U.K.) in conjunction with a flat bed recorder (Smiths Industries Ltd., Industrial Instrument Division, Kelvin House, Wembly Park, Middlesex) was used for the identifying the loci of the radioactively labelled amino acids on TLC plates. The detector carrier gas was a mixture of argon (98 percent) and propane (29 percent) and the flow rate was set at 50 ml per minute. The Geiger Muller detector voltage was set at 1,040 V: the detector dead time at 200 msec. and the discriminator bias at 10 mV. A time constant of 100 seconds and a scanning speed of 30 mm per hour were used. The detector was adjusted to a height of approximately 1 mm above the thin layer plate and all plates were scanned using the 15 x 1 mm detector aperture. The range of peak heights varied with the amounts of radioactivity in the areas of the test sample.

Gamma Counter. A Panax Autobioscint Counter (ESI Panax, Rotheroe and Mitchell Ltd, Ruislip, Middlesex, England) was used for measuring the radioactivity in plasma samples assayed for somatostatin (^{125}I).

A

B

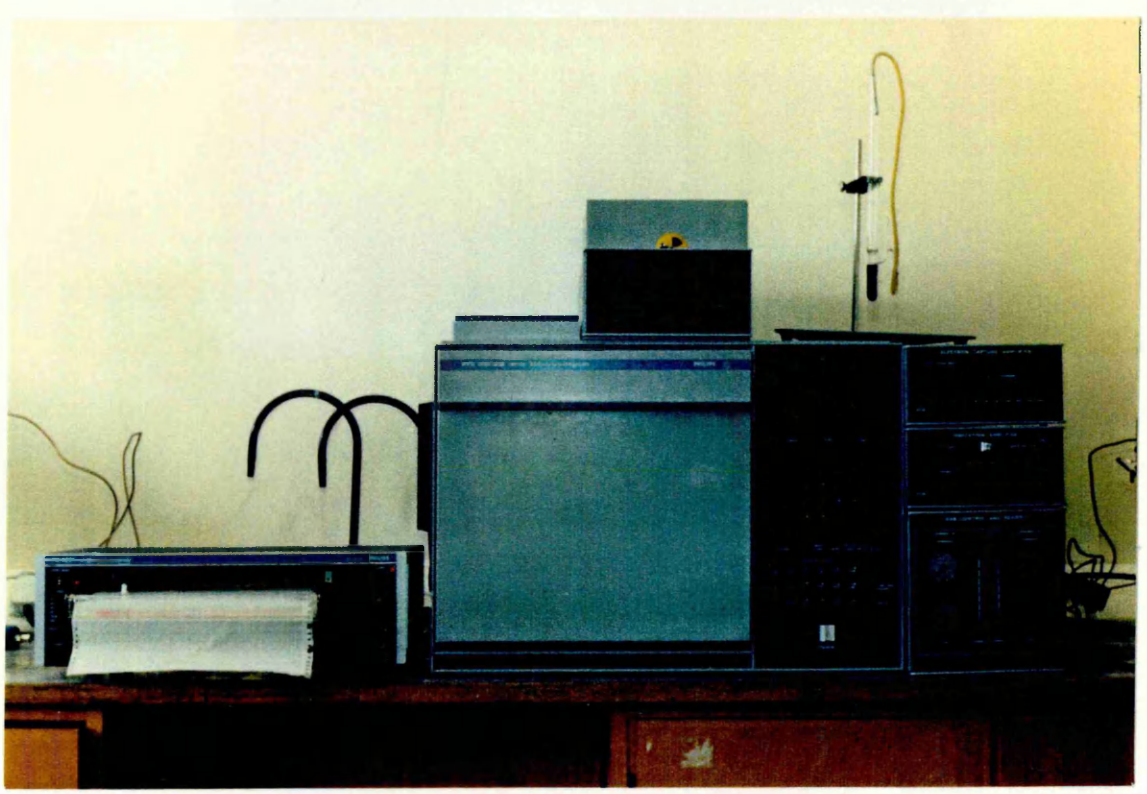


Figure 2.1 A.Karimeter

B. Gas Liquid Chromatography

Freeze Drier. A freeze drying machine, Edwards High Vacuum 9 (subsidiary of BOC Ltd, Manor Royal, Crawley, Sussex, England.) was used for sample drying.

Water Deionization Apparatus. An ELGA Spectrum (System D. SCI) with mixed bed deionization cartridge (ELGA Products Ltd. Lane End, Buckinghamshire, England) was employed for water deionization.

Refrigerated Centrifuge. A Supermagnum refrigerated centrifuge (MSE, Manor Royal, Crawley, Sussex, England) was used for sample centrifugation during somatostatin assay.

Thermostatically controlled Oven. A Gallenkamp Super Hot Spot Furnace (FR 520) was obtained from Gallenkamp Co. Ltd., Sun Street, London, England.

Glassware. Glass vacuum thimbles were purchased from McQuilkin and Co., 21 Polmadie Avenue, Glasgow. These thimbles consist of a scintered glass filter disc incorporated into a tube of glass with expanded walls at either side of the scintered filter. These were used during the elution of samples of amino acids from silica. A Pyrex glass column (3m x 6.35mm OD, 2mm ID) was obtained from Phase Separation LTD., and was used in the Gas Liquid Chromatography Unit.

SGE syringes (5ul and 10ul) were obtained from Scientific Glass Engineering (U.K.) Ltd., Potters Lane, Kiln Farm, Milton Keynes, U.K.

Sealable Glass Ampoules (10ml) were obtained from Adelphi Manufacturers Co. Ltd., Duncan Terrace, London, U.K. Trident Vials were bought from Scientific Supplies, Scientific House, Vine Hill, London, U.K. Graduated pipettes and Pasteur pipettes were purchased from Macfarlane

Robinson Ltd., Hedgefield House, Blavddon-on-Tyne, Tyne and Weir, U.K.

Preparation Procedures

Preparation of Working Solutions

Acetic acid (5M) was prepared by adding glacial acetic acid (1.049 g. per ml)(286 ml) to deionized water (714 ml). The solution was stored in a glass bottle with a glass stopper.

Ammonium hydroxide (2M) was prepared from ammonia solution (Analar Grade) (SG 0.88) by adding 112ml to deionized water (888ml). The solution was stored in a glass bottle.

Esterifying agent was prepared as described (132) by adding acetyl chloride (275 ul) dropwise to isobutanol (1ml) at ^{o}C in a dry glass tube supported in an ice bath.

Hydrochloric acid. (1.0 and 5.0 molar solutions were prepared from concentrated hydrochloric acid (Analar Grade - SG 1.18) by diluting 86 ml and 430 ml each in 1 litre of deionized water. These solutions were stored in a glass stoppered glass bottles.

Scintillation fluid, toluene based contained PPO (2.5-diphenyl-oxazol) (5g.per litre) and POPOP {1,4-di[2-(5-phenyl-oxazalyl)-benzene} (0.05 g. per litre). This solution was used to suspend all radioactive labelled amino acids while their radioactivity was being assessed in a liquid scintillation spectrophotometer. The counts per minute (cpm's) were converted to dpm's (disintegrations per minute) by taking account of the efficiency of the machine for counting ^{14}C-labelled substrates. Sodium hydroxide (1.0

M) was prepared by dissolving sodium hydroxide (40g.) in one litre of deionized water.

Standard solutions of single amino acids (50 nmol/100 ul) were prepared by dissolving separate crystalline amino acids (2.5 umol) in hydrochloric acid (5 ml) (0.1 M). These solutions were stored at -20 ^{o}C.

Thin Layer Chromatographic Plates. These were purchased from Inderman and Co. Ltd., Central Avenue, East Molesly, Surrey, England, and were thin layers (2.0mm) 20 cm x 20 cm of silica.

Purification of ^{14}C-labelled amino acids. Valine, Leucine, Isoleucine and Cycloleucine. Prior to use of these amino acids they were purified by TLC.. Of each (stock standard) 10ul was spotted using disposable microsampling pipettes on a thin layer using a TLC tray. Rapid drying was effected by plying a stream of hot air over the plates. Non-radioactive standards were spotted on the side lanes. The plates were developed in various solvent systems (Table 2.1) until a single peak of Gaussian form was obtained on radiochromatographic scanning. The side-lanes of each plate were sprayed with ninhydrin to locate individual amino acids (unlabelled). Using these loci in conjunction with the chromatographic peaks a precise locus for each radioactive amino acid was identified.

The silica over each radioactive amino acid as indicated by the base of each peak, was removed by scraping and suction into an elution thimble and the amino acid eluted with aqueous ethanol (70 percent) (5 x 2ml). The effluent collected in tubes was blown to dryness under a stream of nitrogen, the tubes being placed in a hot water bath at 40 ^{o}C. The dried individual amino acids were dissolved in aqueous ethanol (2 percent ethanol in water) (2ml) and stored at -20 ^{o}C as working standards.

Table 2.1 Rf values of the amino acids included in the study in different solvents

Amino acid	Solvent A	Solvent B	Solvent C
Alanine	0.149	0.255	0.430
Glycine	0.101	0.235	0.398
Valine	0.268	0.361	0.547
Threonine	0.165	0.243	0.479
Serine	0.125	0.230	0.456
Leucine	0.392	0.480	0.610
Isoleucine	0.335	0.471	0.579
Cyclo-leucine	0.394	0.459	0.612
Lysine	0.100	0.070	0.031
Tyrosine	0.321	0.485	0.598

A---n-butanol/acetic acid/water (60/15/25)

B---n-butanol/acetic acid/water (80/20/20)

C---ethanol/water (70/30)

An aliquot (100 ul) of the working standard was taken for counting by liquid scintillation spectrophotometry and by calculation the new specific activities of the individual solutions were determined. The example for ^{14}C - Cycloleucine is given below.

10 ul stock standard (spotted on T.L.C) contain 1.23×10^6 dpm's

100 ul working standard (after purification) contained 59500 dpm's

Thus the working standard (2ml) contains 20×59500 dpm's= 1.19×10^6.

Thus the recovery rate after purification = $1.19 \times 10^6 / 1.23 \times 10^6 = 96.75$ percent.

Preparation of Dowex 50 W X8 (H^+) Resin

The resin as purchased was suspended in water (deionized) and allowed to settle after gentle shaking. The supernatant fluid containing amino acids, peptides and proteins was removed by suction. This procedure was repeated three times using Dowex (20 g) in deionized water (100 ml). Then followed washing of the resin with sodium hydroxide solution (1M: 3ml) and its removal by suction followed again by water washing (50 ml) and its removal by suction. Thereafter the resin was washed with hydrochloric acid (5M : 30 ml) then with deionized water until the washings were neutral to litmus paper. The prepared Dowex was kept under water until required for use.

Preparation of Ion-Exchange Column

Pasteur pipettes (133, 134, 135) have been recommended as suitable for columns and I found this practical as an adopted practice in the

Laboratories of the Department of Child Health. Short columns are at any rate recommended for the clean-up of samples when using ion exchange resins in the quantitation of amino acids (136).

The narrow end of the pipette was partially occluded with a pledgelet of glass wool so as to allow a flow rate of 15-20 drops per minute. The column was supported in a wooden rack (Figure 2.2). The aqueous suspended resin was placed in the pipette to a height of 1.5 cm and the walls of the pipette were washed down with water. When the Dowex settled a small disk of Whatman No 1 filter paper was placed on top of the support so that the surface would not be disturbed during the application of the test material. The resin was then washed with hydrochloric acid (1M; 2 ml) followed by washing with water (3 x 2ml) or until the washings were neutral to litmus paper. The column was now ready for sample addition.

A frozen plasma sample was allowed to thaw at room temperature. An aliquot(250 ul) was pipetted into a test tube containing ^{14}C-cycloleucine (200 ul of working standard or 119000 dpm's). Acetic acid (5M; 500 ul) was then added so as to adjust the plasma pH to between 2.0 to 2.5. The contents of the tube were transferred quantitatively on to the surface of the exchange resin in the Pasteure pipette. Since the free amino acids are strongly adsorbed by the sulphone group (SO_3-) of the resin, the nonabsorbed materials were removed by simple deionized water washing (4 x 2 ml). Meantime the column eluate was being collected in 2 ml aliquots. Next ammonium hydroxide solution (2 M; 5 x 2 ml) was added to the column to elute the adsorbed amino acids and again 2 ml aliquots of the eluate were being collected. The 2 ml aliquots were collected in counting vials. Each aliquot was blown to dryness using a stream of nitrogen while the vials

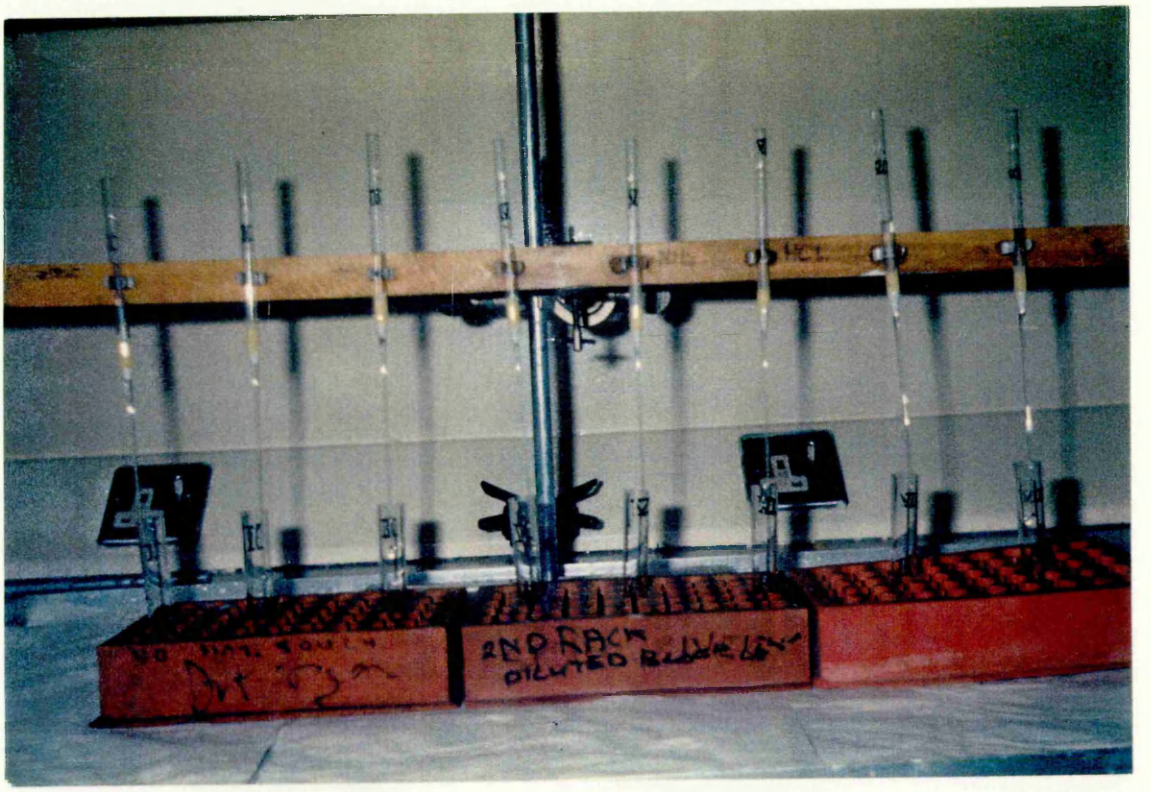


Figure 2.2 Ion-exchange Chromatography

were held securely in a water bath at 40 ^{o}C. The contents of the vials were then exsiccated for four hours in a dessicator. Scintillant fluid (10 ml) was then added for counting in the liquid scintillation spectrophotometer. The radioactivity (expressed as dpm's) in each aliquot of the column is shown in Table 2.2. It will be seen that the radioactivity is eluted in the ammonium hydroxide aliquots No's 1,2 and 3 only and the total recovery is around 99 percent of that added to the column originally. This experiment was repeated but using 20ul (11900 dpms) and varying volumes of patient's plasma so as to test the loading of the column and its capacity to adsorb varying masses of amino acids. The results from this exercise are shown in Table 2.3.

Derivatisation of Amino Acids and percentage derivatisation

Because of the high melting point of free amino acids they cannot be applied directly to the column in a GLC system. They must be derivatised. Impurities in a mixture of free amino acids do interfere with the efficiency of derivatisation, hence the purification procedure of the Dowex 50 column.

Heptafluorobutyrates have been found to be suitable esters of amino acids for GLC separation and quantitation (132, 137, 138). The total formation of suitable derivatives involves the esterification of the amino acids with isobutyl alcohol followed by acylation with heptafluorobutyric anhydride. So is formed the heptafluorobutyryl isobutyl amino acid esters. These amino acids taken from the Dowex 50 column (duplicate samples) were taken to absolute dryness using nitrogen and dessication and to the sealable ampoules was added acidified isobutanol (250 ul). Acidified isobutanol was prepared by the addition at 0` ^{o}C of acetyl chloride (275 ul)

Table 2.2 Percentage recovery of the ammonium hydroxide elute from ion-exchange column of ^{14}C-Cycloleucine (119000 dpms).

Aliqouts	Dpms	Percent recovery
1	94010	79
2	20230	17
3	3451	2.9
Total	117691	98.9

Table 2.3 Percentage recovery of ^{14}C-Cycloleucine(11900 dpms) from the ion-exchange column contained in different amounts of pooled plasma (duplicate).

Plasma	100		200		400		500	
volume (ul)	A	B	A	B	A	B	A	B
dpms in elute	11724	11650	11519	11868	11763	11622	11762	11531
Recovery %	98.52	97.90	96.80	99.73	98.85	97.66	98.84	96.90
Mean of duplicate	98.21		98.27		98.26		97.78	
Mean ±SD	98.15±1.02							

dropwise to isobutanol (1 ml). The ampoules were flushed with nitrogen, immediately sealed and after mixing, the ampoules were incubated at 130 ^{o}C for 30 minutes. The samples were allowed to cool and the mixtures were freeze dried. To the dried residues heptafluorobutyric anhydride (100 ul) was added, the contents mixed, flushed with nitrogen, the ampoules sealed and incubated again at 150^{o}C for 10 minutes. By this the heptafluorobutyryl-isobutyl esters were formed. After cooling the contents of the ampoules were completely dried and to the residues ethyl acetate in appropriate amount was added to each. To test the percentage derivatisation 11900 dpms were added to varying volumes of column purified pooled plasma and the samples derivatised in the usual manner described above. After derivatisation the dried samples were taken up (in dichloromethane) for spotting on TLC plates. These were then developed in solvent system:- chloroform/isobutanol/acetic acid (95/5/1) followed by scanning, and the silica over the ^{14}C-cycloleucine was removed, eluted and the residue subjected to scintillation counting. The recovery values are shown in Table 2.4 giving an average of 97.76 per cent.

Column Separation of the Derivatised Plasma Samples

Although I have used ^{14}C-cycloleucine for recovery experiments related to the Dowex column and the derivatisation procedure, my final quantitation method was by internal standard and relative molar response. Internal standardization is the best method for obtaining accuracy, precision and reliability in GLC analyses. By this the internal standard is added to the sample at the beginning of the procedure and taken through the entire steps. Certain criteria for the internal standard must obtain. For example its chemical structure and properties should be close to those of the test material. Its retention time should be separate

Table 2.4 The assay of the efficiency of derivatisation procedure

(11900) dpms originally added to the column as ^{14}C-cycloleucine)

Plasma volume in	100		300		400		500	
duplica (ul)	A	B	A	B	A	B	A	B
dpms after derivatisation	11722	11616	11796	11443	11360	11767	11732	11514
%derivatisation	98.50	97.61	99.13	96.16	95.46	98.88	99.59	96.76
Mean of duplicate	98.06		97.65		97.17		97.68	
Total mean % ± SD	97.76±1.51							

from those of the sample but the difference should not be great. Finally it must be stable under the GLC conditions used. Cycloleucine answered these requirements. The internal standard method of assay eliminates the need for corrections due to evaporation, spillage, recovery rates etc., and is not affected by minor changes in the GLC conditions (139).

In this work unlabelled cycloleucine (20-50 nmol) was added to each unknown plasma sample as the internal standard and taken through the whole procedure. Additionally this internal standard (50 nmol) was added to the mixture of standard amino acids (of each 2.5 umol per ml) before the derivatisation. Following derivatisation the samples were brought to dryness and reconstituted with ethyl acetate (250 ul). Thus the mixture had a concentration of 1nmol per 1ul. Aliquots of the derivatised standards (2.5 ul) and of the unknown samples similarly reconstituted (2.5 - 5ul) were injected onto the GLC. column under the following running conditions.

Nitrogen flow rate	25 ml per minute.
Hydrogen flow rate	30 ml per minute.
Air flow rate	300 ml per minute.
Injector temperature	250 ^{0}C.
Detector temperature	300 ^{0}C.
Column Oven temperature	100 ^{0}C for 3 minutes
programmed between	100^{0}C and 250^{0}C increasing at 2^{0}C per minute.

