INFLUENCE OF SELECTED AMINO ACID DEFICIENCIES ON SOMATOMEDIN AND GLYCOSAMINOGLYCAN METABOLISM

by

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To

My parents and sister whose love, encouragement and sacrifices made my education possible.

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INTRODUCTION

Normal growth depends upon the regulated influence of both hormonal and nutritional factors, though nutrition perhaps, could be singled out as the most important factor affecting growth. Malnutrition is frequently associated with growth retardation, but the exact mechanism for the growth failure has not been clearly established.

Historical Perspective

One of the classical discoveries in the field of endocrinology has been the growth-promoting activity of the anterior part of the pituitary gland. Growth hormone, of pituitary origin, has been recognized for over 50 years as a factor critically important for growth (1). Disorders of growth are the most common problems relating to the skeletal system encountered in children. By the use of purified preparations and treatment of growth hormone-deficient animals (2) or children (3), growth hormone, in vivo, has been shown to promote skeletal growth. This is done largely through proliferation of growth cartilage at the epiphyseal plates of long bones (2). However, accumulating evidence has shown that growth hormone itself does not stimulate linear growth, but rather induces the formation of a secondary growth-promoting factor, or factors.

In recent years, rapid progress has been made in characterizing this growth factor. In studying the mechanism by which growth hormone promotes growth, Salmon and Daughaday (4) made the first observations that the effect of growth hormone on the growth of skeletal tissue is through a biologically active factor. This substance had originally been termed

"sulphation factor" (SF) to describe its stimulatory action on the incorporation of radioactive sulphate by the costal cartilage of hypophysectomized rats. However, as more information became available, apparently this term was too restricted. Since findings showed this substance to play a much more profound role in cell growth than initially suspected, investigators proposed that the operational term SF be discarded in favor of the more generic name "somatomedin" (5). The prefix 'somato' connotes both a hormonal relationship to somatotropin and also to the soma, which is the target tissue of this agent. The suffic 'medin' was chosen to indicate that it mediates the action of growth hormone.

The somatomedin activity of serum is at least partially growth hormone dependent, and normally parallels growth hormone concentration (6). However, there are exceptions, and several conditions have been described in which somatomedin levels are related inversely to growth hormone levels (7,8). In children with kwashiorkor and other nutritional disturbances, several investigators have reported depressed somatomedin activity, despite elevated growth hormone (7). This altered relationship is reversed by protein feeding (9). Acute studies performed in fasted rats demonstrated marked reduction in plasma somatomedin activity as well as decreased sulphate uptake by cartilage, partial recovery usually being evident within 6-12 hours after refeeding (10). These data certainly suggest that nutrition is an important modulator of serum somatomedin activity and cartilage growth, independent of growth hormone secretion. Additionally, serum somatomedin and cartilage sulphate uptake appear to reflect major changes in nutritional status. However, the extent to which somatomedin is dependent, both upon growth hormone and nutritional factors, has yet to be established.

Since malnutrition is widespread in the world today, the need for further investigations into nutritionally related growth disorders grows stronger with the passage of time. This study was part of a U.S.D.A. Grant Project (Proposal No. 7900641) entitled "Nutritional Factors Regulating Somatomedin and Growth." The overall objective and long-term goal of the project is to gain further understanding of the role that specific nutrients and nutrient interrelationships have on normal growth and development, as well as growth retardation associated with malnutrition. We are aware that a deficiency of certain dietary constituents such as proteins, specific amino acids, vitamins and minerals cause impaired growth, but the mechanisms of their actions and interactions remain obscure, and their effect on growth hormone levels, somatomedin activity or cartilage metabolism is not known. Nor is there data which suggest specific nutrient requirements in relation to these growth parameters during recovery from malnutrition. If an understanding of the role that known required nutrients have on growth via their modulation of somatomedin activity can be sought, and if it can be demonstrated that certain nutritional deficiencies cause specific alterations at the cartilage level, this may provide important clues, as well as strengthen our understanding of nutritional needs for normal growth, and the growth retardation that follows malnutrition.

Despite the great need for further studies, not much research has been conducted on the effects of protein depletion on somatomedin levels in the body. Little data is available regarding deficiencies of protein or specific amino acids on plasma somatomedin activity. Some previous data does suggest that severe growth retardation, in addition to a marked decrease in plasma somatomedin, in the face of normal or elevated growth

hormone concentration, also occurs with decreased dietary protein (11). Similarly, dietary deficiencies of valine, tryptophan and arginine resulted in marked suppression of serum somatomedin activity in rats compared with the activity of those rats on complete diets (12). But a great deal of further investigation is necessary before any facts can be established.