Typical tracings of both standards and unknown samples are shown in Figures 2 (3 and 4). The reason for using temperature programming is that each compound tends to be eluted at its optimum temperature. Throughout the period of this work the stability of the GLC apparatus and

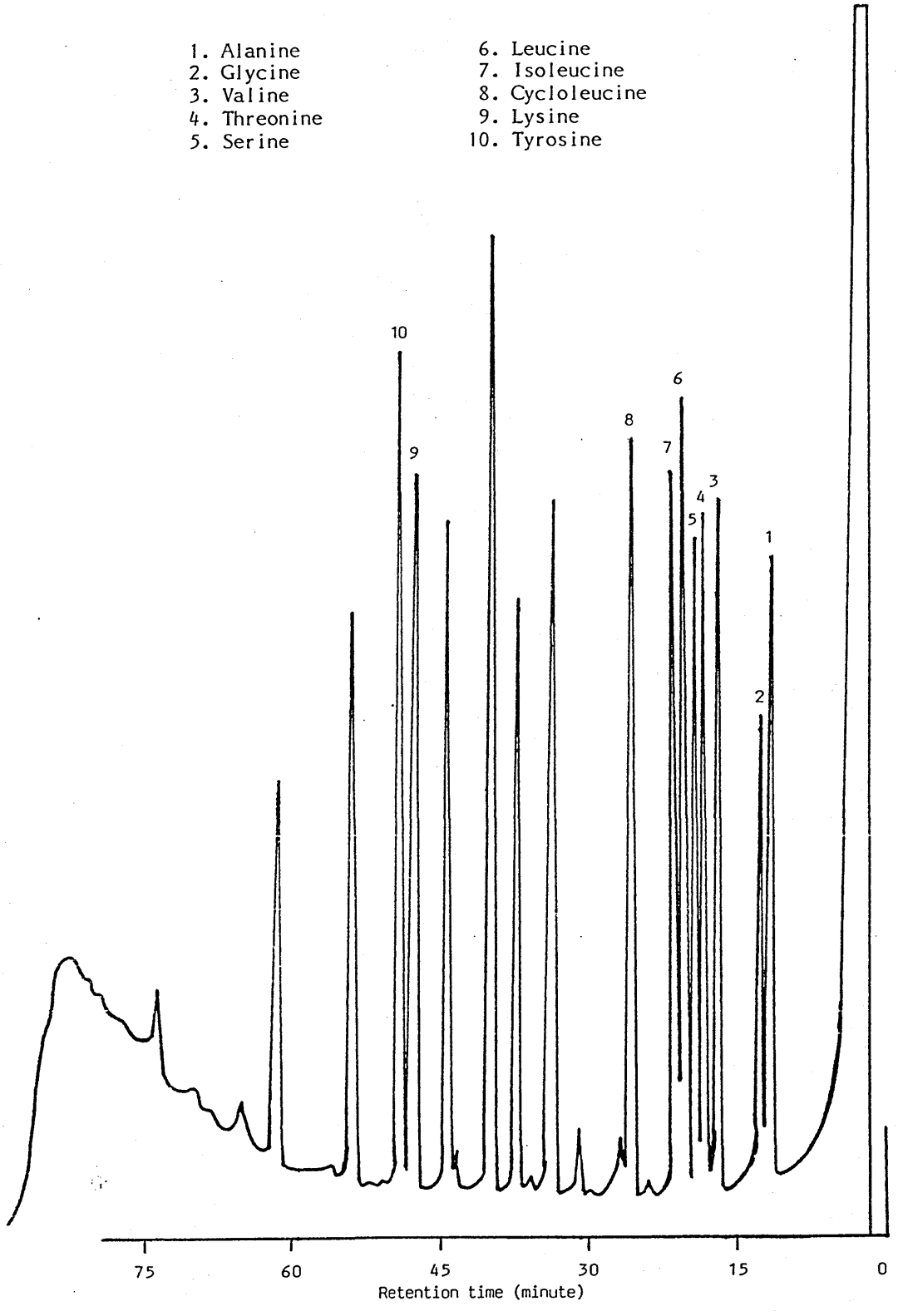


Figure 2.3 Tracing of the amino acids in the standard solution.

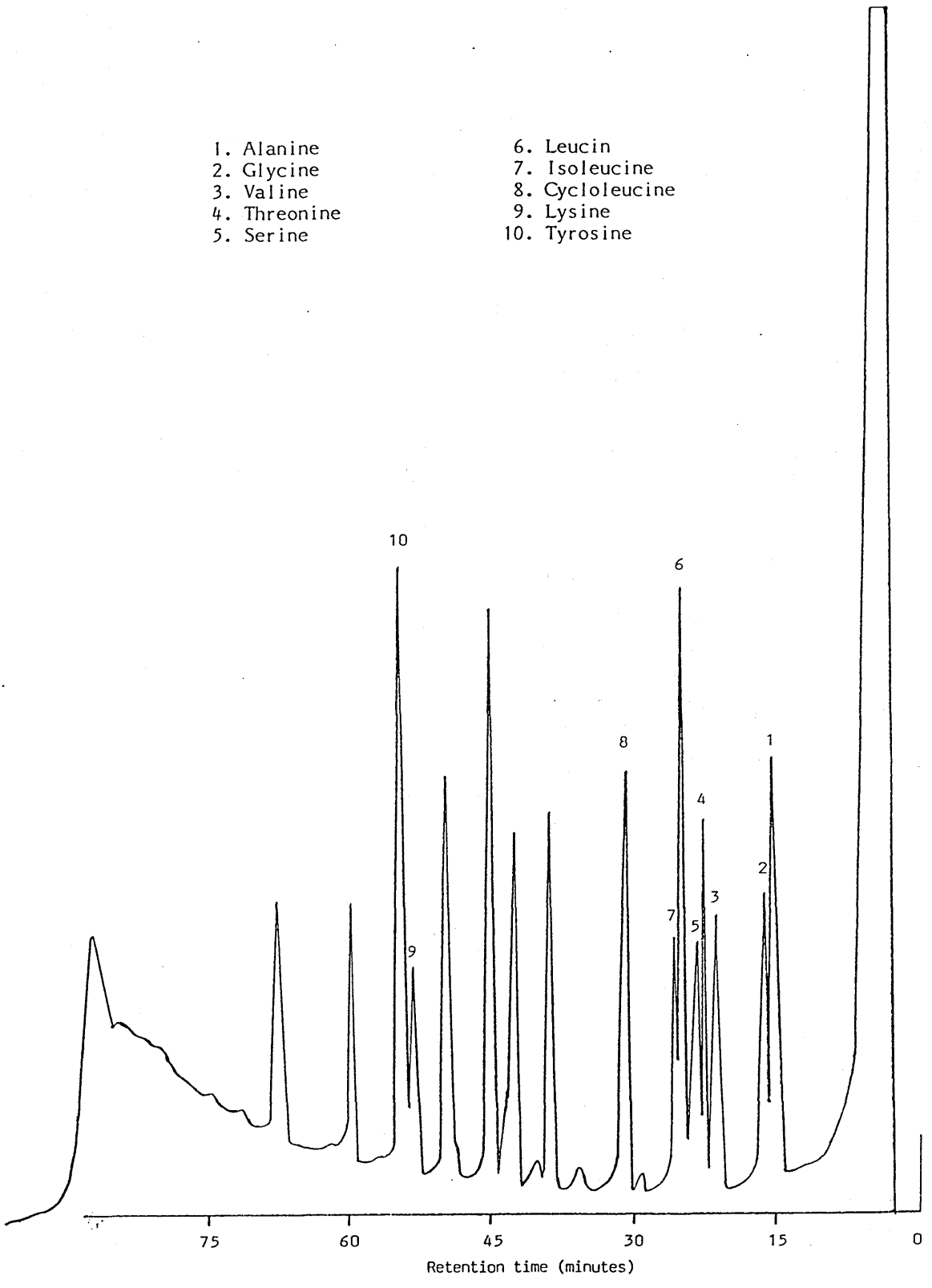


Figure 2.4 Tracing of the amino acids in plasma sample

the satisfactoriness of the running conditions were occasionally checked by processing varying amounts (nmols) of the amino acid standard esters. The linearity of the detector is confirmed by the data of Figure 2.5. I have used the method of the peak height for it has been found acceptable (138).

The order in which each amino acid was registered by the detector and the corresponding peak was studied by processing individual amino acid esters. Thus the composite profile of the total amino acid peaks was built up for the routine work. The quantitation of the amino acids in the plasma samples was calculated by comparison of the relative molar response (RMR) of the individual amino acids to the internal standard cycloleucine (138). The RMR values of the amino acids studied in relation to the internal standard (cycloleucine) are shown in Table 2.5.

Amino acid molar response = peak height per mole of amino acid.

Relative molar response =Amino acid molar response per internal standard molar response. Using the relative molar response of the individual amino acids in relation to cycloleucine as the internal standard it was possible to calculate the plasma amino acid concentration in each sample depending on the plasma volume originally available and the amount of the internal standard added to the unknown plasma sample

$$\text{RMR of a.a (X)} = \frac{\text{Response of 1mol of a.a(X)}}{\text{Response of 1mol of cycloleucine(i.s)}}$$

$$= \frac{\text{Peak Ht.of(X)/moles of (X) injected}}{\text{Peak Ht. of (i.s)/moles of (i.s) injected}}$$

Nanomoles in the unknown sample=

$$\frac{\text{RMR of (X) in the sample}}{\text{RMR of (X) in the standard solution}} \times \text{nmoles of (i.s) added to the sample}$$

So the concentration of the amino acid in the plasma=

$$\frac{\text{nmoles in the plasma sample X 1000}}{\text{volume of plasma taken (ul)}} = \text{nmol/ml (umol/l)}$$

Statement of the Method

1. Cycloleucine (20-50 nmol) and acetic acid (5 M; 500ul) were added to plasma (100-250 ul) in a test tube and the contents mixed.

2. The mixed contents were applied quantitatively to the Dowex 50 W X8(H^+) column.

3. Deionized water (4 x 2ml) was passed through the column and these washings were discarded.

4. Ammonium hydroxide (2M; 4 x 2ml) was used to elute the amino acids from the support and the total collected in a glass ampoule.

This eluate was blown to dryness and the residue excicated in a dessicator.

6. Hydrochloric-isobutanol (250 ul) was added to the dried residue in the ampoule, air expelled with a stream of nitrogen and the ampoule sealed immediately. The contents were mixed

7.---and placed in an oven at 130 ^{0}C for 30 minutes.

8. Cooling was then allowed till room temperature was reached.

9. The excess reagents were then removed by freeze drying.

10. Heptafluorobutyric anhydride (100 ul) was then added to the completely dried sample, the ampoule again flushed with nitrogen, sealed, the contents mixed and the ampoule incubated at 150^{0}C for 10 minutes.

11.The ampoule was then allowed to cool to room temperature before opening.

12.Excess reagents were again removed by freeze drying - a process requiring care because of frothing.

13. Ethyl acetate (100-250ul) was then added to the dried residue and 2-5 ul taken for G.L.C. analysis.

14.The peak heights of the individual amino acids were measured and the calculations were made as above.

15.Accompanying each batch of samples a new set of standards was processed similarly because after esterification certain amino acids may lose their detectability e.g. lysine and cystein lose 15 percent of their detectability within 12 days while that of methionine and arginine is lost within a few days.

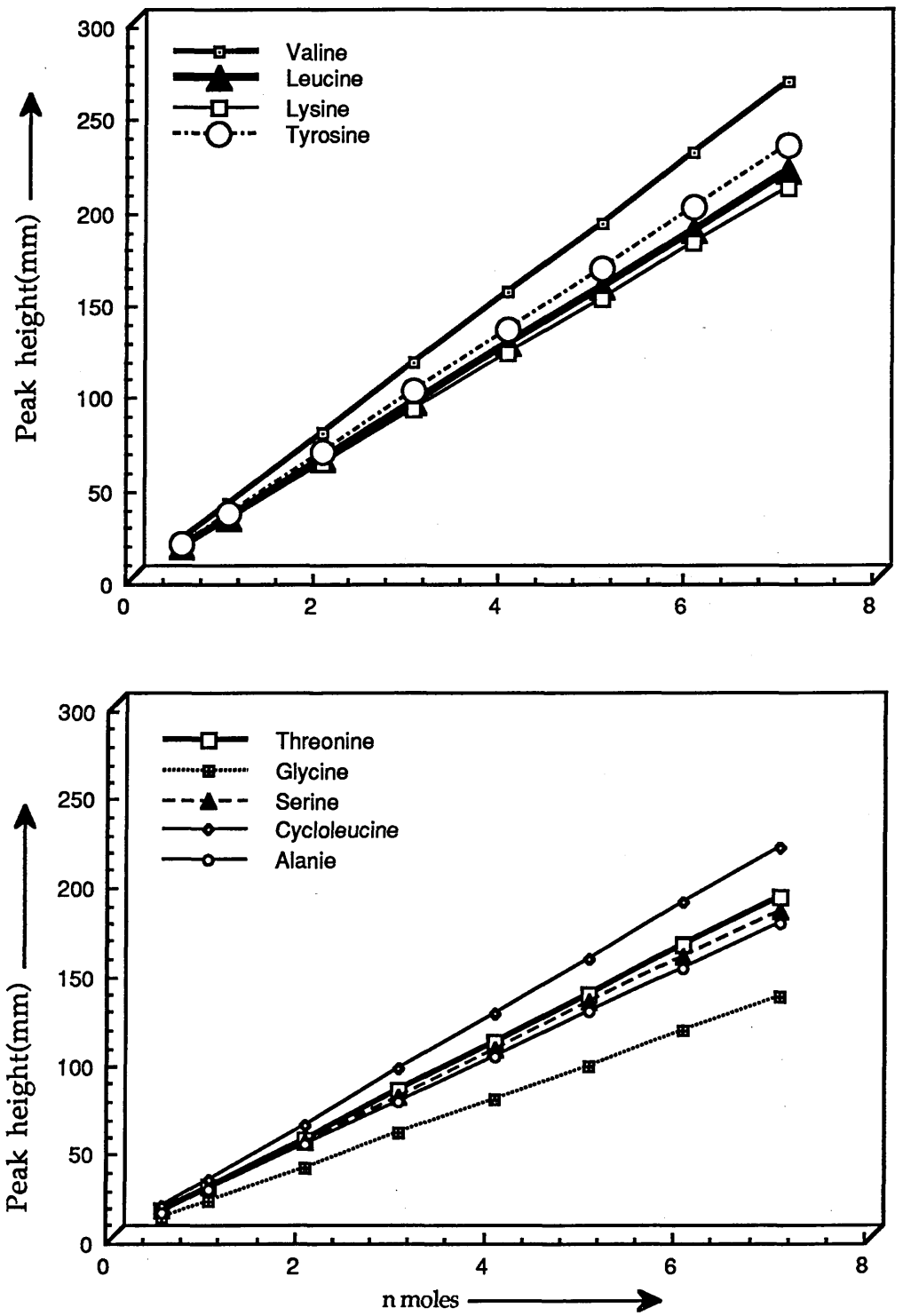


Figure 2.5 Detector linearity for the heptafluoro-butyrylisobutyl amino acid derivatives.

Table 2.5 The relative molar responses of the heptaflourobutyryl-isobutyl esters of the amino acids in relation to cycloleucine as the internal standard.

Amino acid	RMR mean value (n=35)	Standard Deviation
Alanine	0.811	0.057
Glycine	0.610	0.184
Valine	0.901	0.032
Threonine	0.877	0.04
Serine	0.839	0.038
Leucine	1.011	0.116
Isoleucine	0.988	0.132
Lysine	0.957	0.087
Tyrosine	1.076	0.188

Principles of Somatostatin Assay

The availability of synthetic somatostatin made it possible to generate antiserum against it and this led to the development of various radioimmunoassay techniques. Reliable and easy methods for somatostatin assay in tissue extracts, culture media and tissue perfusates are available (140) but its assay in plasma presents some difficulties. It has been proved by several investigators that proteases in unextracted plasma could degrade both native somatostatin and labelled analogues used as tracers in the assay (141,142,143). Additionally large somatostatin molecules may interfere with the specificity of the method.

Moreover plasma contains somatostatin binding protein (144) and other as yet poorly characterized substances which interfere with antibody binding (145,146). Extraction of plasma before performing the radioimmunoassay has gone a long way to solving these problems (145,146). The method of assay used in this work utilized extracted plasma, acetone and petroleum ether being the extractant (147). Thus after extraction, the cleaned-up plasma is subjected to a radioimmunoassay to which is added simultaneously rabbit anti-somatostatin antibody and ^{125}I somatostatin followed by overnight incubation at 4^0C. The somatostatin in the plasma sample is allowed to react with the rabbit antisomatostatin serum. The remaining free moieties of the rabbit antisomatostatin then reacts with the ^{125} I-somatostatin. Phase separation is effected by the addition of a complex of goat anti-rabbit serum, carrier rabbit serum and a polyethylene glycol(GAR-PPT). After 15-25 minutes of incubation, centrifugation is carried out resulting in the formation of a precipitate which contains the radioactive somatostatin which has reacted with the rabbit anti-somatostatin serum. Radioactivity in the precipitate may then be counted.

The higher the radioactivity count in the precipitate the greater is the antibody which reacts with the ^{125}I somatostatin, and thus the less is the somatostatin in the test plasma sample.

Procedures of the assay

A- Extraction procedure. Plasma (1 ml) was pipetted into a chilled glass tube. Cold acetone (2 ml) was added dropwise while continuously shaking the tube. After thorough mixing, the sample was centrifuged for 15 minutes. The supernatant phase was decanted into another chilled glass tube. Petroleum ether (4 ml) was added and the contents mixed followed by centrifugation. The upper ether layer was aspirated and discarded. The aqueous layer was freeze dried and stored at -20^0 C.

B. Radioimmunoassay procedures

Somatostatin assay was by a KIT procedure and the reagent used were:-

1. 1% BSA- Borate Buffer. BSA-borate buffer with merthiolate added (lyophilized) was reconstituted with deionized water (50 ml) and allowed to stand for 15-20 minutes until completely dissolved. This was then stored at -20^0 C.

2. Rabbit antisomatostatin serum. This was diluted in BSA-borate buffer with merthiolate added (lyophilized). The contents of the vial were reconstituted with deionized water (10 ml) and allowed to stand for 15-20 minutes until completely dissolved and then stored at -20^0 C.

3. ^{125}I-somatostatin. Synthetic (Tyr-1) somatostatin labelled with ^{125}I and diluted in BSA-borate EDTA buffer with merthiolate added (lyophilized) was reconstituted with de-ionized water (10 ml) and was allowed to stand for 15-20 minutes until completely dissolved and then stored at -20^0C..

4.Goat anti-rabbit precipitating complex. Normal rabbit serum pre-precipitated with goat anti-rabbit serum and polyethylene glycol (PEG) was diluted in BSA-borate buffer with merthiolate added (lyophilized). Each vial was reconstituted with deionized water (35 ml) and the contents mixed thoroughly until the suspension appeared homogeneous. Each vial was then allowed to stand at room temperature for a minimum of 30 minutes with occasional mixing. The vials were then stored at -20^0 C.

5. Somatostatin standard (500 pg per ml). Synthetic somatostatin diluted in BSA-borate buffer with merthiolate added to obtain a concentration of 500 pg per ml (lyophilized) was provided. The vial was reconstituted with deionized water (2ml) and allowed to stand for 15-20 minutes until completely dissolved.

Steps of the assay procedure

1. In order to obtain a standard curve, serial dilutions of the somatostatin standard were prepared according to Table 2.6

2. The freeze dried plasma residues were then dissolved in BSA-borate buffer (1 ml). The tubes containing them were placed in an incubator at 37^0 C. for 10 minutes, then placed in racks over crushed ice.

3. The above-mentioned reconstituted reagents were then allowed to thaw completely and then mixed gently. They were then placed over crushed ice until use.

4. Numbered tubes (12x75mm) were set up in duplicate according to the protocol in Table 2.7 and the rack of tubes was placed on crushed ice.

5. Reagents were added as shown in Table 2.7 and then incubated at 4^0 C for 24 hours.

6. After incubation goat anti-rabbit precipitating complex(500ul) was added to all the tubes except the total count tubes. The contents were well mixed.
7. Thereafter followed an incubation period of 25 minutes at 25^0 C.
8. Centrifugation at 760 x g for 20 minutes then followed at 20^0C. The supernatant layer was immediately and completely decanted from all the tubes except for the total counts tubes. This was effected by inverting the tubes for a maximum of 2 minutes. The rims of the tubes were blotted to remove any drops of supernatant that may have remained.
10. In a Gamma Scintillation counter the precipitate of each tube and the total counts tubes were assayed for radioactivity (dpm's).

Procedure for calculating values of unknowns

Firstly a calibration curve was constructed by plotting the degree of binding against the stated concentrations of the calibration standards Figure 2.6 . To obtain the percentage binding the average cpm's of each standard was calculated. This was divided by the average cpm's of the total count tubes.

$$\text{Percentage binding} = \frac{\text{cpm's of standards or unknown}}{\text{cpm's of total count tubes}} \times 100$$

The concentration of somatostatin in the unknown plasma samples was then determined from the plotted curve.

Recovery rate of somatostatin from the extracted plasma

This was done by adding varying amounts of synthetic somatostatin (25-100pg/ml) to plasma samples containing known values of somatostatin the samples were then extracted and processed as described before.The mean of the recovery rates was 95.9 % as shown in Table 2.8

Table 2.6 Preparation of somatostatin standard tubes.

Procedures	concentration obtained
1. Add 500 ul of 500 pg/ml of standard to 500 ul of BSA-borate buffer and mix well.	250 pg/ml
2. Add 500 ul of 250 pg/ml of standard to 500 ul of BSA-borate buffer and mix well.	125 pg/ml
3. Add 500 ul of 125 pg/ml of standard to 500 ul of BSA-borate buffer and mix well.	62.5 pg/ml
4. Add 500 ul of 62.5 pg/ml of standard to 500 ul of BSA-borate buffer and mix well.	31.2 pg/ml
5. Add 500 ul of 31.2 pg/ml of standard to 500 ul of BSA-borate buffer and mix well.	15.6 pg/ml
6. Add 500 ul of 15.6 pg/ml of standard to 500 ul of BSA-borate buffer and mix well.	7.8 pg/ml

Table 2.7 Somatostatin assay procedures (set up assay on ice).

	Total Count	NSB	Standards pg/ml								Control & unknown samples			
			0	7.8	15.6	31.2	62.5	125	250	500	1	2	3	4
Tube number	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	etc.
BSA-Borate Buffer		300	200											
Standards				200	200	200	200	200	200	200				
Control&unknown samples											200	200	200	200
Rabbit anti-somatostatin			100 ul											
125Isomatostatin	100 ul													
	Vortex gently. Incubate for 16-24 hours at 2-8°C.													
Goat anti-rabbit(GAR-PPT)		500 ul												
all volumes		Vortex gently for 15-25 minutes at 20-25 °C.												
are		Centrifuge using 760 X g for 20 minutes. decant or aspirate supernatant.												
in microliters	Count pricipitate of each tube and total count tubes for 60 seconds or longer													

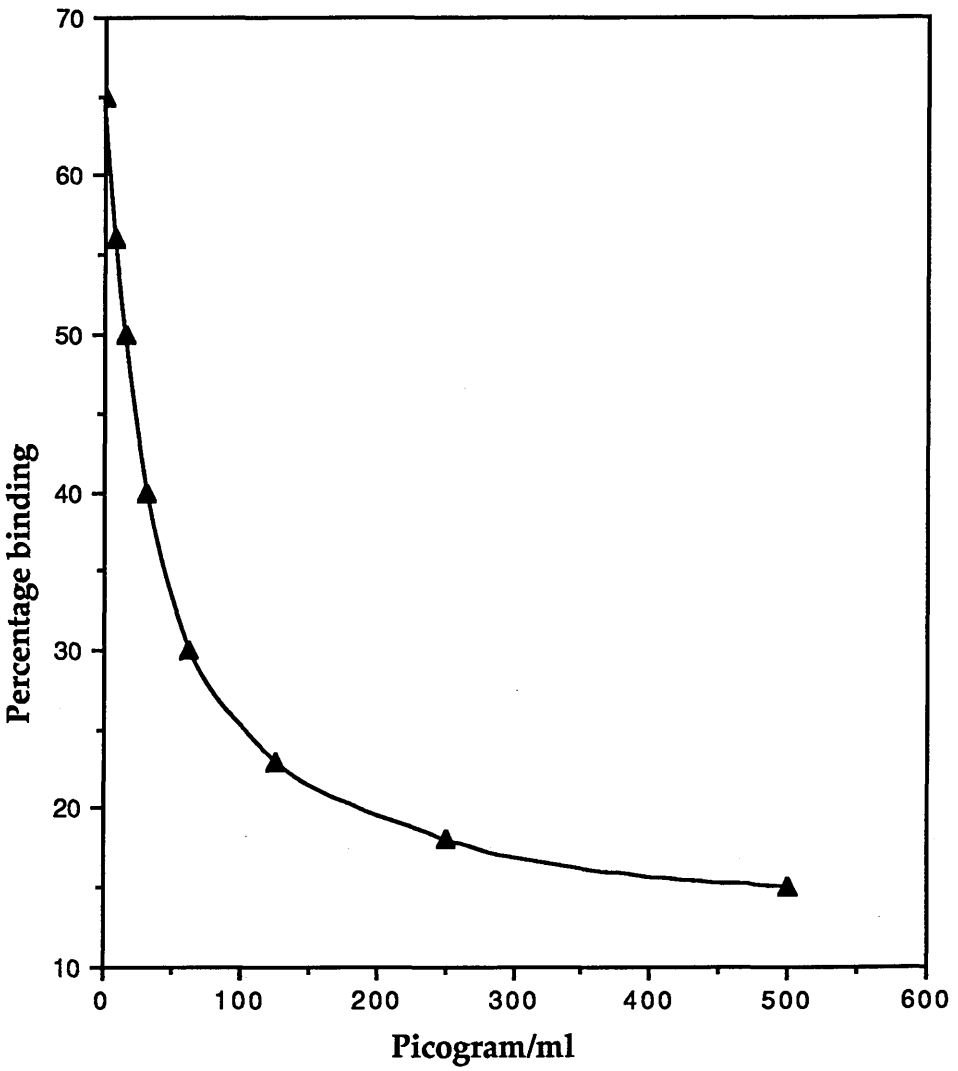


Figure 2.6 Somatostatin standard curve

Table 2.8 Recovery rate of the synthetic somatostatin extracted from the plasma.

Background (pg)	standard added (pg)	expected value (pg)	measured value(pg)	percent recovery
5	25	30	29	96.7
5	50	65	62	95.4
5	100	105	107	101.9
8	25	33	30	90.9
8	50	58	54	93.1
8	100	108	101	93.5
12	25	37	35	94.6
12	50	62	59	95.2
12	100	112	114	101.8
mean recovery rate%		95.9 $\pm$3.75		

CHAPTER THREE

Results

In this Chapter I shall present firstly the effects of withdrawing cadaveric growth hormone from the patients studied and their response to restarting recombinant growth hormone after a lapse of nine months. Following this presentation there will be a statement of the corresponding trends in plasma somatostatin concentrations and this will be followed by a study of the effects of both growth hormone administration and plasma somatostatin on the plasma amino acid concentrations with particular reference to some of the indispensable amino acids.

In Table 3.1 and 3.2 (derived from the anthropometric data in Appendix Tables A1 & 2) are data which highlight the changes in the height standard deviation scores (SDS) consequent on the withdrawal and restarting of growth hormone in the boys and girls who were judged to have idiopathic growth hormone deficiency. These patients had all been accepted by the National Health Service Growth Hormone Committee as suitable for growth hormone therapy and variously had between two weeks and eight years of treatment prior to withdrawal of cadaveric growth hormone. Nonetheless at that point of withdrawal the SDS's are recorded without reference to their former period of treatment.

To remind the reader of the meaning and significance of the SDS, a brief explanation is offered. The SDS for height is the relationship of the recorded patient height to the mean for the patient's age according to the formula:

$$\text{SDS} = \frac{\text{observed height - mean height for patient's age}}{\text{one standard deviation}}$$

Table 3.1 Height standard deviation scores for male children with idiopathic growth hormone deficiency (IGHD) during the period of review. (See Appendix Table A. 1).

		Height Standard Deviation Scores		
PATIENT	Ch. age (Year)	April 1985	Jan 1986	Jan 1987
1-AB	2.16	-4.51	-4.87	-3.74
2-JC	9.39	-2.47	-2.99	-2.51
3-AG	10.88	-3.68	-3.69	-3.07
4-ALB	11.49	-3.54	-3.61	-3.40
5-NMcQ	12.57	-3.63	-3.95	-3.96
6-CMcG	12.92	-2.43	-2.64	-2.24
7-RH	12.94	-3.28	-3.60	-3.49
8-CG	13.65	-2.56	-2.77	-2.56
9-NC	13.92	-1.46	-1.75	-1.50
10-SS	14.15	-3.87	-4.48	-4.81
11-BL	15.02	-4.51	-5.10	-5.41
12-IMcC	15.32	-2.93	-3.55	-3.37
13-DB	16.73	-2.95	-2.96	-2.82
14-MMcG	17.01	-4.47	-4.11	-4.02
Mean		-3.30	-3.57	-3.35

Table 3.2 Height standard deviation scores for female children with idiopathic growth hormone deficiency (IGHD) during the period of review.

		Height Standard Deviation Scores		
PATIENT	Ch.a/Year	April 1985	Jan 1986	Jan 1987
1-SS	5.77	-3.80	-4.25	-2.66
2-CS	6.51	-1.32	-1.61	-1.29
3-LB	6.55	-3.16	-3.79	-2.76
4-TG	9.95	-1.62	-2.14	-1.83
5-AQ	15.40	-4.95	-4.15	-3.20
Mean		-2.97	-3.19	-2.35

A negative value means that the patient's height is less than the mean or 50th centile. The more negative the score, the further is the patient's height below the mean. A SDS which becomes less negative means that the patient's height is coming nearer to the 50th centile. A patient's height could be increasing at the average annual rate, while the actual height is below the 50th centile but increasing parallel to it. Such a patient's SDS for height will not change. It follows that a SDS which improves minimally indicates that only small gains are being achieved over the annual growth velocity. For easy understanding the reader may think of one standard deviation as being approximately 6 cm but one standard deviation throughout the period of child growth ranges from 3.30 cm at an age of 2 years to 8.31 cm at 14 years of age(131).

A study of Tables 3.1 and 3.2 shows that at the time of withdrawal of cadaveric growth hormone the height SDS's were all negative ranging from –4.51 to –1.46 (boys) and for girls –4.95 to –1.32. Also in Tables 3.1 and 3.2 the means for these SDS's are shown and it is of considerable interest that for the boys an initial (1985) SDS of -3.3 worsened on withdrawal of cadaveric growth hormone to -3.57 at January 1986 with some recovery following a year of treatment with recombinant growth hormone to -3.35 (Jan 1987). Thus the restarting of growth hormone did no more than correct the failure to gain height during the withdrawal period. A similar conclusion can be made from the data for girls (Table 3.2) where the mean height SDS's dipped from -2.97 in April 1985 to -3.19 at Jan 1986 and recovered to -2.35 at Jan 1987. An immediate conclusion is that growth hormone therapy once started should not be interrupted. Subsequent years of treatment will therefore be required to bring the actual heights to as near normal as possible.

In Tables 3.3 and 3.4 are the height SDS from the same boys and girls respectively shown alongside the achievement in the annual growth velocities (cm/year) for the same three phases of review. Apart from patients AG, MMcG and BL (boys) and SS and AQ (girls), all patients showed a "fall off" in their annual growth velocities. Patients SS and AQ only received cadaveric growth hormone for 2 weeks before withdrawal of therapy and the fact that their AGV's increased during the withdrawal period may reflect the phenomenon of the increased growth velocity formerly observed in the 'run in' pretreatment year, thought to be due to increased parental attention to the child. Patient RH who only had replacement therapy for two weeks showed a falling AGV during 1986 which is surely the progressive effects of growth hormone deficiency.

Now if it had been expected that following the recommencement of treatment with recombinant growth hormone, a catch-up growth would have followed, then this only occurred in three patients AB and CMcG (boys) and SS (girl). It should be understood that catch-up growth here means a growth velocity sufficient to compensate for the loss of growth during the withdrawal period plus a normal annual increment for the year of renewed treatment. If we now consider the mean annual growth velocities for both boys and girls, it will be seen that for boys a mean annual growth velocity of 5.5 cm/year (range 1.8 - 10.0) fell to 2.8 cm/year (range 0.8 to 4.2 cm/year) and was restored to 6.07 cm/y (range 0.9 to 10.0 cm/yr) (Table 3.3). Similarly significant means for girls (Table 3.4) are 5.76 cm/yr (range 1.5 to 10.10 cm/yr) falling to 3.6 cm/yr (range 1.1 – 7.1 cm/yr), recovering to 8.14 cm/yr (range 5.7 – 13.4 cm/yr).

If we now look at the individual patient response to the withdrawal restart episode, it will be seen that some patients did better than others.

Table 3.3 Height standard deviation scores for males with idiopathic growth hormone deficiency related to their annual growth velocities (AGV) during the period of review.

	Height SDS.			AGV. Cm/Y.		
Patients	Apr/85	Jan/86	Jan/87	Apr/85	Jan/86	Jan/87
1-AB	-4.51	-4.87	-3.74	8.5	4.0	10.0
2-JC	-2.47	-2.99	-2.51	5.1	4.13	5.00
3-AG	-3.68	-3.69	-3.07	2.4	3.2	8.50
4-ALB	-3.54	-3.61	-3.40	10.0	2.7	6.20
5-NMcG	-3.63	-3.95	-3.96	5.6	0.8	6.00
6-CMcG	-2.43	-2.64	-2.24	5.0	3.3	10.50
7-RH	-3.25	-3.60	-3.49	3.6	2.0	8.00
8-CG	-2.56	-2.77	-2.56	8.7	4.2	5.70
9-NC	-1.46	-1.75	-1.50	5.6	4.0	7.90
10-SS	-3.87	-4.48	-4.81	6.1	1.7	7.20
11-BL	-4.51	-5.10	-5.41	1.8	2.8	4.20
12-IMcC	-2.93	-3.55	-3.37	3.3	1.9	3.70
13-DB	-2.95	-2.96	-2.82	8.7	0.9	1.20
14-MMcG	-4.47	-4.11	-4.02	2.6	3.5	0.90
Mean	-3.30	-3.57	-3.35	5.5	2.8	6.07

Table 3.4 Height standard deviation scores for girls with idiopathic growth hormone deficiency related to their annual growth velocities (AGV) during the period of review.

	Height SDS.			AGV. Cm/Y.		
Patients	Apr/85	Jan/86	Jan/87	Apr/85	Jan/86	Jan/87
1-SS	-3.80	-4.25	-2.66	1.50	3.87	13.4
2-CS	-1.32	-1.61	-1.29	7.70	4.00	6.60
3-LB	-3.16	-3.79	-2.76	10.10	2.10	7.80
4-TG	-1.62	-2.14	-1.83	7.60	1.10	7.20
5-AQ	-4.95	-4.15	-3.20	1.90	7.10	5.70
Mean	-2.77	-3.19	-2.35	5.76	3.60	8.14

It is now convenient to separate these two classes.

In Tables 3.5 and 3.6 are data for those children whose annual growth velocities were sufficiently good on restarting recombinant growth hormone to improve their 1987 height SDS's beyond those of April 1985. The respective mean AGVs for boys (Table 3.5) are 6.19, 2.98 and 6.40 cm per year, with the mean height SDS's improving from -3.42 to -3.17 (an improvement of +0.25). For the girls in Table 3.6 the corresponding values are 5.3, 4.27 and 8.4 cm/year, with height standard deviation scores moving from a mean of -3.31 to -2.48 (gains +0.83). In Table 3.7 are the corresponding data for those children who fared less well. Note that their mean annual growth velocities, having fallen from 5.00 cm/year to 2.35 cm/year during the withdrawal period, seemed to increase slightly during the period of recombinant growth hormone treatment to 5.89 cm/year i.e above the April 1985 level, but this was not sufficient to improve their height SDS's of -3.34 (Jan 1987) compared with -2.93 in April 1985 (loss of -0.41). The important feature between these good and poor responding groups is that the loss of linear growth was greater during the withdrawal period in those whose progress fared less well (cf Table 3.5 (2.98 cm/year) and Table 3.6, (4.27 cm/year) with 2.35 cm/year in Table 3.7.

I will now present data for the plasma somatostatin concentrations in these two groups of patients. Firstly however the reader is reminded that in the healthy paediatric subjects the plasma concentrations of somatostatin are inversely correlated with chronological age. The regression line for plasma somatostatin concentrations in 89 normal subjects is shown in Figure 3.1. The age range is from 2.16 to 20 years.

The reader is reminded that the plasma somatostatin SDS is :-

The Patient's plasma SRIF value - the mean for age / 1 SD.

Table 3.5 Height standard deviation scores related to the annual growth velocities (AGV) for boys with idiopathic growth hormone deficiency who had improved their height standard deviation scores during the period of review.

	AGV Cm/Year			Height SDS		
Patients	Pre 4/85	1/86	1/87	4/85	1/86	1/87
1-AB	8.50	4.00	10.00	-4.51	-4.87	-3.74
3-AG	2.40	3.20	8.50	-3.68	-3.69	-3.07
4-ALB	10.00	2.70	6.20	-3.54	-3.61	-3.40
6-CMcG	5.00	3.30	10.50	-2.43	-2.64	-2.24
7-RH	3.60	2.00	8.00	-3.25	-3.60	-3.49
8-CG	8.70	4.20	5.70	-2.56	-2.77	-2.56
13-DB	8.70	0.90	1.20	-2.95	-2.96	-2.82
14-MMcG	2.60	3.50	0.90	-4.47	-4.11	-4.02
Mean	6.19	2.98	6.40	-3.42	-3.53	-3.17

Table 3.6 The Annual growth velocities of the girls with idiopathic growth hormone deficiency (IGHD) who at the end of the review period had improved their height SDS.

	AGV cm/Year			Height SDS		
Patients	Pre/85	1/1986	1/1987	4/1985	1/1986	1/1987
1 . SS	1.50	3.87	13.40	-3.80	-4.25	-2.66
2 . CS	7.70	4.00	6.60	-1.32	-1.61	-1.29
3 . LB	10.10	2.10	7.80	-3.16	-3.79	-2.76
5 . AQ	1.90	7.10	5.70	-4.95	-4.15	-3.20
MEAN	5.30	4.27	8.40	-3.31	-3.45	-2.48

Table 3.7 Height standard deviation scores related to annual growth velocities (AGV) in those children with idiopathic growth hormone deficiency who fared less well during the period of review.

	Height SDS.			AGV. Cm/Y.		
Patients	Apr/85	Jan/86	Jan/87	Apr/85	Jan/86	Jan/87
2-JC	-2.47	-2.99	-2.51	5.10	4.13	5.00
5-NMcQ	-3.63	-3.95	-3.96	5.60	0.80	6.00
9-NC	-1.46	-1.75	-1.49	5.60	4.00	7.90
10-SS	-3.87	-4.48	-4.80	6.10	1.70	7.20
11-BL	-4.51	-5.10	-5.41	1.80	2.80	4.20
12-IMcC	-2.93	-3.55	-3.37	3.30	1.90	3.70
4-TG(F)	-1.62	-2.14	-1.83	7.60	1.10	7.20
Mean	-2.93	-3.42	-3.34	5.00	2.35	5.89

It will be remembered when thinking about standard deviation scores that ±2.5 SDS ranges from above the 97th centile to below the 3rd centile. Abnormal plasma somatostatin concentrations will therefore be associated with SDS's = +2.5 and more. The actual plasma somatostatin concentrations together with their SDS's in my cohort of children are shown in Table 3.8 for the good responders and Table 3.9 for the poor responders.

In this light consider the data in Table 3.10 (good responders) where the now familiar annual growth velocities for the three periods of the survey are shown with the plasma somatostatin SDS's for the same three periods. It will be noticed that at no time were the plasma somatostatin concentrations outside the normal range (except LB"F" during 1986). Perhaps during the withdrawal periods there were slight but insignificant increases in the plasma concentrations as reflected by the SDS's.

A vastly different picture is shown by those patients whose linear growth did less well during the withdrawal period. These data are in Table 3.11. Notice firstly that the mean annual growth velocity fell markedly from 5.00 cm/year to 2.34 cm/year during the withdrawal period. For the same period the mean plasma somatostatin SDS's increased slightly from +2.32 to +2.76 before falling again to +2.24 after recommencement with recombinant growth hormone. The SDS of +2.76 is a significantly abnormal level. The means in Table 3.11 are for boys with data for one single girl added.

At this stage it is worthwhile posing some questions:

1). Do the differences in plasma somatostatin separate two groups of patients?

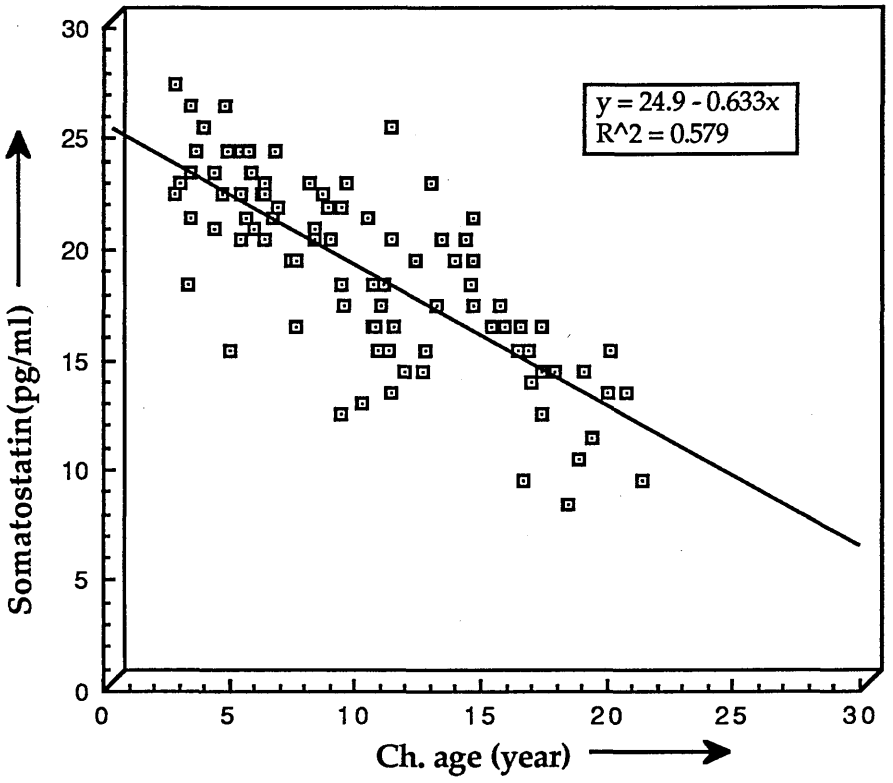


Figure 3.1 Regression line between plasma somatostatin and chronological age for all the normal group.

Table 3.8 Actual plasma somatostatin concentrations (pg/ml), and their standard deviation scores for those children with idiopathic growth hormone deficiency who fared well during the period of review.

	April 1985		Jan 1986		Jan 1987	
Patient	SRIF conc.	SDS	SRIF conc.	SDS	SRIF conc.	SDS
1-AB	21	- 0.67	25	+0.80	19	- 1.01
3-AG	25	+2.38	18	+0.17	16	- 0.30
4-AlB	20	+0.80	21	+1.31	23	+2.18
6-CMcM	23	+2.11	21	+1.57	19	+1.11
7-RH	17	+0.10	18	+0.57	15	- 0.23
8-CG	16	- 0.10	18	+0.70	17	+0.57
13-DB	15	+0.17	16	- 0.67	09	- 1.34
14-MMcG	16	+0.57	17	+1.04	12	+0.25
1-SS(F)	23	+0.57	27	+2.37	21	+0.38
2-CS(F)	20	- 0.38	21	+0.23	24	+1.60
3-LB(F)	24	+1.14	28	+2.90	24	+1.64
5-AQ(F)	19	+1.56	15	+0.27	16	+0.92
Mean		+0.69		+0.94		+0.48

Table 3.9 Actual plasma somatostatin concentrations (pg/ml), and their standard deviation scores for those children with idiopathic growth hormone deficiency who fared less well during the period of review.