The specific objective of this study was to investigate the influence of selected amino acid deficiencies on somatomedin and cartilage glycosaminoglycan metabolism. The specific amino acids chosen for this study were lysine, methionine and histidine. The reasons for the selection of those particular amino acids were as follows: lysine was included because it is one of the most limiting amino acids in cereal and grain products, methionine was selected as one of the sulphur containing amino acids, and finally the reason for selecting histidine was its importance in relation to growth in young children and growing animals.

REVIEW OF LITERATURE

In the recent past, considerable efforts have been made to investigate and explain the characteristics and actions of somatomedin, a relatively unknown and puzzling anabolic substance.

Classification of Somatomedin

With increasing sophistication of analytic and chemical techniques, evidence has shown that plasma contains more than one growth-promoting substance under growth hormone control (13). To avoid confusion and the proliferation of new names, Hall and Van Wyk (14) proposed that the term somatomedin be retained, and that different members of this class of compounds be identified by letter suffixes. To date, at least three somatomedins have been described with different chemical and biological properties: Somatomedins A, B and C. In addition, two other growth-promoting polypeptides have been isolated from human plasma; insulin-like growth factor 1 (IGF-1), formerly known as non-suppressible insulin-like activity (NSILA), and insulin-like growth factor 2 (IGF-2) (15). Yet another growth-promoting polypeptide, multiplication-stimulating activity (MSA) has been purified from calf serum (16) and more recently from liver (17). Measurements of circulating somatomedin activity are based on the ability of serum samples to stimulate sulphate uptake by cartilage, in vitro, in bioassays (18).

Purification and Chemical Properties

During the past few years, considerable progress has been made toward the isolation and chemical characterization of the somatomedins (13, 19, 20). Starting with somatomedin-rich plasma from acromegalic

subjects, the biological activity in native plasma was found to be associated with high molecular weight proteins of 50,000 daltons or more. But after extraction with cold acid ethanol, about 20-40% of the original activity could be recovered, while precipitating 99% of the plasma proteins (21). After acid ethanol extraction, further purification is achieved using gel filtration and ion exchange chromatography (22). The greatest resolution has been obtained using isoelectric focusing. When this procedure is utilized, somatomedin activity is recovered in an acid, neutral and (or) basic pH range (22). All have molecular weights ranging from 6,000 to 11,000 daltons (22).

The neutral peptide has been termed somatomedin A and has been found to be most active in chick embryo system (15), stimulating sulphate uptake by chick cartilage. The acidic peptide, termed somatomedin B, has a molecular weight of approximately 6,000 daltons, slightly less than somatomedin A. It stimulates thymidine incorporation by human fibroblasts and glial-like cells (13). The basic peptide has been termed somatomedin C (22). It is an arginine-rich peptide of about fifty amino acid residues and is very active in stimulating isotope-labelled sulphate and thymidine uptake in hypophysectomized rat cartilage (17). The molecular weight is very similar to somatomedin A.

The somatomedin peptides identified to date appear to circulate bound to large carrier proteins of approximately 60,000-70,000 daltons molecular weight (23, 24). "Free" unbound somatomedins comprise less than 5% of total serum levels. Although the carrier proteins exhibit some growth hormone dependence (25, 26), their regulation and biological function is still unclear. The carrier proteins appear to prolong the half-life of

circulating somatomedins (27), and may also modulate their action on target tissues. "Free" somatomedin is cleared by the body within minutes, whereas the half-life of bound somatomedin is 3-5 hours in rats (28). The development of sensitive and precise radioreceptor and radioimmunoassays has resulted from partial purification of somatomedins (29). Measurements with these procedures have confirmed the growth hormone dependence of somatomedins, and have reinforced the concept that the somatomedins are a family of circulating factors.

Somatomedin activity may also be measured by other properties of somatomedins, such as insulin-like activity. Somatomedin has markedly insulin-like properties, and strong evidence has been advanced that not only is somatomedin insulin-like in its biological actions, but that highly specific cellular receptors in certain tissues are incapable of distinguishing between insulin and somatomedin (30).

Despite differences among the various somatomedins, they all, however, meet the criteria established for a somatomedin (22), that is:

1) They are to some extent under the control of growth hormone.
2) They have been shown to be insulin-like in their activity.
3) They stimulate cell growth in one or more tissues.
4) They stimulate sulphate in at least one species.