	April 1985		Jan 1986		Jan 1987	
Patient	SRIF conc.	SDS	SRIF conc.	SDS	SRIF conc.	SDS
2-JC	25	+2.08	24	+1.88	22	+1.41
5-NMcG	25	+2.71	27	+3.52	24	+2.71
9-NC	18	+0.60	21	+1.78	17	+0.64
10-SS	24	+2.68	26	+3.48	23	+2.68
11-BL	26	+3.52	27	+3.99	24	+3.18
12IMcC	22	+2.24	21	+2.04	23	+2.91
4-TG(F)	25	+2.44	25	+2.63	23	+2.14
Mean		+2.32		+2.76		+2.24

Table 3.10 Plasma Somatostatin concentrations expressed as standard deviation scores and annular growth velocities in patients with idiopathic growth hormone deficiency who fared well during the period of review.

	AGV Cm/Year			SRIF SDS		
Patients	Apr/85	Jan/86	Jan/87	Apr/85	Jan/86	Jan/87
1-AB	8.50	4.00	10.00	-0.67	+0.80	-1.01
3-AG	2.40	3.20	8.50	+2.38	+0.17	-0.30
4-AlB	10.00	2.66	6.20	+0.80	+1.31	+2.18
6-MMcG	5.00	3.30	10.50	+2.11	+1.57	+1.11
7-RH	3.60	2.00	8.00	+0.10	+0.57	-0.23
8-CG	8.70	4.20	5.70	-0.10	+0.70	+0.57
13-DB	8.70	0.93	1.20	+0.17	-0.67	-1.34
14-NMcG	2.60	3.50	0.90	+0.57	+1.04	+0.25
1-SS	1.50	3.87	13.40	+0.57	+2.37	+0.38
2-CS	7.70	4.00	6.60	-0.38	+0.23	+1.60
3-LB	10.10	2.10	7.80	+1.14	+2.90	+1.64
5-AQ	1.90	7.10	5.70	+1.56	+0.27	+0.92
Mean				+0.69	+0.94	+0.48

Table 3.11 Plasma Somatostatin concentrations expressed as standard deviation scores and annular growth velocities in patients with idiopathic growth hormone deficiency who fared less well during the period of review.

	AGV Cm/Year			SRIF SDS		
Patients	Apr/85	Jan/86	Jan/87	Apr/85	Jan/86	Jan/87
2-JC	5.10	4.13	5.00	+2.08	+1.88	+1.41
5-NMcG	5.60	0.80	6.00	+2.71	+3.52	+2.71
9-NC	5.60	4.00	7.90	+0.60	+1.78	+0.64
10-SS	6.10	1.70	7.20	+2.68	+3.48	+2.68
11-BL	1.80	2.80	4.20	+3.52	+3.99	+3.18
12IMcC	3.30	1.90	3.70	+2.24	+2.04	+2.91
4-TG(F)	7.60	1.10	7.20	+2.44	+2.63	+2.14
Mean	5.00	2.34	6.00	+2.32	+2.76	+2.24

In other words are there two groups of children with growth hormone deficiency i.e good and poor responders to therapeutic growth hormone administration? or could it be that the division is into those with neurosecretory growth hormone deficiency and those with pituitary failure of growth hormone synthesis?

2) Are there two groups of people, one with normally low plasma somatostatin and another one with high plasma level (cf. hypo and hyper-cholesterolaemia)? If growth failure for either reason develops do those with high plasma somatostatin concentrations grow less well than those with low concentrations, or do they require a higher replacement dosage of growth hormone?

3) Could it be that high plasma somatostatin concentrations reflect high concentrations at the pituitary gland thus impeding growth hormone release? Other possibilities come from a fertile mind if perhaps imaginative and these will be considered later in discussion.

Somatostatin is recognised as a general inhibitory substance (Table 1.5). In this respect my interest is in the inhibition of absorption of amino acids from the intestines. Can it be shown that in those children who had high plasma concentrations of somatostatin there was an accompanying lowering of plasma amino acid concentrations?

Plasma amino acids in IGHD children

The actual plasma amino acid concentrations for the three periods of this study are shown in Appendix (Tables B.1-9) and the concentrations are expressed in micromoles per litre (umol/L). So as to be able to express these data as SDS's with a view to eliminate an age-dependent factor, I

show in Table 3.12 and Table 3.13 the regression analyses data derived from the normal subjects for the indispensable and dispensable amino acids respectively and also in Figures 3.2 and 3.3 (p. 137-138) are examples of some regression lines showing the relationship between some of the amino acid concentrations and the chronological age. From these the SDS's for the indispensable and dispensable amino acids for the children with IGHD were calculated and are shown in Appendix Tables C 1&2. For convenience and ease of understanding the means of the SDS's for the 'good' and 'poor' responders for the indispensable and dispensable amino acids are shown in Tables 3.14 & 3.15.

It will be seen in Table 3.14 (indispensable amino acids) that all the means are certainly within the 3rd and 97th centiles i.e. being within ±2.5 standard deviations. Nonetheless during the period of no treatment (Jan. 1986), the SDS's had worsened for all amino acids except threonine, indicating that at that time the actual concentrations of the individual amino acids had on average fallen. These changes are statistically significant except for threonine (Table 3.14). If this deficiency in these amino acids is maintained over long periods, then it is understandable that even with maximum utilization there will be a reduction in structures and functions which require a full complement of these indispensable amino acids. A further point of interest in this Table is that for those children who fared less well the means of the SDS's were more negative except for leucine and lysine at Jan. 1986 than for those children who fared well at that point in time. Additionally on restarting recombinant growth hormone the recovery of the plasma amino acid concentrations including leucine and lysine was greater in the 'good' responders than in the 'poor' responders. Thus those poor responding children had at all times lower plasma amino acid concentrations of the indispensable amino acids than the good ones.

Table 3.12 Regression analysis data of the relationship between plasma indispensable amino acids and chronological age for the normal group.

	Valine	Leucine	Isoleucine	Threonine	Lysine	BCAA's
M	0.54	0.51	0.41	0.49	0.30	0.57
r F	0.51	0.55	0.44	0.57	0.30	0.60
M+F	0.55	0.52	0.45	0.52	0.33	0.60
M	0.29	0.26	0.17	0.24	0.09	0.32
r^2 F	0.24	0.30	0.19	0.32	0.09	0.36
M+F	0.30	0.27	0.20	0.27	0.11	0.36
M	168	83.0	41.2	65.9	96.4	292
a F	158	85.9	38.8	62.4	83.6	283
M+F	162	84.8	39.5	64.7	88.3	286
M	3.02	2.39	1.07	1.61	1.32	6.48
b F	3.27	2.36	0.98	2.25	1.55	6.61
M+F	3.27	2.33	1.10	1.83	1.63	6.69
M	24.81	21.54	12.31	15.22	22.57	49.43
SD.Y F	27.82	18.53	10.27	16.68	24.12	44.24
M+F	26.19	20.02	11.40	15.88	23.62	46.74

M = Male
F = Female
BCAS = Sum of branched chain amino acids

Table 3.13 Regression analysis data of the relationship between plasma dispensable amino acids and chronological age for the normal group.

		Alanine	Glycine	Serine	Tyrosine
r	M	0.57	0.00	0.17	0.24
	F	0.63	0.24	0.30	0.42
	M+F	0.61	0.14	0.22	0.35
r^2	M	0.32	0.00	0.03	0.06
	F	0.40	0.06	0.09	0.18
	M+F	0.37	0.00	0.05	0.12
a	M	244	260	111	48.10
	F	238	237	108	42.80
	M+F	240	248	110	45.20
b	M	5.67	0.193	0.790	48.10
	F	5.54	2.093	1.46	42.60
	M+F	5.75	1.100	1.01	45.20
SD.Y	M	44.02	34.96	24.69	0.403
	F	34.18	40.49	24.05	0.738
	M+F	39.51	37.53	24.25	0.588

M = Male

F = Female

BCAS = Sum of branched chain amino acids

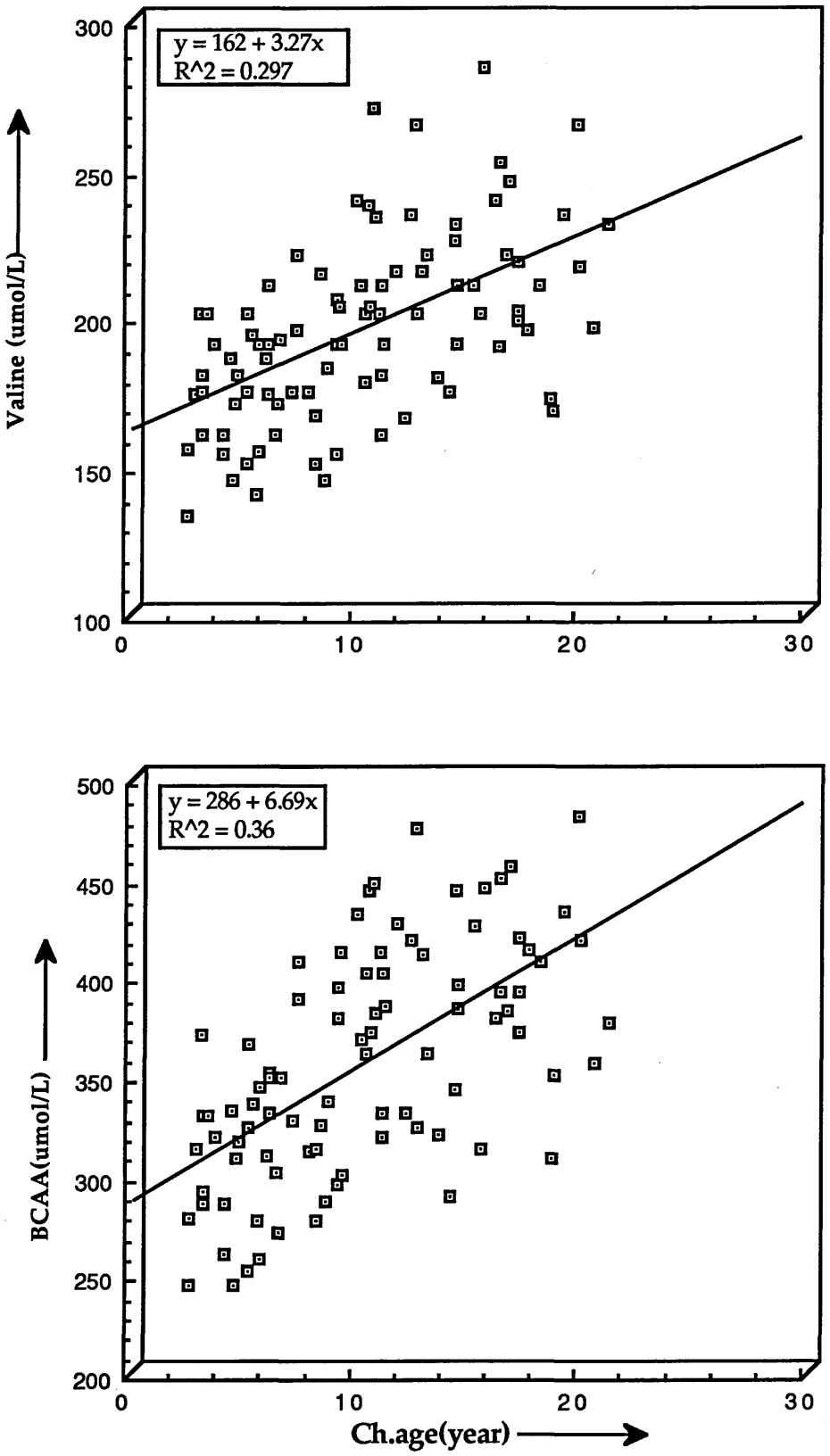
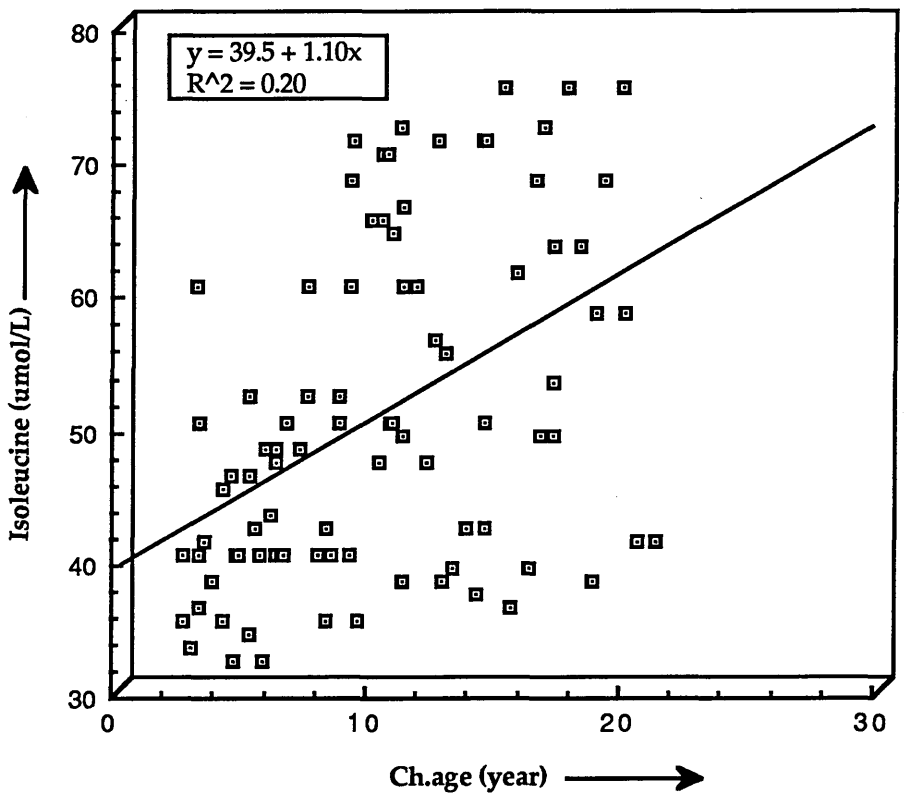


Figure 3.2 Regression line for both BCAA's and valine related to chronological age.



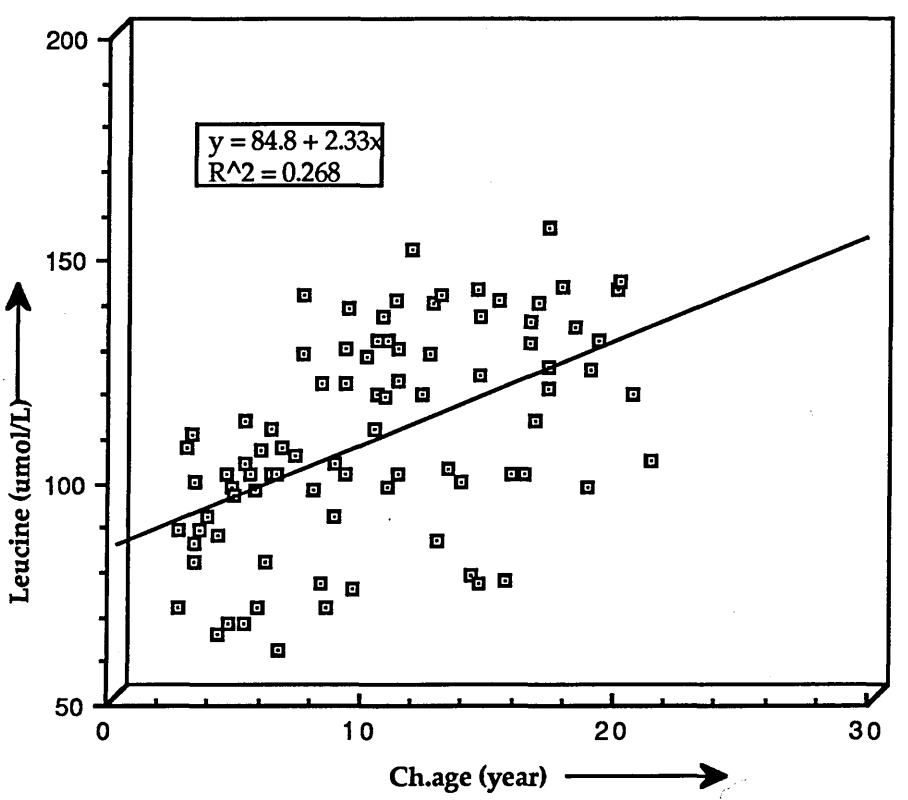


Figure 3.3 Regression line between chronological age and both leucine and isoleucine

Table.3.14 Means of indispensable amino acid plasma concentrations in patients with idiopathic growth hormone deficiency (good and poor responders).

	Valine	Leucine	Isoleucine	Threonine	Lysine	
April/85	+0.09	-0.20	-0.11	-0.34	+0.08	Good
Jan /86	-1.15	-1.08	-0.96	+0.14	-0.83	responders
Jan /87	-0.01	-0.18	+0.25	-0.14	+0.37	
April/85	-0.45	-0.37	-0.18	-0.58	-0.38	Poor
Jan /86	-1.16	-0.85	-1.19	-0.20	-0.61	responders
Jan /87	-0.19	-0.27	-0.34	-0.39	+0.06	

Table 3.15 Means of SDS's of the dispensable amino acid plasma concentrations in patients with idiopathic growth hormone deficiency (good and poor responders).

	Alanine	Glycine	Serine	Tyrosine	
April/85	-0.05	-0.12	+0.12	+0.30	Good
Jan /86	-0.79	-0.45	-0.40	+0.58	responders
Jan /87	+0.13	-0.17	-0.01	+0.15	
April/85	-0.63	-0.21	-0.30	-0.61	Poor
Jan /86	-0.87	-0.62	-0.45	-0.05	responders
Jan /87	-0.57	-0.09	-0.01	-0.35	

It is of extreme interest to correlate these plasma amino acid concentrations with the corresponding higher plasma somatostatin concentration (cf Table 3.9 and Table 3.14).

The question to which we will address ourselves in discussion is the true or theoretical relationship in terms of cause and effect of plasma somatostatin and plasma indispensable amino acid concentrations. If as we have observed that a rise in plasma somatostatin concentrations is accompanied with a lowering of the plasma indispensable amino acid concentrations, then this could be reflect on body status.

We will now consider the SDS's of the plasma concentrations of the dispensable amino acids in both groups. It will be seen from Table 3.15 which brings together from Appendix Tables D 1&2. the means of the SDS's for these amino acid plasma concentrations, that firstly the concentrations of these amino acids was higher in the 'good' responders during the three periods of review than in the 'poor' responders, except for glycine and serine at Jan. 1987. Secondly on withdrawal of growth hormone the concentrations in both groups fell except for tyrosine whose plasma concentrations increased. On recommencing growth hormone all the dispensable amino acids rose from the lower 1986 value with exception of tyrosine which actually decreased. An attempt to explain this will be given later.

Nutritional Ratios

The reason for selecting the indispensable and the dispensable plasma amino acids above referred to was on their usage by other authors in the field of nutrition to calculate what has become known as nutritional ratios. Four of the nutritional ratios which have been accorded significance are:

Ratio 1 which relates the total plasma branched chain amino acids to the sum of the plasma concentrations of alanine, glycine and serine.

Ratio 2 relates the total plasma branched chain amino acids to the sum of the plasma concentrations of glycine and serine.

Ratio 3 relates the plasma concentration of valine as an indispensable amino acid to glycine, a dispensable amino acid.

Ratio 4 relates the plasma concentration of tyrosine to that of glycine both of which are dispensable amino acids.

The crude data for this work for both groups (good and bad responders) are contained in the (Appendix Table B 1-9). Hence it will suffice to show the actual nutritional ratios for both groups of patients (good and poor responders) (Appendix Tables E 1-2). The means for these nutritional ratios for the three periods of observation are shown in Table 3.16. It will be seen that the ratios R1 and R2 fell significantly but the fall in R3 was not significant while R4 rose significantly in the good responding group during the period of treatment withdrawal. Following recommencement of growth hormone therapy the recovery of R1, R2 and R3 were highly significant while R4 fell significantly back to the 1985 level.

Table 3.16. shows also the corresponding nutritional ratios for the 'poor responders'. Following the withdrawal of growth hormone only R1 fell significantly, while the falls in R2 and R3 were not significant. Interestingly R4 rose significantly. On resumption of therapy R1, R2 and R3 showed a non-significant rise, while R4 showed a non-significant fall. These changes in the nutritional ratios for the two groups of patients will receive further consideration in discussion.

Table3.16 Means of the nutritional ratios and P values between 1985- 1986 and 1986-1987 for both good and poor responders in children with idiopathic growth hormone deficiency.

	R_1		R_2		R_3		R_4		
	R_1 value	P value	R_2 value	P value	R_3	P value	R_4 value	P value	
1985	0.519	0.004	0.945	0.01	0.787	0.07	0.212	0.04	*Good*
1986	0.481	0.0003	0.859	0.00002	0.715	0.004	0.230	0.04	responders
1987	0.541		0.986		0.801		0.211		
1985	0.539	0.011	0.964	0.07	0.768	0.46	0.187	0.04	*Poor*
1986	0.501	0.06	0.907	0.21	0.746	0.16	0.221	0.06	*Respondes*
1987	0.536		0.962		0.796		0.195		

Short stature in patients with cerebral tumours

Not all children with intracranial tumours present with short stature. Neurological signs and symptoms may be the salient features which bring the patients under scrutiny. Certain tumours like craniopharyngioma because of their site of occurrence frequently interrupt the hypothalamo-pituitary axis to give early reduction in growth hormone secretion which results in early evidence of growth failure. In such patients if there are no neurological signs, the child is commonly noted to be small for age and puberty may be delayed. Some degree of thyroid dysfunction may also be indicated. However if the neurological signs appear early with the tumour growth then it might be expected that linear growth need not be much affected. Such was the case with patients F1 (LB) and F5 (AMcC) whose failing visual acuity was noted by the school teachers and early diagnosis made because of optic atrophy.

In this present series of patients with tumours the general rule was no matter the nature of the individual tumour, patients were near normal in height when the presentation was neurological whereas when short stature was marked, neurological signs and symptoms were absent or not part of the original complaint although elicitable on detailed neurological investigation.

The data regarding these patients will be presented in the same style as formerly since the reader will now be familiar with the deduction theory.

In Appendix Tables F.1&2 are the crude data for all the patients with cerebral tumours, male and female. It is immediately noted that at the time of cadaveric growth hormone withdrawal all but one child "F1" (LB)

were in the pubertal age group chronologically. The maximum number of years of treatment was 8.16 years (range 1.00-8.16 years) post-operatively.

In Table 3.17 are grouped those patients whose height SDS for chronological age were improved at the period of last review when compared with their heights on withdrawal of cadaveric growth hormone. Table 3.18 shows the height SDS's for those patients who did less well. Those who improved their growth status on resumption of treatment with recombinant growth hormone did so by a mean for the group of +0.53 SD's while the others (Table 3.18) lost ground by a mean for the group of -0.82 SD's.

It is of interest that those who improved their linear growth, all except Patient F4 (SD) (Appendix Table F.2) had craniopharyngioma. Thus it might be concluded that in terms of height for chronological age and post-operative improvement with growth hormone therapy, the patients with craniopharyngioma improved on any height deficit present at the time of diagnosis. Scrutiny of these two Tables 3.17 & 18 shows that six patients out of seven who improved on their height status had a cranio-pharyngioma while only one out of six who responded poorly to growth hormone treatment had a craniopharyngioma. Note the good increase in height (+2.08) of patient F5 (AMcC) (Table 3.17 and Appendix Table F.2).

Plasma Somatostatin concentrations (Tumours)

Crude data for these plasma somatostatin concentrations are in Appendix Tables G.1 & 2 and Table 3.19 shows the SDS's for plasma somatostatin concentrations in relation to the annual growth velocities in those patients who improved their height status on treatment. All had normal plasma somatostatin concentrations except Patient F4 (SD) whose plasma value of

Table 3.17 The annual growth velocities related to height standard deviation scores for children with cerebral tumours who fared well to growth hormone treatment during the period of review.

	AGV cm/Year			Height SDS		
Patients	April/85	Jan/86	Jan/87	April/85	Jan/86	Jan/87
1.DH	10.6	7.6	6.7	-0.69	-0.64	-0.68
6.JMcD	5.8	2.1	2.6	-2.25	-2.10	-1.76
7.KT	4.2	1.6	6.5	-2.37	-2.21	-1.23
3.CL'F'	4.5	1.7	4.3	-3.12	-3.46	-3.07
4.SD'F'	4.0	3.2	3.2	-4.36	-4.30	-3.91
5.AMcC'F'	8.1	1.7	6.8	+0.73	+0.95	+2.08
6,PC'F'	4.0	1.3	0.00	-1.70	-1.53	-1.53
Mean	5.9	2.7	4.3	-1.97	-1.90	-1.44

*All the patients had craniopharyngioma except "F4" who had medullo-blastoma.

Table 3.18 The annual growth velocities related to height standard deviation scores for children with cerebral tumours who fared less well to growth hormone treatment during the period of review.

	AGV cm/year			Ht SDS		
Patients	April/85	Jan/86	Jan/87	April/85	Jan/86	Jan/87
2.DB	7	2.7	2,8	-2.25	-2.74	-3.44
3.MM	5	2.1	1.8	-1.76	-2.30	-3.01
4.PC	12	5.7	6	-3.00	-3.16	-3.27
5.TR	4.8	2.5	5.2	-2.09	-2.59	-2.50
1.LB'F'	3	2.3	3	+0.25	-0.78	-0.68
2.AMcE'F'	3.1	2.3	6.3	-3.70	-4.42	-4.57
Mean	5.8	2.9	4.2	-2.09	-2.67	-2.91

somatostatin concentration was exceeding the normally accepted high concentration of +2.5 SD in 1985 and 1987. Interestingly she also had the worst height standard deviation scores, (Table 3.17) being -4.36 at April 1985 and -4.30 at January 1986 and rising to -3.91 at January 1987 but still with markedly abnormal low height standard deviation score. Could it be that this girl was going to be of short stature because of her high plasma somatostatin concentration and that her tumour played little part in the pathology of her short stature? She was already very short statured on initial presentation despite her presenting features being neurological and hence coming early under scrutiny.

The plasma somatostatin concentrations were all within the normal range in those patients who responded poorly to growth hormone treatment (Table 3.20). It is difficult to draw many conclusions from a study of plasma somatostatin concentrations in this group of patients with tumours for their growth hormone deficiency was iatrogenic and not endogenous. Finally relative to this subject it is worth while sighting again the pathology data contained in Appendix Table F. 1&2 which will be dealt with later in the Chapter 4.

Plasma amino acid concentrations

Indispensable amino acids

While we have noted that in these patients with cerebral tumours there were no significant fluctuations in plasma somatostatin concentrations due to the temporary withdrawal of growth hormone therapy, a study of their actual plasma amino acid concentrations for both 'good' and 'poor' responders are shown in Appendix Table H. 1-6. The calculated SDS's for these indispensable amino acids are shown in Appendix Tables I.1 & 2.

Table 3.19 The annual growth velocities related to plasma somatostatin standard deviation scores for children with cerebral tumours who responded well to growth hormone therapy during the period of review.

	AGV cm/year			SRIF SDS		
Patients	April/85	Jan/86	Jan/87	April/85	Jan/96	Jan/87
1.DH	10.6	7.6	6.7	-0.57	-1.07	-0.57
6.JMcD	5.8	2.1	2.6	+0.50	-0.03	-0.84
7.KT	4.2	1.6	6.5	-1.27	+0.20	-0.60
3.CL'F'	4.5	1.7	4.3	+0.76	+0.53	+1.56
4.SD'F'	4.0	3.2	3.2	+2.82	+1.49	+2.86
5.AMcC'F'	8.1	1.7	6.8	-1.34	+0.24	-0.46
6.PC'F'	4.0	1.3	000	-0.57	000	-1.44
Mean	5.9	2.7	4.3	+0.05	+0.22	+0.07

Table 3.20 The annual growth velocities related to plasma somatostatin standard deviation scores for children with cerebral tumours who responded less well to growth hormone therapy during the period of review.

	AGV cm/year			SRIF SDS		
Patients	April/85	Jan/86	Jan/87	April/85	Jan/86	Jan/87
2.DB	7.0	2.7	2.8	-0.37	+0.13	-0.34
3.MM	5.0	2.1	1.8	+0.64	+1.14	+0.67
4.PC	12	5.7	6.0	+1.11	+0.87	+1.74
5.TR	4.8	2.5	5.2	-1.21	-1.71	-0.50
1.LB'F'	3.0	2.3	3.0	+1.53	+1.98	+1.60
2.AMcE	3.1	2.3	6.3	+1.07	-1.27	+0.76
Mean	5.8	2.9	4.2	+0.46	+0.36	+0.67

For convenience the means of these standard deviation scores are shown in Table 3.21.

The interesting but not unexpected point emerges that all plasma concentrations except for threonine in the 'good responders' fell when growth hormone was withdrawn and they rose again when treatment was restarted. In Table 3.21 all the changing values throughout the three observation periods are statistically significant except where indicated (NS).

Dispensable amino acids

The actual plasma concentrations of these amino acids found in these 'tumour patients' are as has been mentioned before in Appendix Tables H1-6. From these data the standard deviation scores for the plasma concentrations have been calculated and are shown in Appendix Tables J.1 & 2 for both good and bad responders. The means for these standard deviation scores are brought together in Table 3.22. It will be noted from this Table that uniformly except for tyrosine in the 'good responders', that the plasma concentrations as reflected by their SDS's fell on withdrawal of growth hormone but rose again on recommencement of treatment. In those poor responders tyrosine concentrations continued to fall even after restarting the recombinant growth hormone.

Nutritional Ratios

The nutritional ratios for both 'good and poor responders' are shown in Appendix Table K. 1 & 2. From these data Table 3.23 has been constructed which shows the means of the nutritional ratios together with P values for the two groups of patients during the three periods of review. In the "good responding" group the fall in nutritional ratios for R1, R2 and R3 on withdrawal of growth hormone treatment were statistically significant; on

the other hand R4 increased during that period although the rise was not significant. The improvement (rise) in the nutritional ratios following the recommencement of growth hormone therapy was significant for R1 and R2 but not significant for R3, while R4 fell again with recommencement of treatment although the fall was not significant.

The corresponding values found for the 'poor responders' again indicated that on withdrawal of therapy the falls in R1, R2 and R3 were significant while R4 showed a rise but that rise was not significant. Following the resumption of treatment the ratios R1, R2 and R3 rose but the rise was not significant, while R4 fell which fall also was not significant.

The relevance of these trends in the nutritional ratios as calculated from the plasma amino acid concentrations will be discussed in Chapter 4.

Table.3. 21 Means of SDS's for indispensable amino acids plasma concentrations in patients with cerebral tumours (good and poor responders).

	Valine	Leucine	Isoleucine	Threonine	Lysine	
April/85	+0.06	+0.15	+0.24	-0.13	-0.16	
Jan /86	-1.18	-1.11	-0.92	+0.12 "ns"	-0.76 "ns"	Good
Jan /87	-0.1	+0.05	+0.71	+0.45 "ns"	+0.21	Responders
April/85	0.00	+0.22	+0.69	+0.21	+0.09	Poor
Jan /86	-1.16 "ns"	-0.88	-0.83	-0.01 "ns"	-0.84	Responders
Jan /87	-0.23	000	+0.40	+0.16 "ns"	-0.12	

Table.3.22 Means of SDS's for dispensable amino acids plasma concentrations in patients with cerebral tumours (good and poor responders).

	Alanine	Glycine	Serine	Tyrosine	
April/85	-0.03	-0.25	-0.21	+0.52	Good
Jan /86	-0.44	-0.82	-0.45	+0.58	Responders
Jan /87	-0.31	-0.21	+0.26	+0.38	
April/85	+0.20	+0.06	+0.09	+0.53	Poor
Jan /86	-0.91	-0.59	-0.34	+0.28	Responders
Jan /87	-0.18	+0.38	+0.46	-0.12	

Table3-23 Means of the nutritional ratios and P values between 1985-1986 and 1986-1987 for both good and bad responders in children with cerebral tumours.

	R_1		R_2		R_3		R_4		
	R_1 value	P value	R_2 value	P value	R_3	P value	R_4 value	P value	*Good*
1985	0.559	0.003	1.045	0.013	0.830	0.034	0.230	0.17	*Respondes*
1986	0.496	0.008	0.932	0.01	0.775	0.14	0.252	0.11	
1987	0.567		1.032		0.818		0.223		
1985	0.548	0.017	1.001	0.007	0.782	0.04	0.218	0.768	*Poor*
1986	0.498	0.16	0.886	0.28	0.715	53	0.225	0.16	*Respondes*
1987	0.529		0.941		0.739		0.190		

CHAPTER FOUR

Before discussing in some detail the results of the investigations as presented in the Tables of Chapter 3, I will revert to some topics which arose in the earlier Chapters.

The Creutzfeldt-Jacob incident in the history of growth hormone as therapy for children with short stature, may have been a fortunate event in that it may have accelerated the introduction of recombinant growth hormone. However to date I have not read incontrovertible proof that those children who allegedly developed the spongiform degenerative neurological disease have produced evidence of an anti-viral titre to the noxious agent nor has that noxious agent been identified. If cows had observed the affected patients would they have labelled them as having 'mad humans' disease ? After all mad cows' disease is also a spongiform degenerative neurological disease and so is scrapie in sheep. It may be in time to come there will be a unifying pathology to account for the various expressions of inexplicable neurological disease which we recognize in children. An example of such unification is the link-up of Letterer-Siwe disease, Hand-Schuller-Christian disease and eosinophilic granuloma of bone. Now these three are regarded as variants of malignant reticuloendotheliosis. The cow may yet say, 'What fools these mortals be ?'

Cadaveric growth hormone of the UK variety was produced in a Cambridge Laboratory associated with that seat of learning. It was given to children by Medical Research Council good intentions but the product was never given a 'contents specification' nor did it ever answer the requirements of the British Standards Institute - not that the product was ever submitted for analyses. However the product is now of historical

interest but with this reserve. The calves born of mothers or presumably fathers with BSE are slaughtered. Can we expect possibly affected children born of parents, one or both of whom received cadaveric growth hormone? Many of these children are now in the marriageable age or in this present era, capable of having and fathering children. Is therefore the monitoring of the British cohort of children who received the now potentially noxious cadaveric growth hormone sufficiently identified at least in some register against future scientific requirements and maybe also legal. This would be better done by the official British Paediatric Association rather than by a Government Department or the British Paediatric Endocrine Society.

The dawn and high noon of the recombinant growth hormone is upon us and with characteristic enthusiasm the endocrinologically orientated clinicians are bent on ' something to observe' - that is to say, give it and see what happens. And if the growth response is not satisfactory, then let us give more - double the dose : a practice detrimental to the coffers of the National Health Service, but advantageous to the pharmaceutical companies which produce it and may be of some value to the patients.

Different Pharmaceutical Companies use different starting cells. Some use a strain of E. coli, others use mouse mammary gland cells. In the final extraction the E. coli bodies have to be ruptured to release the hormone at least but also may be prions, while the mammary cells release the formed growth hormone into the incubation effluent. The extracted product need not be as pure as we would like it to be. Only this, it is unlikely to contain other trophic hormones, a feature of the cadaveric product. Those Firms which use E. coli reassure us that E. coli are free from virus affection. However, even at cellular level there is naught for naught. The bacteria

and the mouse mammary cells have to receive something in return for their producing growth hormone after the appropriate amino acid sequence is inserted into their plasmids. The Pharmaceutical Companies do not reveal the content of their incubation mixtures and what effects the total process has on individual compounds in the incubation effluent. Perhaps we have to await the occurrence of juvenile or young adult Alzheimer's disease or an illness like Huntington's chorea in a child without a family history. This might produce career material for a neuroendocrinologist.

Another point on which I wish to comment is the number of tests available by which isolated growth hormone deficiency may be diagnosed. Historically, children were diagnosed as having growth hormone deficiency if they did not grow, that is if their annual gains were less than 60 percent of the normal for their age. Added to such an observation was the insulin unresponsiveness test in which the child was given intravenously soluble insulin 0.1 iu per kg body weight and the blood sugar concentration followed over a 2.5 hour period. Coma, convulsions or profound hypoglycaemia not recovering at 2.5 hours was taken as supportive evidence of growth hormone deficiency. These were the days before growth hormone assay was possible. The advent of RIA method for growth hormone assay has not made the definition of growth hormone deficiency any more universly agreed. Firstly, there is no agreement on the degree of hypoglycaemia to be achieved before the test is valid. How then can the response as measured by the subsequent plasma growth hormone levels be compared patient with patient or age group with age group if the glucoprivation stimulus varies from patient to patient? Secondly , there is no great agreement on what minimum plasma growth hormone concentrations which must be achieved to justify the terms

complete and partial growth hormone deficiency. The relevance of this latter diagnosis has even been questioned.

In testing for growth hormone lack, as opposed to testing for other growth factors such as IGF-I & II , GRF and somatostatin, we are really attempting to discover whether or not the problem is in the hypothalamus (neurosecretory) or in the pituitary (synthetic). This distinction is academic, for growth hormone is the current therapy for both conditions. It is true that GRF is available but the present experience is that there is a deteriorating response with time.

However in this work I have been concerned with two groups of patients with growth hormone deficiency namely those whose deficiency was sui generis and the other iatrogenic. My clinical observations strongly suggest that the growth hormone deficiency is the only common feature. There does seem to be a basic "unwillingness ' of those with the deficiency sui generis, to grow whereas those with the deficiency iatrogenic 'want ' to grow. In other words there seems to be indefinable impedimenta in those sui generis patients which are absent in the iatrogenic group.

Finally on growth hormone, we recognize that it has a plurality of functions. I am concerned here with its growth promoting action and its interaction with both amino acids and somatostatin. Its other actions may be given only brief mention.

There are a few points regarding somatostatin on which I wish to retrench or dilate. Somatostatin is very readily destroyed by proteases and hence to minimize this trasylol was added to the blood withdrawn for somatostatin assay. Nonetheless any losses due to proteases must be common to all investigators. But it is surprising that clinical endocrinologists have been

slow to study CSF somatostatin for here is a biological fluid with an exceedingly low concentration of protein and thus eminently suitable for study of its somatostatin content.

The actions of somatostatin are many and varied but in this work my interest is in its possible reciprocal relationship with growth hormone and its direct relationship with plasma amino acid concentrations. But at the pituitary level it does in its main action suppress the release of growth hormone presumably thereby causing less linear growth when in excess. This seems to be the basis for the administration of a somatostatin analogue (SMS 201-995) to patients with acromegaly. Indeed there is a current trial of administering SMS 201-995 to children with tall stature in an attempt to restrict the growth velocity and thus curtail height achievement. (148). Additionally high plasma concentrations of somatostatin are thought to advance bone maturation (149) and to reduce plasma concentrations of IGF-I (150). Since menses in the female is more related to osseous maturation than to chronological age then it will be of interest when large series of tall children treated with somatostatin are published, to observe if the somatostatin induced an earlier onset of puberty. This was the problem of oestrogens for tall girls and an androgenic anabolic hormone for tall boys although many fewer tall boys are offered treatment.

My penultimate statement on somatostatin here concerns the finding that infused somatostatin causes an immediate fall in plasma growth hormone concentration followed by a rebound peak. Patients with either neurosecretory or synthetic growth hormone deficiency would not show this phenomenon. If however a short statured child in whom growth hormone deficiency were suspected, were firstly tested with the 1-hour

GRF test and did not respond positively then it could be concluded that there was a lack of preformed growth hormone in the pituitary. No further test need be done and growth hormone replacement would be indicated. If however there was an increase in the plasma growth hormone concentration following the GRF test then somatostatin could be infused and the expected fall followed be a rise in the plasma growth hormone concentration could be sought. A failure to observe the phenomenon in a child known to respond to GRF would indicate that the original growth hormone deficiency is due to a hypothalamic cause.

The readers of this thesis are respectfully requested to consider this suggestion as 'copyright' for a period of 3 years until I have had an opportunity to investigate the idea in my own hospital in Tripoli, Libya.

Finally there is work going on in the veterinary field (151)whereby sheep are immunized against their own somatostatin. This has resulted in a more rapid increase in carcase mass and height. Were this work to be extended to children with short stature then it must be essentially reserved for those children in whom is demonstrated pathologically high plasma concentrations of somatostatin .

Simple comment but interesting enough are the findings that somatostatin inhibits the absorption of xylose and that in patients with cystic fibrosis there is a relative increase in the somatostatin-producing tissue in the pancreas in both diabetic and non-diabetic patients (152). I might hazard a statement on the effects of this latter finding. If this histochemically identified increase in somatostatin producing cells are active then the increased plasma somatostatin concentrations in patients with cystic fibrosis can do nothing but worsen their clinical status for insulin, chymotrypsin and trypsin are all inhibited by somatostatin and so

is the gut absorption of nutrient. But the delay in gut motility and hence the onward passage of nutrient through the gut may provide a longer period for gut absorption to make good the enzymic deficiency.

On the subject of plasma amino acids I wish to highlight if perhaps reiterate some points . The indispensable amino acids lysine and threonine are the only amino acids which are truly nutritionally indispensable for they cannot be replaced like other indispensable amino acids by their α–keto analogues. The branched chain amino acids are like wise important for they are not significantly metabolized in the liver. Of the total amino acid nitrogen in the hepatic effluent blood after a meal 70 percent is due to the branched chain amino acids. These amino acids are metabolized and degraded mainly in muscle, kidney and even in the brain tissue. Leucine on the other hand is important in that it is a regulator of protein turn over, low plasma concentrations stimulating protein synthesis while high concentration inhibiting catabolism of tissue proteins. The metabolism of these branched chain amino acids in muscles results in alanine which is returned to the liver as a glucogenic amino acid. It might be expected that when children receive growth hormone as therapy that the expected rise in plasma amino acids would be in favour of the branched chain amino acids as well as of lysine and threonine and that if these are concurrently being built into muscle then there might be an increase in the circulating alanine.

Now regarding the cohort of patients studied in this work, 32 were chosen from a total of 40 patients. The reason for this selection was that the 8 patients excluded were from irresponsible families in which either a single parent mother who was out working and the child cared for by an irresponsible grandmother, or a single parent father who was totally

inadequate for the task. Others of the 8 children just had unreliable parents as judged by the number of non-attendances at the growth clinic and by the long periods of time between each request for a further supply of growth hormone. It was felt that these 8 children would not be wholly representative of good treatment and anthropometric measurements from them, if included would certainly skew the data adversely.

We now come to the interesting consideration of the data provided in the several Tables of Chapter 3. Let the reader be reminded that almost throughout, standard deviation scores were used to express the deviation from the mean or 50th. centile which existed for each patient whether with reference to height , plasma somatostatin or plasma amino acid concentrations. This system was used to eliminate the variations due to the age factor. Further while equating patient data with the normal mean or 50th. centile I have also used each patient as his own mean as it were as he moves with time in relationship to the events taking place at each time interval.