Relation of Somatomedins to Growth

The concept that somatomedins mediate human skeletal growth is supported largely by correlations of somatomedin levels with growth velocity in children. In hypopituitary children, treated with growth hormone, Hall and Olin (3) demonstrated the increase in height to be significantly correlated with the rise in their serum somatomedin activity. When the

growth rate inevitably decreased after one to seven years of therapy, the somatomedin levels followed the same downward trend, substantiating the age dependency of plasma somatomedin. Van den Brande and Du Caju (7) reinvestigated the dose response relationship between the log of the amount of growth hormone administered and plasma somatomedin levels in two hypopituitary patients, and found the correlation to be very high ($r = 0.91$). These same authors also found that normal children tall for their age tend to have relatively higher serum somatomedin activity (1.34 ± 0.41), while those short for their age manifest relatively lower levels (0.80 ± 0.15). They found no sex differences to be apparent. In children with obesity, plasma somatomedin was not different from normal (0.94 ± 0.15) contrasting with the low plasma growth hormone levels found in three of the five obese children (7). A hypothesis suggested by Van den Brande and Du Caju as a result of these findings, is that the growth capacity of some essential parenchymatous organs, such as the liver, may determine the production of somatomedins, which in turn may serve as messengers to the growing supporting tissues, thus ensuring harmonious growth of the whole organism. However, such a construction can only partially explain the change in growth rate and plasma somatomedin activity with increasing age.

Infants and very young children have lower levels of somatomedins and serum somatomedin activity despite their faster growth rates than older children (31, 32). Whether this is due to the altered levels of carrier proteins, has not been clarified. In animal studies, cartilage from younger rats or pigs appears more sensitive to stimulation by somatomedins (33, 34). These observations suggest that the rapid growth in young children may be due in part to increased tissue responsiveness (29).

Information on somatomedin during pregnancy is still limited, and the role of somatomedins in the rapid growth phase of fetal development is not clearly defined. Bioassayable somatomedin in pregnant women has been reported either normal (6, 35) or low (36) as compared to normal adult levels. In contrast, fetal somatomedin levels in human cord blood have been found invariably low by all workers, both by bioassay and radioreceptor assay (31, 36, 37). However, levels of somatomedin activity and somatomedin C in cord blood are correlated with fetal length, fetal weight and placental weight (37, 38). Since somatomedin in the fetal or neonatal circulation does not correlate with either growth hormone or human chorionic somatotropin levels (39), investigators have concluded that somatomedin is controlled by factors other than growth hormone during this period. Baumann (40) suggests that somatomedins may be regulated in utero by both insulin and nutrition, factors considered important for intrauterine development.

A genetic defect in somatomedin synthesis has been identified in Laron's dwarfism (41). Laron's syndrome is characterized by severe dwarfism, high circulating levels of immunoreactive growth hormone, but very low levels of somatomedin (41, 42). Significantly, the latter did not respond to administered growth hormone by increasing their somatomedin activity (42, 43), and failed to produce an acute metabolic response as measured by mineral retention, nitrogen retention or release of free fatty acids from adipose tissue; however, an unsustained growth response to long-term administration of human growth hormone was observed (42). Elders et al. (42) suggest that this could be due to the absence of growth hormone receptors in the target tissue, or alternatively the inability of the target tissue to synthesize somatomedin. Presumably, these patients could successfully be treated with purified somatomedin.

Despite increasing circumstantial evidence for the association of somatomedins with growth, until recently there has not been any direct evidence that administration of somatomedins to humans or animals can stimulate growth (29). However, there is some evidence now, that growth hormone-deficient mice have a substantial increase in growth cartilage activity as well as body length and weight when they are treated with somatomedins partially purified from human plasma (44). This preparation of somatomedins contains no other recognized anabolic factors such as growth hormone, insulin, testosterone or thyroxine. Therefore, if these findings are confirmed, they will constitute the strongest evidence to date that somatomedins contribute directly to mammalian growth (29).

Origin of Somatomedins

Identification of the site of somatomedin production has been difficult, in that extraction procedures have not revealed a tissue particularly enriched in somatomedin content (29). So far, the mechanism by which growth hormone stimulates somatomedin production remains unclear. Extraction of many organs has revealed no source richer than plasma itself (30). However, the evidence available so far, does favor the liver and possibly the kidneys as tissues of origin. Bioassayable somatomedin activity is reduced in humans with chronic liver and kidney disease (45, 46). Whether this is due to a decrease in somatomedin production, an accumulation of somatomedin inhibitors (47) or just a non-specific effect of altered nutritional balance due to chronic illness is unclear. Retarded growth and absence of acromegalic features were observed in patients with chronic renal failure, in spite of increased somatomedin A and growth hormone levels (48). Takano et al. (48) attributed this to inhibitory factors present in the sera or to a decreased

sensitivity of the target organs. They suggested that the elevated somatomedin A levels in uremic patients can be partly attributed to a longer biological half-life for somatomedin, since the kidneys are believed to be one of the organ systems involved in the degradation of somatomedin (49).

A study by Saenger et al. (50) indicated the presence of low serum somatomedin levels (0.39 $\pm$ 0.10 U/ml) in children with end stage renal disease and growth retardation. Somatomedin rose toward normal (0.84 $\pm$ 0.14 U/ml) after renal transplantation. Post-transplant growth velocity was correlated directly ($P<0.05$) with serum somatomedin levels, suggesting that the growth failure in these uremic patients was due, in part, to lack of serum somatomedin.

Perfusion studies have contributed more direct evidence that liver is a major site of somatomedin production (51-53). Addition of growth hormone to the perfusion fluid led to a dramatic increase ($P<0.01$) in the somatomedin content of the effluent (51). Similar findings were obtained by perfusing rat kidney (54). Apparently the somatomedins are released by the liver under regulation by the growth hormone (52, 53) and this process requires protein synthesis (55). Studies by Moses et al. (56) suggest that the somatomedin-binding proteins, like somatomedin itself, may also be synthesized in the liver. Spencer (57) demonstrated the production of the binding proteins by hepatocytes, establishing that the liver is their site of synthesis.

Nutritional status and insulin also appear to regulate production of somatomedins. Daughaday et al. (58) found fasted animals to have a decreased hepatic release of somatomedin activity. Direct addition of insulin appears to enhance release of somatomedin (53). In another study

Shapiro et al. (59) demonstrated that the livers of well-nourished rats, perfused with a hormone-free medium, showed a significant generation of somatomedin activity over two hours (124.1 ± 5.6). But the livers of protein malnourished rats showed much less activity (108.4 ± 4.9) ($P<0.05$). These same authors also found that insulin caused a greater stimulation of somatomedin generation by the livers of well-nourished rats in vitro (211.5 ± 10.4) (P 0.005). The reduced somatomedin activity during protein malnutrition may be a specific homeostatic mechanism by which the growth stimulating action of growth hormone might be modulated at a time when growth is inappropriate for survival. On the other hand, the reduced somatomedin activity may be a response to alterations in liver function and the general synthesis of proteins by the liver (59).

Despite identification of the liver and kidney as organs capable of generating somatomedin-like activity when stimulated by growth hormone, little growth hormone is actually stored in these organs (30). And the exact mechanism for the formation and secretion of somatomedin still remains obscure.

Action of Somatomedin on Body Tissues

Somatomedins have broad anabolic actions on body tissues, which were summarized by Phillips and Vassilopoulou-Sellin (29) (Table 1).

The best known biological effects of somatomedin are on the cartilage cell. That the actions of growth hormone on cartilage are attributable to somatomedin, is well accepted now (60). Somatomedin is transported via the plasma, from its site of synthesis, to the cartilage cell where a number of anabolic events are initiated (61). These include the incorporation of ^{3}H-thymidine into DNA (62-64) and ^{3}H-uridine into RNA (63, 64), the

TABLE 1. Somatomedin Effects on Tissues (29).

Tissue	Effects
Cartilage	Increased sulphate uptake
	Increased DNA and RNA synthesis
	Increased protein synthesis
	Increased collagen synthesis
Muscle	Increased protein synthesis
	Increased transport and uptake of amino acids
	Increased glucose uptake
	Increased incorporation of glycine into glycogen
Adipose	Increased DNA synthesis
	Increased glucose oxidation
	Decreased lipolysis
	Increased lipid synthesis
Cell cultures	Increased replication

incorporation of ^{14}C-leucine into protein-polysaccharide complexes (63-65), $^{35}SO_4$ into chondromucoprotein, the conversion of ^{14}C-proline into hydroxyproline of collagen (67), the biosynthesis of glycosaminoglycans (GAGS) and increased thickness of the epiphyseal growth plate with resultant long bone growth (68). In addition, the somatomedins stimulate amino acid transport and synthesis of RNA, DNA, protein and chondroitin sulphate in cartilage (69).