Table 3.1 containing data for boys with IGHD shows how each patient's height deviated from the normal mean over the 1.75 years of the survey. Each patient, following a nine month period without growth hormone treatment fell in height further from his mean for chronological age (except Patient 14- MMcG who had a better growth rate according to his chronological age at that time) through the 'off' period. In so far as the height SDS's for January 1986 are not worsened by a factor of -1.0, these children therefore did grow but their growth velocity was not sufficient to maintain their 'treatment' height SDS's of April, 1985. The seriousness of this is that when treatment with growth hormone was restarted, then catchup growth had to make good what had not been achieved during the

period without treatment and in addition to promote further increments representing growth for the second treatment year (to January 1987.). This was not satisfactorily achieved so that several of the children were even shorter at the end of the survey period that is to say- a more negative SDS at January 1987 than at April 1985 with reference to the 50th. centile for their chronological age at January 1987. Taking the group as a whole it will be seen that the mean height SDS of -3.3 at April 1985 after a dip to -3.57 at January 1986, did not achieve the April 1985 score and was only -3.35, that is to say an overall worsening.

In Table 3.2 are similar data for 5 girls with IGHD. Here it will be observed again that the mean height SDS at January 1986 had worsened again not by a factor of -1.0 but less and the recovery of the mean height SDS at January 1987 was greater than 1.0 in Patients 1(SS) and 3 (LB). However the mean SDS's for these 5 girls improved during the 1.75 years of observation by +0.62 SD's . This is very unimpressive but salutary.

If we now consider the nett changes in annual growth velocities relative to the changes in the height SDS's, then in Table 3.3 for males and Table 3.4 for females it will be seen that during the "off" growth hormone period their annual growth velocities were on average less than 4 cm (ie. at January 1986) but that on restarting growth hormone the annual growth velocities exceeded 6 cm per year. The mean for each group was 6.07 cm per year for boys and 8.14 cm per year for girls. But the important point is that for the period April 1985 to January 1987 the mean annual growth velocity for boys was 4.44 cm per year and for girls 5.87 cm per year. Thus the girls seemed to gain more than did boys but neither achieved the mean full annual growth velocity over the withdrawal /restart period.

Another point to be derived from these two Tables is that the mean annual growth velocities for the first year of the recombinant growth hormone treatment, as well as exceeding those of the withdrawal period where also greater than the last year of cadaveric growth hormone therapy. Two meanings could be derived from this observation. Firstly it could be claimed that recombinant growth hormone is a better stimulus to linear growth than was the cadaveric growth hormone. Pharmaceutical companies producing the new product would be delighted to think that this was entirely so. But it is recognised that the annual growth velocity is usually greater in the first year of treatment and it could be considered that after nine months without treatment , the patients were as though they had not been treated at all. Also the last year of cadaveric growth hormone treatment was for several the nth. year of treatment in which the annual growth velocity was not expected at any rate to be maximal.

The reader will have observed from the foregoing Tables that some children responded better than others. Now consider the "good" and the 'poor' responders. These two groups are collected firstly into Tables 3.5 and 3.6 as the good responding boys and girls. From Table 3.5 it will immediately be appreciated that the height SDS's at January 1987 were improved when compared with the corresponding height SDS's at April 1985. The means were -3.17 for January 1987 compared with -3.42 at April 1985. This value of -3.42 at April 1985 fell to -3.53 at January 1986. Thus the improvement in height SDS from January 1986 to January 1987 was by +0.36 SD's. Thus there was an improvement in height when compared to the appropriate 50th centile for age of +0.25 SD's. Bearing in mind that these boys all had lost ground during the "off" period the new treatment was expected to make good these losses and at the same time to continue

to produce as normal as possible a growth velocity for the first year of treatment with recombinant growth hormone.

In Table 3.6 are the corresponding data for the girls who did well. The mean height SDS's for the two periods are -3.31 (April 1985) and -2.48 (January 1987) which represent a better improvement of +0.83 SD's when compared with that for the boys of +0.25 SD's at the same period. These girls also showed a worsening of their height data to a mean of -3.45 i.e. a fall of -0.14 SD's thus the improvement between January 1986 and January 1987 was +0.97 SD's. This is even a better response than that for the boys of +0.36 SD's.

When we consider the actual losses and gains in terms of the annual growth velocities then the"good" responding boys only achieved a mean of 2.98 cm per year during the "off" period and this must be attributed to the lack of administered growth hormone. When growth hormone was reintroduced the annual growth velocity improved to a mean of 6.4 cm per year. Thus over the 1.75 years i.e. April 1985 to January 1987, the mean annual growth velocity was 4.69 cm per year which is just above the 60 per cent level recognised as representing significant growth failure. The efficacy of the recombinant growth hormone may be judged by its ability to correct a reduced annual growth velocity and to restore it to normal. While this was achieved during the one year of treatment the deficit sustained during the "off" period was responsible for the continuing negative SDS's.

A similar pattern of annual growth velocity is seen in Table 3.6 (good responding girls). A slight fall at January 1986 to 4.27 cm per year was restored to 8.40 cm per year at January 1987 due to the restarting of recombinant growth hormone. Interestingly here the mean annual

growth velocity for the 1.75 years of the "off/on" period was 6.34 cm per year which clearly is a very good response. This is reflected in the marked improved height SDS of -2.48 at January 1987 representing an improvement of +0.97 SD's.

We now consider the status of these "poor" responders whose data are in Table 3.7. Firstly note that their mean height SDS fell from -2.93 at April 1985 to -3.42 at January 1986 and rose to -3.34 at January 1987. Even so, the mean height SDS at January 1987 was worse than that at April 1985 by a mean SDS of -0.41. Thus this group lost in height a SDS of -0.49 through lack of growth hormone therapy and on recommencement of therapy only gained +0.08 SD's. In summary then these "poor" responders lost more through lack of growth hormone and gained less after restarting than did either the "good" group of patients (boys and girls).

When we look at their annual growth velocities during the 'off' period the means were 2.35 cm per year rising to 5.89 cm per year, thus averaging 4.12 cm per year over the 1.75 year period. This is almost on the 25th. centile for growth velocity and is typical of growth hormone deficiency continuing. Maybe in this group of patients an increased schedule of growth hormone therapy is required or maybe there are other impeding factors to linear growth which if removed or counteracted would permit improved linear growth in response to normal treatment.

Let us now consider the plasma concentrations of somatostatin in these two groups of patients. These data are in Tables 3.8 & 3.9 where the real plasma concentrations of somatostatin are shown for each child with the appropriate SRIF SDS's. Immediately it will be seen in Table 3.8 that no child had an abnormally high plasma concentration of somatostatin at any of the three periods of the survey except Patient 3 LB (girl) at January 1986

ie at the end of the 'off' period which normalised on recommencement of growth hormone therapy. When one considers the mean SDS's for the plasma concentrations of somatostatin for these three periods on/off/on growth hormone, they were +0.69 at 1985 : +0.94 at 1986 and +0.48 at 1987 i.e normal values well within the +2.5 SD's.

Reference now to Table 3.9 wherein are data for the 'poor 'responders, a vastly different set of findings are seen. Most have much higher plasma concentrations of somatostatin for age and this is reflected in high SRIF SDS's. Several are well above the +2.5 SD level while the means for the three periods, on/off/on growth hormone therapy are +2.32 (1985) ; +2.76 (1986) and +2.24 (1987). This immediately suggests that there are two types of children with short stature, one with high concentrations of plasma somatostatin and the other with normally low concentrations. The question now is whether or not high plasma concentrations of somatostatin impede normal linear growth? If this were so, then the action of the somatostatin need not only be at the pituitary level for these children have already growth hormone deficiency. Is it that the high plasma somatostatin concentrations act at gut level to prevent the absorption of amino acids which themselves are the building bricks for protein metabolism and the provision of substrate on which the linear growth of bone and muscles depend?

Note that during the 'off' period of treatment the mean plasma somatostatin SDS was abnormally high. If this concentration of somatostatin were to act at the pituitary level then there would be a suppression of any releasable growth hormone from the pituitary and if one adverts to Table 3.11 the annual growth velocity for this 'poor 'responding group of children fell to 2.34 cm per year during the 'off

treatment' period. This was the lowest mean annual growth velocity observed. Thus there would be a prima facie case to attribute failure to grow to the high plasma concentrations of somatostatin.

This idea is not altogether fantasy for IGF-I plasma concentrations have been used as a marker to indicate which group of children will grow in response to growth hormone therapy. Combine this with the recent finding (149) that administration of somatostatin analogue (SMS 201995) results in a fall in the plasma concentration of IGF-I then a reduction in linear growth associated with high plasma somatostatin concentrations is easily accepted. Somatostatin analogue when given to acromegalic patients also results in a decrease in the plasma concentration of IGF-I. My finding is that high plasma concentrations of somatostatin are associated with failure to grow in response to growth hormone. The question now is whether or not the patients' plasma somatostatin status is the all important item in this difficult situation.

Interestingly on this score, are the animal experiments (151) which show that immunization against somatostatin resulted in increased weight and height in lambs so immunized and at the human level somatostatin analogue (SMS 201995) has recently been used to control height in tall children (148, 149).

It is easy to suggest that those children with growth hormone deficiency who do not respond well to growth hormone therapy and who have high plasma concentrations of somatostatin should be immunized against somatostatin? There are dangers in such a thought for surely some of the good inhibitory effects of somatostatin are necessary for the body economy. Nonetheless if it were possible to suppress the plasma concentrations of somatostatin in these children in whom the concentrations are high then

an opportunity for increased linear growth would be won. Certain amino acids infused intravenously have been reported to suppress plasma concentrations of somatostatin (153, 154). Herein may be another approach to the treatment of children with short stature. Thus it emerges that the assay of the plasma concentration of somatostatin must be included in the standard investigations of children with short stature when growth hormone deficiency and replacement therapy are contemplated.

But to provide some information on the relationship of plasma somatostatin concentrations and those of plasma amino acids I now present plasma amino acid concentrations for the patients considered in this work.

In Table 3.14 are the mean SDS's for the indispensable amino acids for both the 'good' and 'poor' responders and in Table 3.15 are the corresponding mean values for the dispensable amino acids. From these Tables we note that at no time were the plasma concentrations of either group of amino acids, in either the 'good' or 'poor' responders significantly abnormal. Nonetheless during the 'off' growth hormone period the plasma indispensable amino acid concentrations were reduced in all except for threonine whose plasma concentration increased. A similar trend was noted for the plasma dispensable amino acids in all except for tyrosine which rose during the 'off' period.

On restarting growth hormone, uniformly the concentrations of all the indispensable amino acids rose except for threonine in both 'good' and 'poor' responders. A similar trend is seen for the dispensable amino acids all of which except tyrosine increased in their plasma concentrations. Additional to the above observation it is notable that in the 'poor'

responders these plasma concentrations of amino acids were lower than in the 'good' responders.

Interestingly some of these findings agree with published work. It has been shown (155), before that following a one year period of treatment of IGHD patients with growth hormone, plasma concentrations of threonine were reduced while the concentrations of other amino acids were significantly increased.

At this stage it might be reasonable to make some conclusions concerning the relationship between the plasma concentrations of somatostatin and those of the amino acids. As we have noted, some patients with high plasma concentrations of somatostatin do not grow as satisfactorily as those with normal plasma concentrations even with the administration of growth hormone. Those patients with high plasma somatostatin concentrations have lower plasma concentrations of amino acids than do those children whose plasma concentration of somatostatin is normal. The means of the SDS's for the amino acids for both the indispensable and dispensable amino acids have been collected together with the respective SDS's for plasma somatostatin into Tables 4.1&4.2 (p.181-182) so as to show the relationships between the plasma somatostatin and the plasma amino acids. It will be remembered that all the patients experienced a period of 'on/off/on' growth hormone therapy. What did vary between the two groups was that the 'good' responders had on average low normal plasma somatostatin SDS's whereas the 'poor' responders had high normal or even abnormally high plasma somatostatin SDS's especially during the 'off' growth hormone period. Thus plasma somatostatin must act in these children at two sites viz. to inhibit the release of growth hormone at the

pituitary level and to restrict the absorption of nutrient in the form of amino acids. Clearly there are two types of patients in my cohort.

Concerning growth hormone therapy I have not found any reference to this very interesting and important observation that first, some growth hormone deficient children have a high plasma somatostatin concentration while others do not and in this regard, those whose plasma somatostatin concentration are high have lower concentrations of plasma amino acids while those with a normal plasma concentration of somatostatin have higher plasma amino acid concentration. This cannot be without significance in terms of substrate for linear growth. But it might be relevant to consider here that those children whose plasma somatostatin concentration is high may require an increased dosage of growth hormone as a therapy when compared with their counterparts. Or would it be for this group that immunization against somatostatin should be explored?

Now regarding the Nutritional Ratios the reader is referred again to the Appendix Tables B 1-6 for the 'good' responders and to Appendix Tables B 7-9 for the 'poor' responders. Herein are the actual plasma amino acid concentrations. In Appendix Tables E 1&2 are the Nutritional Ratios calculated from the real plasma amino acid concentrations. Let us now consider the significance of these ratios. In a sense these make a tale that is told.

During the 'off' growth hormone period Ratios 1, 2 & 3 were reduced and we know that the actual plasma concentrations of amino acids used to calculate these ratios had fallen during that period. The numerator in Ratios 1&2 are the sums of the branched chain amino acids and in Ratio 3 the numerator is valine. The denominator in Ratios 1, 2&3 are

dispensable amino acids and since the ratios fell during the 'off' period it follows that most affected by the withdrawal of growth hormone was the concentrations of the branched chain amino acids. It is therefore likely that this fall in the ratio values is due to the unopposed (by growth hormone) action of the plasma somatostatin. Does this finding indicate that there is a competitive antagonism at the gut level between growth hormone and somatostatin? This suggestion is novel.

Ratio 4 is interesting in that the tyrosine increased during the 'off' period and this was reflected in a higher value at that stage with a fall when growth hormone therapy was current. Could this indicate that another metabolic hormone was being brought into action viz. thyroxine requiring more tyrosine, to compensate for the loss of growth hormone? It is of interest that it has been found that if tyrosine is fed in excess, phenylalanine requirement is reduced.(156) This means that a conditionally indispensable amino acid like tyrosine can compensate to some extent for the parent amino acid.

When we come to consider those patients who were short stature due to their having had a cerebral tumour, it is interesting that we are dealing with an older age group of patients. The total number of "tumour" patients was 13 (7 boys& 6 girls) whose age range was 9.37 years to 17,94 years. There were no cases in this group who had had leukaemia. Nonetheless the fact that there were 19 patients with IGHD, and 13 patients with tumours, highlights the trend in the nature of paediatric illness, namely the increase in tumour/malignant disease. But another reason for the absence of post-treatment leukaemia patients is that it is customary to have at least 5-year survival period for children with leukaemic malignant disease before considering the disease "cured" so as to justify growth

hormone treatment. The greatest number of these "tumour" patients (7 out of 13) had craniopharyngioma. Three had medulloblastoma, two had glioma (optic and pontine) and one had a retinoblastoma. This is not in agreement with general experience for the commonest intracranial tumour in childhood is the cerebellar medulloblastoma. Indeed it is stated that only 5 to 13 per cent are account for by craniopharyngioma. But my series is small enough not to show the recognised trends.

Intracranial tumours may affect the infundibular stalk. Such is the case with craniopharyngiomas, but in addition subsequent irradiation is likely to damage the hypothalamic nuclear aggregates which elaborate the releasing hormones, whether the irradiation is given for craniopharyngiomas or for other tumours most commonly for the cerebellar medulloblastoma .

Once again the "tumour"patients were divided into those who over the period of review improved their height SDS's and those who did not. Firstly in those who improved (Table 3.17) (6 of 7 had craniopharyngioma), some certainly were extremely short statured at the beginning of the review period (patients 6 & 7 "boys" and 3&4 "girls") but on average with a mean height SDS of -1.97. They were not as short statured as the good group of the idiopathic patients whose mean height SDS was -3.42. During the "off" growth hormone period there was no loss of height status and on restarting growth hormone a small mean catchup growth was reflected for each patient in a mean of -1.44. height SDS.

The "poor" responding group (Table 3.18) (only one craniopharyngioma) with a starting height SDS of -2.09 worsened during the "off" growth hormone period to -2.67 and even on restarting growth hormone their mean height SDS worsened further to -2.91. All of those children at 1987

were significantly short statured (except patient LB "girl"). Thus it would appear that patients with a craniopharyngioma are likely to fare better in terms of height in response to growth hormone than those with medulloblastoma or glioma. Why this should be is unclear

From my reasoning regarding the IGHD patients one might expect that the "good"and "poor" responders of the tumour group would show the same trends in their plasma somatostatin concentration. This was not so (Tables 3.19.and 3.20) for throughout the period of review (1.75 years) the SDSs' for plasma somatostatin were entirely normal except for patient 4.SD (female) whose plasma somatostatin concentration was significantly abnormally high at April 1985 and January 1987 these being periods of growth hormone administration. Combine this observation with her height SDS's at these periods, it will be seen that she is grossly underheight. It is therefore suggested that this girl was on course for short stature due to high plasma somatostatin concentrations before her cerebral tumour developed. This girl had only had two years of growth hormone therapy and after removal of her medulloblastoma she had irradiation therapy but had she been of normal height, then it is unlikely that within two years she lost so much height to have a height SDS of -4.36 when growth hormone was withdrawn. This patient's example reinforces the earlier suggestion that all patients who are short statured, without other obvious reason should have the plasma somatostatin concentration assayed.

In Tables 3.21.and 3.22 are the mean SDS's for the indispensable and dispensable amino acids in this tumour group of patients. It will be seen that at all times these values were normal. But there was a lowering of the concentration both for the indispensable and the dispensable amino acids

except for threonine at January 1986 and tyrosine at the same period. These latter trends were only seen in the 'good 'responders.

Thus for this tumour group there is (a) normal plasma concentration of somatostatin (b) normal plasma amino acid concentrations and despite these similarities we have two subgroups behaving differently in relation to growth, one improving and one worsening. The only differentiating factor is the pathology in that those who do show some linear growth during the period of observation have a craniopharyngioma. Only one of those children who did not grow satisfactorily had a craniopharyngioma. The question is whether or not medulloblastoma and glioma have as yet indefinable growth restricting actions.

In Table 3.23 are the changes in the nutritional ratios of the "tumour" group. A similar trend is here appreciated like unto IGHD group. The fall in the ratios at January 1986 was due to greater fall in the indispensable amino acids than occured in the dispensable amino acid concentrations. For what the comparison is worth, the 'good" responders had a higher ratios in general. The p-values show where the changes were statistically significant.

To summarize the review of the data which were available to me, I have tried to assess the advantage to each of the subgroups ("good"and "poor" responders within the two main groups of patients "IGHD" and "tumours") of the introduction of recombinant growth hormone.

In Appendix Tables L. 1&2 are the annual advances in osseous maturation experienced by each patient during the first year of the new treatment. I have abstracted the mean annual bone maturation score for each group of patients into Table 4.3.(p.183) The data are of considerable interest. It will be seen that the children with IGHD who responded well in their linear

growth had a bone advancement of 1.23 years per year while those children who had a poor growth response advanced their bone age by only 0.74 years per year. Those children with cerebral tumours whether "good" or "poor" responders advanced their bone age by 0.94 and 0.86 years per year respectively.

I concluded that those "poor" responders in the IGHD group, although their linear growth were unsatisfactory, their slow bone maturation compensated to a certain extent by permitting more bone age years for growth. Thus in a sense the "good"responders were disadvantaged to some extent by their rapid osseous maturation. It is very worthy of note that these "poor" responders in linear growth and osseous maturation were the only children with high plasma concentrations of somatostatin. Can it be concluded that somatostatin may antagonise the action of growth hormone at the epiphyseal growth plate? Although I am concerned with the interactions of growth hormone and somatostatin I am aware that other factors may require to be considered such as thyroxine and androgenic anabolic hormones but these cannot be discussed here. However a fairly recent article from Japan (157) suggests that in the management of children with growth hormone deficiency especially of those who are nearing puberty, there is a role for a drug which will suppress gonadal function (cyproterone acetate and medroxyprogesterone) so reducing bone maturation and permitting an increased period for linear growth in response to growth hormone. These authors have reported that such treatment prolongs the duration of puberty and so improves the final height. According to my findings as above given it may be of some advantage to those children who although growing well but with an advancing bone maturation that they may should also have an antigonadotrophin so as to prevent or reduce the advancement in bone

age giving them more time for linear growth in terms of bone age years . By this we may be able to improve their final height.

It is tempting to continue a discussion by raising suggestions new and old and to apply these to the argument. Little is to be achieved however by so doing. But at the end of this work it is pertinent that I ask myself if I have been guilty of a manner of research which I earlier criticized :- "Let us do something and watch the effects ".

The Creutzfeldt-Jacob incident did happen rightly or wrongly. Perforce the cadaveric growth hormone had to be withdrawn. There was no substitute available widely and necessarily those affected children had a period without growth hormone therapy. Then came the recombinant product, methionyl growth hormone and continuous treatment was again available. The dosage schedule was similar to that used with the cadaveric product. That is where I came in - as it were and I have simply observed these children throughout their 'on/off/on' growth hormone treatment. Something was done and I have followed the effects. Hopefully however I have added something to our knowledge of the behaviour (clinical and biochemical) of some aspects of the changes which took place. It is probably a truism, but one finding was that once growth hormone is started as treatment it must be continued otherwise to stop it will result in a return to a phase of growth failure which in some will only be reversed with difficulty.

Clinicians managing children of short stature who are being treated with growth hormone are aware that some respond better than others. I have offered a reason for this. In my cohort of patients those who did not show the same degree of linear growth as their peers had higher and sometimes

abnormally high plasma concentrations of somatostatin. But someone may say , "plasma somatostatin ?' Yes, for the pituitary is bathed with systemic blood via the long and short pituitary portal vessels which are radicles of the hypophyseal arteries. Hence the adenotrophs under a more intense continuous tonic suppression of growth hormone release may not respond to our insulin hypoglycaemia test which may wrongly cause us to label these children as having growth hormone deficiency. How can we prevent either these high plasma concentrations of somatostatin or the actions of these high concentrations?