Although sulphate incorporation is the final step in GAGS biosynthesis, an alteration at any step in the biosynthesis could result in decreased sulphate uptake. At present it is not known which of these steps somatomedin specifically stimulates or which may be altered by other factors (60). GAGS is known to be synthesized by the sequential addition of two galactose residues and one glucuronic acid residue (70). These biological effects were ascribed previously to growth hormone; however, the somatomedin mediated effects of growth hormone have been amply demonstrated now. Growth hormone in vitro is without effect on cartilage (4).

In muscle, somatomedins enhance protein synthesis and glucose uptake (71). The action of protein synthesis is immediate and requires only minimal amounts of the hormone, whereas the action of growth hormone on muscle, in vitro, is very weak and delayed (72). The same is true for the effects of somatomedin on liver (73).

In the adipose tissue, somatomedin promotes glucose oxidation (72, 74), stimulates lipid synthesis, but opposes the effect of epinephrine on stimulating lipolysis (75). Somatomedin depresses the basal adenylate cyclase levels in membranes from spleen lymphocytes, fat cells, liver cells and chondrocytes (76). Somatomedin also blocks the adenylate cyclase normally produced in lymphocytes and fat cells by epinephrine and in chondrocytes by parathormone.

Although the above mentioned actions are insulin-like, they are not suppressed by antibodies to insulin (77). Somatomedins can also inhibit insulin degradation (49). Cartilage can be stimulated by insulin itself too, but only at supraphysiological concentrations of the latter (64, 65). Similarity of action between insulin and the somatomedins is

thought to be due in part to overlapping occupancy of receptor sites on target tissues. Competition for the same receptor site occurs particularly with fat cells; less overlapping occurring with receptors on placental, hepatocyte, monocyte and fibroblast plasma membranes, and very little with receptors on cartilage cells (49, 78-80). The cartilage cell is much more sensitive to somatomedin than to insulin.

Somatomedins and Somatomedin Inhibitors

As measured in bioassays, somatomedin activity reflects not only the presence of somatomedins, but of somatomedin inhibitors as well (29). Whole serum from normal humans or animals has net cartilage stimulating activity (2). On the other hand, serum from severely malnourished humans (7, 81) or from hypophysectomized, starved or severely diabetic rats (82-84) not only provides no cartilage stimulation, but may actually suppress somatomedin activity when added to normal serum. Phillips (85) has attributed this to the presence of somatomedin inhibitor(s); stimulatory sera are rich in somatomedins and inhibitory sera rich in somatomedin inhibitors.

In a subsequent study, Phillips et al. (86) showed that at pH 7.0 (somatomedins presumably bound to carrier proteins) only somatomedin activity was found in serum from normal rats, while only somatomedin inhibitory activity was found in serum from diabetic rats. In contrast, at pH 2.4 (somatomedins presumably dissociated), somatomedin activity and somatomedin inhibitory activity was found in both sera. Serum from diabetic rats contained more somatomedim inhibitory activity and less somatomedin activity than serum from normal rats. The somatomedin inhibitory activity had an apparent molecular weight less than carrier-bound somatomedin, but greater than dissociated somatomedins. These observations further demonstrate that

somatomedin activity in whole serum reflects the presence of both somatomedins and somatomedin inhibitor(s). It also reflects that the decrease in somatomedin activity in serum from diabetic rats is apparently a result of both a decrease in somatomedins and an increase in inhibitor(s).

Salmon (82) found this inhibitory factor(s) to be non-dialyzable, probably a peptide, and possibly obscuring the presence of low levels of somatomedins, as are present in hypophysectomized rats. The destruction of this inhibitory activity by incubation with trypsin is consistent with dependence on a peptide component. Although theories for a physiological role of somatomedin inhibitor(s) have been postulated, none have been clearly elucidated yet. However, as more information becomes available on them, these inhibitory factor(s) could provide an additional mechanism for limiting growth and conserving metabolic fuel in conditions of nutritional and (or) hormonal deficiency.

Factors Influencing Somatomedin Activity

Various factors have been shown to effect somatomedin activity to varying degrees. These include age, nutritional status, constitutional and other as yet unidentified factors, summarized in Table 2 (60).