The plasma amino acids studied in this work were basically within normal during the changing routine, but for all the patients studied here during the "off" period of growth hormone treatment the indispensable amino acids valine, leucine, isoleucine and lysine concentration fell but recovered on restarting growth hormone therapy. The same was true of the dispensable amino acids alanine, glycine and serine. Has this any significance? I have concluded that this fall in plasma amino acids was due to the unopposed action of the plasma somatostatin during the withdrawal of growth hormone therapy. However in those children with IGHD who fared less well, the fall in the plasma amino acid concentrations was greater and the rise in plasma somatostatin also greater.

Protein is necessary for normal growth in children, and the amino acids are the the building blocks for proteins. It may then not be surprising that the period of reduced linear growth of these patients was associated with a lowering of the plasma concentration of some amino acids. The therapeutic challenge would therefore seem to be that rational therapy for children with growth hormone deficiency can only be maximized if the plasma somatostatin status is known for each child. There is no logic in

increasing the growth hormone schedule if there is a built-in mechanism which is obviating optimum utilization of growth hormone. Even although I have only shown gains in mean height SDS's of +0.25, and +0.83 in the good responders for both males and females respectively(Table 3.5 and 3.6 p 124&125), that has to be compared with a loss of 0.41 SDS's when plasma somatostatin was high (Table 3.7 p 126). I therefore concluded that high plasma somatostatin concentration not only prevent optimum linear growth in response to growth hormone therapy, but attempts must be made to reduce the effects of these high concentrations. Does this mean immunization against somatostatin for at least some of these children ?

Table 4.1 Means of SDS for indispensable amino acids in relation to SRIF SDS for both good and poor responders during the period of review.

	Valine	Leucine	Isoleucine	Threonine	Lysine	SRIF	
Apr/85	+0.09	-0.20	-0.11	-0.34	+0.08	+0.69	Good
Jan/86	-1.15	-1.08	-0.96	+0.14	-0.83	+0.94	responders
Jan/87	-0.01	-0.18	+0.25	-0.14	+0.37	+0.48	
Apr/85	-0.45	-0.37	-0.18	-0.58	-0.38	+2.32	Poor
Jan/86	-1.16	-0.85	-0.19	-0.20	-0.61	+2.76	responders
Jan/87	-0.19	-0.27	-0.34	-0.39	+0.06	+2.24	

Table 4.2 Means of standard deviation scores for dispensable amino acids related to SRIF SDS in both good and poor responders during the period of review.

	Alanine	Glycine	Serine	Tyrosine	SRIF	
April/85	-0.05	-0.12	+0.12	+0.30	+0.69	Good
Jan /86	-0.79	-0.45	-0.40	+0.58	+0.94	responders
Jan /87	+0.13	-0.17	-0.01	+0.15	+0.48	
April/85	-0.63	-0.21	-0.30	-0.61	+2.32	Poor
Jan /86	-0.87	-0.62	-0.45	-0.05	+2.76	responders
Jan /87	-0.57	-0.09	-0.01	-0.35	+2.24	

Table 4.3 Mean of the bone age (years) advancement during one year of recombinant growth hormone treatment.

	IGHD		Tumours	
	Good responders	Poor responders	Good responders	Poor responders
Males	1.03	0.80	0.83	0.89
Females	1.65	0.40 (one girl)	1.03	0.80
Total	1.23	0.74	0.94	0.86

APPENDIX TABLES

Table I Plasma amino acid values (umol/L) and somatostatin (pg/ml) in normal girls.

NO	Age	Ala	Gly	Val	Thr	Ser	Leu	Iso	Lys	Tyr	SRIF
1-	14.2	312	215	231	92	141	141	71	81	62	18
2-	14.3	345	265	210	132	129	135	50	110	60	17
3-	15.5	261	310	283	110	115	100	61	136	49	16
4-	16.0	371	315	239	78	165	100	39	71	57	15
5-	16.25	305	225	189	115	120	134	68	75	55	9
6-	17	317	298	201	97	131	155	63	129	49	14
7-	17	335	236	218	101	118	124	49	105	46	12
8-	18.5	350	318	172	79	145	97	38	125	54	10
9-	20	347	297	196	98	139	118	41	128	61	13

Table 2 Plasma amino acid values (umol/L) and somatostatin(pg/ml) in normal boys.

No	Age	Ala	Gly	Val	Thr	Ser	Leu	Iso	Lys	Tyr	SRIF
1-	13.5	257	289	179	75	107	98	42	105	38	19
2-	14	322	236	174	116	88	77	37	118	46	20
3-	14.25	314	279	225	70	135	75	42	126	48	19
4-	14.3	291	310	198	96	150	122	71	79	51	21
5-	15	297	283	210	108	140	139	75	150	59	16
6-	15.34	263	298	200	65	126	76	36	108	47	17
7-	16.2	393	252	251	109	97	129	68	142	62	16
8-	16.5	272	314	220	95	170	112	49	105	49	15
9-	16.6	405	289	245	68	139	138	72	95	69	13.5
10-	17	364	244	198	99	89	119	53	146	52	16
11-	17.5	327	323	195	84	163	142	75	87	48	14
12-	18	364	200	210	110	133	133	63	116	61	8
13-	18.65	347	273	168	95	151	123	58	95	52	14
14-	19	324	253	234	83	100	130	68	157	66	11
15-	20	425	246	264	97	128	141	75	122	53	13
16-	21	348	217	216	80	121	143	58	107	68	15
17-	22	315	263	231	105	84	103	41	131	45	9

Table A. 1 Anthropometric data of male patients with idiopathic growth hormone deficiency during the period of review.

			April 1985			January 1986			January 1987		
Patient	D.O.B	Ch.age	Ht/Cm	B.A/Y	Ht. SDS	Ht/Cm	B.A/Y	Ht. SDS	Ht/Cm	B.A/Y	Ht. SDS
1-AB	10/3/83	2.16	72.0	1.0	-4.51	75	1.2	-4.87	85	2.50	-3.74
2-JC	23/11/75	9.39	118.7	8.0	-2.47	121.8	9.2	-2.99	126.8	9.8	-2.51
3-AG	9/5/74	10.88	117.0	9.0	-3.68	119.4	9.3	-3.69	127.9	9.9	-3.07
4-ALB	3/10/79	11.49	120.0	6.5	-3.54	122.2	8.1	-3.61	128.2	9.0	-3.40
5-NMcQ	4/9/72	12.57	123.0	11.2	-3.63	124	12.9	-3.95	130	13.5	-3.96
6-CMcG	29/4/72	12.92	134.0	10.3	-2.43	136.5	10.9	-2.64	147	13.4	-2.24
7-RH	24/4/72	12.93	127.5	11.0	-3.25	129	11.7	-3.60	137	13	-3.49
8-CG	20/8/71	13.65	137.1	10.5	-2.56	140.3	10.8	-2.77	146	11.7	-2.65
9-NC	8/5/71	13.92	148.0	14.0	-1.46	152	14.1	-1.75	159.9	15.0	-1.50
10-SS	7/2/71	14.15	129.5	8.7	-3.87	130.8	8.7	-4.48	138	9.0	-4.81
11-BL	24/3/70	15.02	131.4	11.2	-4.51	133.5	11,6	-5.1	137.7	11.8	-5.41
12-IMcC	4/12/69	15.32	146.4	11.8	-2.93	147.8	11.8	-3.55	151.5	14.4	-3.37
13-DB	10/7/68	16.73	154.1	14.5	-2.95	154.8	17.7	-2.96	156	18	-2.82
14-MMcG	3/4/68	17.01	144.5	15.0	-4.47	147.1	16.6	-4.11	148	17	-4.02

Table A.2 Anthropometric data of female patients with idiopathic growth hormone deficiency during the period of review.

			APRIL 1985			JANUARY 1986			JANUARY 1987		
Patient	D.O.B	C.A at Apr/85	Ht/C m	B.A/Y	Ht. SDS	Ht/C m	B.A/Y	Ht. SDS	Ht/C m	B.A/Y	Ht. SDS
1-SS	8/3/79	5.77	92.9	4.0	-3.80	95.8	5.0	-4.25	109.2	6.2	-2.66
2-CS	28/9/78	6.51	109.4	5.3	-1.32	112.4	5.6	-1.61	119.0	7.3	-1.29
3-LB	14/9/78	6.55	101.4	3.9	-3.16	103	4.8	-3.79	110.8	7.2	-2.76
4-TG	18/4/75	9.95	125.9	10.9	-1.62	126.7	11.8	-2.14	133.9	12.2	-1.83
5-AQ	8/11/69	15.40	132	10.5	-4.95	137.3	10.6	-4.15	143.0	11.9	-3.20

Table B.1 Plasma amino acid concentrations (umol/l) in male patients with idiopathic growth hormone deficiency who responded well (January 1985) i.e at the withdrawal of the cadaveric growth hormone.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1-AB	285	235	170	60	118	89	51	98	51
3-AG	274	241	176	65	109	95	50	113	49
4-AlB	350	275	240	92	135	118	68	140	59
6-CMcQ	290	256	198	66	140	115	48	82	61
7-RH	320	238	231	72	105	77	45	89	53
8-CG	351	247	229	84	135	127	51	131	49
13-DB	345	257	221	102	116	138	49	125	62
14-MMcG	324	245	197	96	137	111	42	99	55

Table B.2 Plasma amino acid concentrations (umol/l)in male patients with idiopathic growth hormone deficiency who responded well (January 1986) i.e at commencement of recombinant growth hormone.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1-AB	249	233	153	63	108	75	38	114	43
3-AG	277	231	163	79	111	76	46	100	55
4-ALB	277	251	174	96	118	98	45	97	48
6-CMcQ	283	253	167	110	138	97	41	81	60
7-RH	311	229	198	82	129	83	39	74	56
8-CG	295	237	193	87	128	100	38	94	52
13-DB	333	255	198	102	110	101	49	103	59
14-MMcG	312	273	179	86	101	108	45	84	66

Table B.3. Plasma amino acid concentrations (umol/l) in male patients with idiopathic growth hormone deficiency who responded well (Jan 87) i.e after one year of recombinant growth hormone treatment.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1-AB	298	245	179	69	120	92	60	110	53
3-AG	338	232	187	72	115	97	59	105	62
4-ALB	287	253	223	75	125	107	57	130	47
6-CMcQ	289	264	203	110	131	109	61	152	58
7-RH	321	249	219	95	128	144	72	134	55
8-CG	299	251	212	75	139	117	54	98	49
13-DB	379	278	204	94	120	130	60	114	54
14-MMcG	369	269	210	83	116	122	49	121	64

Table-B.4 Plasma amino acid concentrations (umol/l) in four girls with idiopathic growth hormone deficiency who responded well (April 1985) i.e on withdrawal of cadaveric growth hormone.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1-SS	292	275	159	73	121	91	49	87	54
2-CS	265	270	198	79	128	108	45	115	44
3-LB	279	292	225	82	123	93	56	137	56
5-AQ	315	262	183	78	127	111	50	98	58

Table B.5 Plasma amino acid concentrations (umol/l) in four girls with idiopathic growth hormone deficiency who responded well (Jan 86) i.e at the commencement of the recombinant growth hormone treatment.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1-SS	243	213	165	85	97	73	37	82	52
2-CS	255	231	161	74	106	87	41	76	46
3-LB	213	237	147	88	90	76	40	87	58
5-AQ	284	243	160	105	110	106	39	81	56

Table B.6 Plasma amino acid concentrations (umol/l) in four girls with idiopathic growth hormone deficiency who responded well (Jan 87) i.e one year after commencement of recombinant growth hormone treatment.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1-SS	285	253	202	87	110	112	51	98	51
2-CS	297	249	192	84	119	105	49	121	51
3-LB	259	259	221	66	121	89	54	128	49
5-AQ	398	261	198	116	123	109	58	112	53

Table B.7 Plasma amino acid concentrations (umol/l) in six boys and one girl with idiopathic growth hormone deficiency (Apr.85 i.e on withdrawal of cadaveric growth hormone.) who responded less well during the period of review.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
2-JC	287	234	174	91	123	99	53	106	39
5-NMcG	293	261	190	69	120	104	47	98	48
9-NC	325	250	201	81	89	96	55	112	45
10-SS	265	297	221	74	124	112	65	107	62
11-BL	270	255	175	80	101	125	46	99	42
12-IMcC	310	249	207	73	121	112	48	110	41
4-TG.(F)	275	234	196	75	122	98	59	90	56

Table B.8 Plasma amino acid concentrations (umol/l) in six boys and one girl with idiopathic growth hormone deficiency (Jan 86) i.e at the commencement of the recombinant growth hormone treatment) who responded less well during the period of review.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
2-JC	268	228	162	93	107	92	34	102	56
5-NMcG	282	244	185	90	127	84	44	93	50
9-NC	292	241	191	94	102	96	47	109	45
10-SS	276	254	179	86	131	104	45	100	60
11-BL	290	248	175	73	99	112	37	95	59
12-IMcC	300	238	198	84	110	100	41	110	44
4-TG(F)	274	226	162	76	104	99	39	85	56

Table B.9 Plasma amino acid concentrations (umol/l) in six boys and one girl with idiopathic growth hormone deficiency (Jan 87) i.e one year after the commencement of the recombinant growth hormone treatment who responded less well during the period of review.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
2-JC	279	253	180	93	112	106	41	118	45
5-NMcG	295	269	213	69	141	97	53	104	50
9-NC	298	271	217	93	121	124	64	118	49
10-SS	312	225	207	82	119	120	55	108	45
11-BL	329	283	222	76	135	91	51	130	61
12-IMcC	325	291	209	75	119	118	53	120	53
4-TG.(F)	284	235	198	102	115	112	49	112	52

Table C 1 Standard deviation scores for plasma indispensable amino acids in children with idiopathic growth hormone deficiency who fared well during the period of review.

Pat	Valine			Leucine			Isole ucine			Threonine			Lysine		
no.	1985	1986	1987	1985	1986	1987	1985	1986	1987	1985	1986	1987	1985	1986	1987
1.AB	-0.18	-0.96	-0.03	+0.04	-0.69	-0.02	+0.61	-0.51	+1.43	-0.62	-0.50	-0.21	-0.06	+0.61	+0.37
3.AG	-1.00	-1.62	-0.73	-0.65	-1.61	-0.75	-0.23	-0.62	+0.35	-1.21	-0.37	-0.94	+0.10	-0.52	-0.36
4.AlB	+0.93	-1.25	+0.60	+0.35	-0.66	-0.36	+1.18	-0.76	+0.13	+0.50	+0.68	-0.81	+1.26	-0.69	+0.71
6.CMcG	-0.36	-1.71	-0.38	+0.05	-0.87	-0.42	-0.57	-1.21	+0.33	-1.36	+1.44	+1.34	-1.39	-1.48	+1.60
7.RH	+0.96	-0.46	+0.26	-1.71	-1.52	+1.20	-0.82	-1.37	+1.22	-0.97	-0.39	0.35	-1.08	-1.79	+0.80
8.CG	+0.80	-0.74	-0.10	+0.53	-0.81	-0.13	-0.30	-1.49	-0.87	-0.25	-0.14	-1.03	+0.73	-0.95	-0.83
13.DB	+0.10	-0.93	-0.80	+0.70	-1.12	+0.13	-0.82	-0.90	-0.08	+0.60	+0.51	-0.11	+0.29	-0.74	-0.30
14.NMcG	-0.90	-1.72	-0.59	-0.59	-0.81	-0.27	-1.42	-1.24	-1.00	+0.18	-0.56	-0.86	-0.88	-1.59	0.01
1.SS.F	-0.64	-0.56	+0.65	-0.46	-1.57	+0.41	+0.44	-0.83	+0.43	-0.14	+0.43	+0.41	-0.23	-0.51	+0.09
2.CS.F	+0.67	-0.75	+0.25	+0.36	-0.87	-0.02	-0.02	-0.49	+0.20	+0.12	-0.29	+0.18	+0.88	-0.78	+1.02
3.LB.F	+1.64	-1.26	+1.29	-0.45	-1.47	-0.89	+1.05	-0.59	+0.68	+0.29	+0.55	-0.91	+1.79	-0.33	+1.30
5.AQ.F	-0.92	-1.83	-0.58	-0.62	-0.98	-0.94	-0.39	-1.53	+0.22	-1.16	+0.37	+0.90	-0.40	-1.15	+0.07
Mean	+0.09	-1.15	-0.01	-0.20	-1.08	+0.61	-0.11	-0.96	+0.25	-0.34	+0.14	+0.14	+0.08	-0.83	+0.37

Table C.2 Standard deviation scores for plasma indispensable amino acids in children with idiopathic growth hormone deficiency who fared less well during the period of review.

Pat	Valine			Leucine			Isoleucine			Threonine			Lysine		
no.	1985	1986	1987	1985	1986	1987	1985	1986	1987	1985	1986	1987	1985	1986	1987
JC	-0.90	-1.48	-0.87	-0.30	-0.71	-0.17	+0.14	-1.47	-0.99	-0.66	+0.71	+0.60	-0.12	-0.35	+0.30
5.NMcQ	-0.63	-0.93	+0.06	-0.42	-1.43	-0.94	-0.62	-0.93	-0.29	-1.13	+0.71	-1.31	-0.66	-0.93	-0.50
9.NC	+0.41	-0.86	+0.07	-0.94	-1.07	+0.16	-0.82	-0.81	+0.49	-0.48	+0.29	+0.12	-0.12	-0.30	+0.04
10.SS	-1.55	-1.37	-0.37	-0.22	-0.68	-0.05	+0.70	-0.99	-0.27	-0.47	-0.26	-0.63	-0.36	-0.71	-0.42
11.BL	-0.29	-1.63	+0.13	+0.25	-0.40	-0.50	-0.92	-1.71	-0.67	-0.66	-1.19	-1.12	-0.76	-0.98	+0.51
12.IMcC	+0.20	-0.75	-0.43	-0.38	-1.00	-0.28	-0.78	-1.42	-0.53	-1.15	-0.51	+1.21	-0.29	-0.34	+0.04
4.TG F	-0.45	-1.12	+0.06	-0.61	-0.66	-0.09	+1.02	-1.01	-0.13	-0.59	-0.64	+0.79	-0.37	-0.63	+0.42
Mean	-0.45	-1.16	-0.19	-0.37	-0.85	-0.27	-0.18	-1.19	-0.34	-0.58	-0.20	-0.39	-0.38	-0.61	+0.06

Table D.I Standard deviation scores for plasma dispensable amino acid concentrations in children with idiopathic growth hormone deficiency who fared well during the period of review.

Pat. No.	Ala			Gly			Ser			Tyr		
	1985	1986	1987	1985	1986	1987	1985	1986	1987	1985	1986	1987
1.AB	+0.65	-0.26	+0.72	-0.73	-0.79	-0.45	+0.21	-0.25	+0.24	+0.23	+0.42	+0.38
3.AG	-0.72	-0.75	+0.51	-0.60	-0.89	-0.87	-0.43	-0.43	-0.24	-0.39	+0.25	+1.00
4.AlB	+0.93	-0.83	-0.74	+0.37	-033	-0.27	+0.60	-0.12	+0.14	+0.71	+0.57	-0.51
6.CMcG	-0.62	-0.88	-0.87	-0.19	-0.28	+0.03	+0.76	+0.75	+0.34	+0.87	+0.72	+0.45
7.RH	+0.06	-0.25	-0.14	-0.70	-0.96	-0.40	-0.66	+0.33	+0.22	-0.04	+0.27	+0.12
8.CG	+1.12	-0.69	-0.73	-0.45	-0.73	-0.34	+0.54	+0.26	+0.64	-0.52	+0.22	-0.60
13.DB	+0.14	-0.24	+0.68	-0.18	-0.24	+0.41	-0.33	-0.69	-0.23	+0.81	+0.43	+0.18
14.NMcG	-0.66	-0.75	+0.42	-0.52	+0.27	+0.15	+0.51	+1.12	-0.40	0.00	+1.21	+0.95
1.SS F	+0.64	-0.97	+0.10	+0.64	-0.95	-0.01	+0.19	+0.87	-0.40	+0.89	+0.53	+0.30
2.CS F	-0.27	-0.69	+0.39	+0.48	+0.53	-0.13	+0.44	-0.53	-0.04	-0.46	-0.28	+0.27
3.LB F	+0.14	-1.92	-0.74	+1.02	-0.38	+0.11	+0.23	-1.20	+0.03	+1.07	+1.25	-0.12
5.AQ F	-0.79	-1.28	+1.90	-0.53	-0.69	-0.29	-0.57	-0.90	-0.42	+0.48	+1.31	+0.32
Mean	+0.05	-0.79	+0.13	-0.12	-0.45	-1.17	+0.12	-0;40	+0.01	+0.30	+0.58	+0.15

Table D.2 Standard deviation scores for plasma dispensable amino acid concentrations in children with idiopathic growth hormone deficiency who fared less well during the period of review.

Pat.	Alanine			Glycine			Serine			Tyrosine		
No.	1985	1986	1987	1985	1986	1987	1985	1986	1987	1985	1986	1987
2.JC	-0.23	-0.76	-0.64	-0.80	-0.79	-0.26	+0.19	-0.49	-.32	-1.46	+0.43	-0.86
5.NMcQ	-0.51	-0.86	-0.69	-0.04	-0.53	-0.18	-0.04	+0.22	+0.76	-0.59	-0.59	-0.44
9.NC	+0.05	-0.80	-0.79	-0.36	-0.62	+0.23	-1.34	-0.83	-0.10	-0.99	-0.99	-0.61
10.SS	-1.35	-1.20	-0.51	+0.98	-0.25	-1.09	+0.07	+0.33	-0.19	+0.93	+0.93	-1.08
11.BL	-1.34	-0.98	-0.24	-0.23	-0.43	+0.56	-0.89	-0.99	+0.43	-1.38	-1.38	+0.69
12.IMcC	-0.47	-0.80	-0.58	-0.40	-0.72	+0.79	-0.09	-0.56	-0.22	-1.50	-1.50	-0.23
4.TG"F"	-0.53	-0.69	-0.56	-0.59	-0.83	-0.66	-0.02	-0.82	-0.41	+0.75	+0.75	+0.07
Mean	-0.63	-0.87	-057	-0.21	-0.62	-0.09	-0.30	-0.45	-0.01	-0.61	-0.61	-0.35

Table E. 1 Nutritional ratios for children with IGHD who responded well to treatment during the period of review.

Pat. No.	April 1985				Jan 1986				Jan 1987			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
1. AB	0.486	0.878	0.723	0.217	0.451	0.780	0.657	0,227	0.499	0.907	0.731	0.216
3. AG	0.514	0.917	0.730	0.207	0.460	0.833	0.706	0.238	0.501	0.988	0.806	0.267
4. AlB	0.61	1.039	0.873	0.215	0.491	0.859	0.693	0.191	0.581	1.024	0.881	0.186
6. CMcG	0.526	0.912	0.773	0.238	0.453	0.780	0.660	0.237	0.545	0.944	0.769	0.220
7. RH	0.532	1.029	0.971	0.223	0.478	0.894	0.865	0.245	0.623	1.154	0.880	0.221
8. CG	0.555	1.065	0.927	0.206	0.502	0.907	0.814	0.219	0.556	0.982	0.845	0.195
13. DB	0.568	1.094	0.860	0.241	0.499	0.953	0.776	0.231	0.507	0.990	0.734	0.194
14.NMcG	0.496	0.983	0.804	0.224	0.484	0.888	0.656	0.242	0.505	0.990	0.781	0.238
1.SS F	0.435	0.755	0.578	0.196	0.497	0.887	0.775	0.244	0.563	1.006	0.798	0.202
2CS F	0.529	0.882	0.733	0.163	0.488	0.858	0.697	0.199	0.572	0.940	0.771	0.197
3. LB F	0.539	0.901	0.771	0.192	0.487	0.804	0.620	0.245	0.570	0.958	0.853	0.189
5.AQ F	0.489	0.884	0.698	0.221	0.479	0.864	0.658	0.230	0.467	0.951	0.759	0.203
Mean	0.519	0.945	0.787	0.212	0.481	0.859	0.715	0.229	0.541	0.986	0.801	0.211
P value					0.004	0.01	0.07	0.045	0.0003	0.000	0.004	0.04

Table E. 2 Nutritional ratios for children with IGHD who responded poorly to the treatment during the period of review.

Pat.	April 1985				Jan 1986				Jan 1987			
NO.	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
2.Jc	0.506	0.913	0.745	0.167	0.478	0.860	0.711	0.246	0.508	0.896	0.711	0.178
5.NMcQ	0.506	0.895	0.728	0.184	0.479	0.844	0.758	0.205	0.515	0.885	0.792	0.186
9.NC	0.530	1.038	0.804	0.180	0.526	0.974	0.793	0.187	0.578	1.033	0.801	0.181
10.SS	0.580	0.945	0.744	0.209	0.496	0.852	0.705	0.236	0.582	1.110	0.920	0.200
11.BL	0.553	0.972	686	0.164	0.509	0.938	0.706	0.238	0.487	0.871	0.784	0.216
12.IMcC	0.540	0.992	0.832	0.165	0.523	0.974	0.832	0.185	0.507	0.910	0.718	0.182
4.TG F	0.559	0.992	0.838	0.239	0.497	0.909	0.717	0.248	0.566	1.026	0.843	0.221
Mean	0.539	0.964	0.768	0.187	0.501	0;907	0.746	0.221	0.536	0.962	0.796	0.195
P value					o.0112	0.067	0.460	0.042	0.064	0.210	0.160	0.059

Table F. 1 Anthropometric data for male patients with cerebral tumours during the period of review.

Patients	D O B	Ch.age/ year	April 1985			January 1986			January 1987			Pathology
			Ht/cm	BA/Y	Ht.SDS	Ht/cm	BA/Y	Ht.SDS	Ht/cm	BA/Y	Ht.SDS	
1. DH	8.4.1972	12.98	147.9	13.1	-0.69	153.6	13.4	-0.64	160.3	13.8	-0.68	Craniopharyngioma
2. DB	23.3.1971	14.06	142	13.6	-2.25	144.0	14.1	-2.74	146.8	15.6	-3.44	Medulloblastoma
3. MM	22.3.1971	14.03	146	14.9	-1.76	147.6	16.3	-2.30	149.4	16.9	-3.01	Medulloblastoma
4. PC	21.9.1970	14.63	140.2	12.7	-3.00	144.5	14.4	-3.15	150.5	15.17	-3.28	Retinoblastoma
5. TR	5.5.1970	14.91	150	12.3	-2.07	151.8	12.3	-2.60	157.0	13.0	-2.50	Craniopharyngioma
6. JMcD	11.7.1968	16.76	158.8	12.9	-2.25	160.4	13.3	-2.10	163.0	14.0	-1.76	Craniopharyngioma
7. KT	25.4.1967	17.94	158.8	12.3	-2.37	160.0	12.5	-2.21	166.5	13.9	-1.23	Craniopharyngioma

Table F. 2 Anthropometric data for female patients with cerebral tumours during the period of review.

Patients	D O B	Ch.age/ 1985	April 1985			January 1986			January 1987			Pathology
			Ht/cm	BA/Y	Ht.SDS	Ht/cm	BA/Y	Ht.SDS	Ht/cm	BA/Y	Ht.SDS	
1. LB	16.11.1975	9.37	134.3	8.8	+0.25	136.0	9.0	-0.78	139.0	10.0	-0.68	Optic glioma
2.AMcE	27.4.1973	11.97	121.0	9.1	-3.7	122.7	9.3	-4.71	129.0	9.9	-4.57	Pontine glioma
3. CL	16.9.1971	13.60	137.7	11.5	-3.12	139.0	11.6	-3.46	143.3	13.0	-3.07	Craniopharyngioma
4. SD	28.1.1971	14.27	133.0	11.3	-4.36	135.5	11.6	-4.30	138.7	12.5	-3.91	Medulloblastoma
5. AMcC	5.4.1969	15.99	166.6	12.5	+0.73	168.0	13.0	+0.95	174.7	13.9	+2.08	Craniopharyngioma
6. PC	28.11.1967	17.41	152.0	12.1	-1.70	153.0	12.2	-1.53	153.0	13.1	-1.53	Craniopharyngioma

Table-G 1. Actual plasma somatostatin concentrations (pg/ml), and their standard deviation scores for those children with cerebral tumours who fared well during the period of review.

	April 1985		January 1986		January 1987	
Patient	SRIF conc.	SDS	SRIF conc.	SDS	SRIF conc.	SDS
1.DH	15	-0.57	13	-1.07	14	-0.57
6.JMcD	16	+0.50	14	-0.03	11	-0.84
7.KT	10	-1.27	14	+0.20	11	-0.60
3.CL 'F'	18	+0.76	17	+0.53	19	+1.56
4.SD 'F'	23	+2.82	19	+1.49	22	+2.86
5.AMcC 'F'	11	-1.34	15	+0.42	12	-0.46
6.PC'F'	12	-0.57	13	000	8	-1.44
Mean		+0.05		+0.22		+0.07

Table-G 2. Actual plasma somatostatin concentrations (pg/ml), and their standard deviation scores for those children with cerebral tumours who fared less well during the period of review.

	April 1985		January 1986		January 1987	
Patients	SRIF conc.	SDS	SRIF conc.	SDS	SRIF conc.	SDS
2.DB	15	-0.37	16	+0.13	14	-0.34
3.MM	18	+0.64	19	+1.14	17	+0.67
4.PC	19	+1.11	18	+0.87	20	+1.74
5.TR	12	-1.21	10	-1.71	13	-0.50
1.LB 'F'	23	+1.53	24	+1.98	22	+1.60
2.AMcC'F'	20	+1.07	16	-0.27	18	+0.76
Mean		+0.59		+0.36		+0.66

Table H-1 Plasma amino acid concentrations (umol/L) in male patients with cerebral tumours (April 1985) i.e on withdrawal of cadaveric growth hormone.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1. DH	328	286	224	84	120	119	73	113	61
2. DB	290	251	188	76	114	113	63	88	64
3. MM	297	269	199	94	141	126	75	93	60
4. PC	379	288	221	107	109	131	56	132	70
5. TR	346	270	229	89	135	135	70	149	51
6. JMcD	346	230	198	97	128	143	59	144	54
7. KT	357	244	237	106	106	120	53	122	60

Table H.2 Plasma amino acid concentrations (umol/L) in male patients with cerebral tumours (Jan/1986) after nine months from the withdrawal of the cadaveric growth hormone.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1. DH	312	249	188	83	129	99	41	107	60
2. DB	268	235	172	82	111	100	39	86	63
3. MM	273	250	181	98	103	110	52	82	52
4. PC	289	264	169	100	118	98	50	108	72
5. TR	302	278	197	87	121	105	49	112	56
6.JMcD	315	220	174	109	125	96	52	100	64
7. KT	321	229	193	98	109	89	46	117	52

Table H.3 Plasma amino acid concentrations (umol/L) in male patients with cerebral tumours (Jan/1987) after one year following the commencement of the recombinant growth hormone.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1. DH	343	259	234	101	121	119	58	126	47
2. DB	291	272	191	77	134	107	69	95	54
3. MM	299	251	200	102	128	116	73	100	57
4. PC	359	283	238	98	137	148	54	139	39
5. TR	354	290	220	82	140	129	63	123	48
5. JMcD	282	241	198	110	121	137	72	111	37
7. KT	364	280	237	117	163	143	69	149	69

Table H.4 Plasma amino acid concentrations (umol/L) in female patients with cerebral tumours (April/1985) i.e at the withdrawal of the cadaveric growth hormone.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1. LB	300	238	201	90	137	119	64	100	43
2. AMcE	308	263	195	93	112	97	46	112	57
3. CL	272	248	177	78	114	117	55	82	59
4. SD	279	218	171	77	110	103	44	85	54
5AMcC	368	325	266	117	138	147	67	107	64
6. PC	343	247	218	86	141	124	58	99	58

Table H.5 Plasma amino acid concentrations (umol/L) in female patients with cerebral tumours (Jan/1986) i.e nine months after the withdrawal of the cadaveric growth hormone treatment.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1. LB	268	239	171	98	132	89	41	71	36
2. AMcE	296	228	176	111	107	96	38	95	57
3. CL	289	234	164	103	110	100	39	79	63
4. SD	275	203	165	83	101	104	41	58	49
5. AMcC	326	254	182	115	120	123	52	106	70
6. PC	331	265	213	94	132	97	46	95	58

Table H.6 Plasma amino acid concentrations (umol/L) in female patients with cerebral tumours (Jan/1987) one year after the commencement of the recombinant growth hormone treatment.

Patients	Alanine	Glycine	Valine	Threonine	Serine	Leucine	Isoleucine	Lysine	Tyrosine
1. LB	268	270	169	99	153	94	63	96	55
2. AMcE	323	296	210	107	124	125	43	108	61
3. CL	303	238	195	108	115	116	70	83	64
4. SD	284	250	185	58	138	121	57	90	57
5. AMcC	347	288	230	110	150	135	70	146	63
6. PC	355	267	225	103	149	119	61	128	55

Table I.1 SDS for plasma indispensable amino acids in children with cerebral tumours who responded well to growth hormone treatment during the period of review.

Patients	Valine			Leucine			Isoleucine			Threonine			Lysine		
	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87
1. DH	+0.68	-0.87	+0.86	+0.23	-0.79	+0.03	+1.45	-1.21	+0.08	-0.18	-0.33	+0.74	-0.02	-0.34	+0.45
6. JMcD	-0.83	-1.89	-1.04	+0.93	-1.34	+0.46	-0.01	-0.65	+0.90	+0.27	+0.98	+0.94	+1.13	-0.86	-0.43
7. KT	+0.60	-1.28	+0.38	-0.27	-1.80	+0.60	-0.60	-1.24	+0.55	+0.74	+0.12	+1.27	+0.09	-0.18	+1.13
3. CL'F'	-0.92	-1.47	-0.47	-0.05	-1.07	-0.33	+0.27	-1.13	+1.57	-0.90	+0.50	+0.67	-0.94	-1.11	-1.01
4.SD'F'	-1.21	-1.51	-0.91	-0.89	-0.93	-0.14	-0.86	-1.02	+0.24	-1.05	-0.79	-0.80	-0.86	-2.02	-0.76
5.AMcC'F'	+2.00	-1.11	+0.50	+1.26	-0.13	+0.39	+1.21	-0.32	+1.33	+1.12	+0.89	+0.46	-0.06	-0.15	+1.45
6.PC'F'	+0.11	-0.16	+0.16	-0.16	-1.71	-0.65	+0.20	-0.87	+0.33	-0.93	-0.55	-0.15	-0.48	-0.69	+0.61
Mean	+0.06	-1.18	-0.10	+0.15	-1.11	+0.05	+0.24	-0.92	+0.71	-0.13	+0.12	+0.45	-0.16	-0.76	+0.21

Table I.2 SDS for plasma indispensable amino acids in children with cerebral tumours who responded poorly to growth hormone treatment during the period of review.

Patients	Valine			Leucine			Isoleucine			Threonine			Lysine		
	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87
2. DB	-0.91	-1.52	-1.00	-0.17	-0.86	-0.64	+0.55	-1.47	+0.88	-0.82	-0.51	-0.94	-1.19	-1.33	-0.99
3. MM	-0.46	-1.25	-0.64	+0.44	-0.39	-0.22	+1.53	-0.41	+1.21	+0.36	-0.54	+0.70	-0.97	-1.51	-0.77
4. PC	+0.36	-1.82	+0.84	+0.60	-1.00	+1.21	-0.07	-0.61	-0.38	+1.15	-0.62	+0.39	+0.72	-0.38	+0.93
5. TR	+0.64	-0.74	+0.06	+0.76	-0.72	+0.28	+1.04	-0.73	+0.32	-0.06	-0.27	-0.71	+1.46	-0.23	+0.20
1. LB'F'	+0.44	-0.80	-0.91	+0.59	-1.21	-0.99	+1.56	-0.68	+1.29	+0.39	+0.68	+0.69	+0.08	-1.22	-0.20
2.AMcE'F'	-0.08	-0.85	+0.26	-0.93	-1.08	+0.36	-0.45	-1.08	-0.91	+0.22	-1.20	+0.8	+0.41	-0.35	+0.13
Mean	-0.00	-1.16	-0.23	+0.22	-0.88	0.00	+0.69	-0.83	+0.40	+0.21	-0.01	+0.16	+0.09	-0.84	-0.12

Table J.1 SDS for plasma dispensable amino acids in children with cerebral tumours who responded to growth hormone treatment during the period of review.

Patients	Alanine			Glycine			Serine			Tyrosine		
	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87
1. DH	+0.24	-0.46	+0.35	+0.67	-0.39	-0.11	-0.05	+0.29	-0.31	+0.87	+0.72	-0.80
6. JMcD	+0.16	-0.64	-1.52	-0.95	-1.24	-0.65	+0.15	+0.01	-0.19	-0.10	+1.00	-0.21
7. KT	+0.26	+0.66	+0.19	-0.56	-0.99	+0.46	-0.78	-0.68	+1.48	+0.47	-0.38	+1.47
3. CL'F'	-1.21	-0.89	-0.58	-0.43	-0.81	-0.77	-0.58	-0.95	-0.64	+0.79	+1.23	+1.26
4.SD'F'	-1.11	-1.35	-1.24	-1.20	-1.61	-0.50	-0.78	-1.20	+0.28	+0.09	-0.62	+0.31
5.AMcC'F'	+1.21	-0.15	+0.31	+1.35	-0.47	+0.34	+0.28	-0.52	+0.67	+1.20	+1.89	+0.91
6.PC'F'	+0.25	-0.22	+0.32	-0.65	-0.25	-0.25	+0.32	-0.10	+0.54	+0.30	+0.23	-0.25
Mean	-0.03	-0.49	-0.31	-0.25	-0.82	-0.21	-0.21	-0.45	+0.26	+0.52	+0.58	+0.38

Table J.2 SDS for plasma dispensable amino acids in children with cerebral tumours who responded poorly to growth hormone treatment during the period of review.

Patients	Alanine			Glycine			Serine			Tyrosine		
	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87	Apr/ 85	Jan/ 86	Jan/ 87
2. DB	-0.22	-1.37	-0.97	-0.33	-0.80	+0.26	-0.33	-0.47	+0.42	+1.16	+1.01	-0.05
3. MM	-0.60	-1.25	-0.79	+0.18	-0.60	-0.34	+0.77	-0.80	+0.18	+0.71	-0.24	+0.29
4. PC	+1.18	-0.94	+0.49	+0.72	-1.20	+0.57	-0.55	-0.21	+0.53	+1.81	+2.01	-1.77
5. TR	+0.40	-0.70	+0.35	+0.20	+0.43	+0.77	+0.49	-0.10	+0.64	-0.35	+0.18	-0.77
1. LB'F'	+0.30	-0.87	-0.41	-0.46	-0.51	+0.24	+0.73	+0.34	+1.19	-0.86	-1.89	+0.45
2.AMcE'F'	+0.11	-0.34	+0.26	+0.04	-0.88	+0.79	-0.56	-0.81	-0.21	+0.69	+0.61	+1.03
Mean	+0.20	-0.91	-0.18	+0.06	-0.59	+0.38	+0.09	-0.34	+0.46	+0.53	+0.28	-0.12

Table K. 1 Nutritional ratios for patients with cerebral tumours who fared well during the period of review.

Patients	April 1985				January 1986				January 1987			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
1. DH	0.567	1.025	0.73	0.213	0.475	0.868	0.755	0.241	0.582	1.108	0.903	0.224
6. JMcD	0.568	1.117	0.861	0.235	0.488	0.933	0.791	0.291	0.632	1.124	0.822	0.154
7. KT	0.580	1.171	0.971	0.246	0.498	0.970	0.843	0.227	0.558	1.103	0.846	0.246
3. CL'F'	0.550	0.964	0.714	0.238	0.469	0.881	0.701	0.269	0.581	1.079	0.819	0.269
4.SD'F'	0.524	0.970	0.784	0.248	0.535	1.020	0.812	0.241	0.540	0.936	0.740	0.228
5.AMcC'F'	0.578	1.037	0.818	0.197	0.510	0.955	0.717	0.276	0.554	0.993	0.799	0.219
6.PC'F'	0.547	1.031	0.882	0.235	0.490	0.897	0.804	0.219	0.525	0.974	0.799	0.219
Mean	0.559	1.045	0.830	0.230	0.496	0.932	0.775	0.252	0.567	1.032	0.818	0.223
P value					0.003	0.013	0.034	0.17	0.008	0.01	0.14	0.11

Table K.2 Nutritional ratios for patients with cerebral tumours who fared less well during the period of review.

Patients	April 1985				January 1986				January 1987			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
2. DB	0.556	0.997	0.749	0.255	0.507	0.899	0.732	0.268	0.527	0.904	0.702	0.199
3. MM	0.566	0.976	0.740	0.223	0.548	0.972	0.724	0.208	0.574	1.026	0.797	0.227
4. PC	0.526	1.028	0.767	0.243	0.472	0.830	0.640	0.273	0.565	1.047	0.841	0.138
5. TR	0.578	1.072	0.848	0.189	0.501	0.880	0.709	0.201	0.526	0.958	0.759	0.166
1. LB'F'	0.569	1.024	0.845	0.181	0.471	0.811	0.715	0.151	0.546	0.771	0.626	0.204
2.AMcE'F'	0.495	0.907	0.741	0.217	0.491	0.925	0.772	0.250	0.521	0.938	0.707	0.205
Mean	0.548	1.001	0.782	0.218	0.498	0.886	0.715	0.225	0.529	0.941	0.739	0.190
P value					0.017	0.007	0.04	0.758	0.16	0.28	0.53	0.16

Table L.1 Bone age (year) at 1986 and 1987 and its advancement during the one year period for the patients with IGHD.

Patients	1986	1987	BA advance/y
1.AB	1.2	2.5	1.3
2.JC	9.2	9.8	0.6
3.AG	9.3	9.9	0.6
4.AlB	8.1	9.0	0.9
5.NMcQ	12.9	13.5	0.6
6.CMcG	10.9	13.4	2.5
7.RH	11.7	13.0	1.3
8.CG	10.8	11.7	0.9
9.NC	14.1	15.0	0.5
10.SS	8.7	9.0	0.3
11.BL	11.6	11.8	0.2
12.IMcC	11.8	14.4	2.6
13.DB	17.7	18.0	0.3
14.NMCG	16.6	17.0	0.4
1.SS"F"	5.0	6.2	1.2
2.CS"F"	5.6	7.3	1.7
3.LB"F"	4.8	7.2	2.4
4.TG"F"	11.8	12.2	0.4
5.AQ"F"	10.6	11.9	1.3

Table L.2 Bone age (years) of tumour patients at 1986 and 1987 and its advancement during the one year of recombinant growth hormone treatment.

Patients	1986	1987	BA advance/y
1.DH	13.4	13.8	0.4
2.DB	14.1	15.6	1.5
3.MM	16.3	16.9	0.6
4.PC	14.4	15.17	0.77
5.TR	12.3	13.0	0.70
6.JMcD	13.3	14.0	0.70
7.KT	12.5	13.9	1.4
1.LB"F"	9.0	10.0	1.0
2.AMcE"F"	9.3	9.9	0.6
3.CL"F"	11.6	13.0	1.4
4.SD"F"	11.6	12.5	0.9
5.AMcC"F"	13.0	13.9	0.9
6.PC"F"	12.2	13.1	0.9

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