Among the factors which have a considerable influence on somatomedin activity, nutritional status has a great deal of importance in the underlying regulatory mechanisms. That nutritional status alters somatomedin activity is a well-established fact by now. Malnutrition, prolonged fasting or protein deficiency, all lead to low somatomedin levels (7-12, 87). Many studies have amply demonstrated that nutrition plays an important role in the generation of somatomedin (10-12, 88-90). Dietary protein is essential to maintain somatomedin activity. Yeh and Aloia (88) recently

TABLE 2. Factors Which Influence Plasma Somatomedin Activity (60)

Factors Studies	Effect on Plasma: Somatomedin Activity	Effect on Plasma: Growth Hormone Concentration	Stature
Endocrine			
Growth hormone			
Excess	High	High	Tall
Deficiency	Low	Low	Short
Limited responders	Low-normal	Low-normal	Short
Thyroid hormone			
Excess	Normal	Normal	Normal-tall
Deficiency	Normal-low	Normal-low	Normal
Insulin			
Excess	Normal	Normal-low	Normal-tall
Deficiency	Normal-low	Normal	Normal
Sex hormones			
Androgens	Normal	Normal	Tall
Estrogens	Decreased	Normal	Normal
Environmental			
Nutritional status			
Fasting	Low	High	---
Malnutrition	Low	Low	Short
Obesity	Normal	Normal-low	Tall
Emotional Deprivation syndrome	Low	High	Short
Constitutional			
Stature			
Short	Low	Normal	Short
Tall	High	Normal	Tall
Dysmorphic dwarfism	Low	Normal	Short
Sex	Normal	Normal	---
Age	Increases	Normal	---
Genetic			
Laron's dwarfism	Low	High	Very short
Turner's syndrome	High	Normal	Short
Noonan's syndrome	High	Normal	Short
Cerebral gigantism	Low	Normal	Tall
Achondroplasia	High	Normal	Short
Drugs			
Estrogens	Decreased	?Normal	---
Glucocorticoids	Decreased	?Normal	Short
Testosterone	No effect	Normal	---
Other			
Free fatty acids	Decreased	Normal	---
Uremia	Decreased	Normal	Short

demonstrated the serum somatomedin activity of rats measured under several dietary conditions. The mean (± SD) serum somatomedin activity (units/ml) was reduced following three days of fasting (0.41 ± 0.12) and increased to prefasting levels in animals fed a balanced diet (0.95 ± 0.11) for two days. The increase in mean somatomedin activity following two days of refeeding a high-protein diet (0.79 ± 0.09) was greater than that observed with a high-carbohydrate (0.56 ± 0.10) or a high fat (0.60 ± 0.10) diet, suggesting that the protein content of the diet plays a major role in the restoration of serum somatomedin activity following refeeding. These findings conflict with those of Phillips and Belosky (91) who found somatomedin activity to be increased most by refeeding fat and least by refeeding protein when the calories consumed were identical. However, the two experiments differed in the dietary composition, caloric consumption, number of days of refeeding and method used to measure somatomedin activity (porcine cartilage as opposed to chick embryo assay). In addition to the importance of dietary protein, a balanced mixture of amino acids is required. The latter is true because stimulation of cartilage sulphate uptake by somatomedin was shown by Salmon (90) to be dependent on the amino acid pool of the tissue, rather than on a single amino acid.

Elevated levels of free fatty acids in the plasma have been shown to be inhibitory to plasma somatomedin in vitro (91-93). At high levels of dietary fat (20%) an inverse relationship to plasma somatomedin activity was found to exist when the protein intake (20%) was adequate (94). The above observations indicate that both the total quantity of calories in the diet and the distribution of dietary nutrients are important for the regulation of circulating somatomedin and the preservation of somatomedin-induced skeletal growth.

Since absorbed nutrients require the presence of insulin for normal utilization in body tissues, the relation between somatomedin activity and insulin is also of great importance. Phillips and Young (84) have shown that despite the abundance of circulating nutrients (glucose, amino acids, free fatty acids and ketoacids), lack of insulin per se leads to a state of lowered somatomedin activity, decreased growth cartilage activity and poor growth. These same investigators suggest that this may occur due to a diminished intracellular utilization of the circulating nutrients, in the absence of insulin. Thus insulin-directed processing of nutrients is a critical step in the maintenance of somatomedin activity, and subsequent normal growth.

Thyroid hormone is another substance essential for normal growth and potentiates the growth-promoting actions of growth hormone in vivo (2). However, there is not much evidence to indicate that it does so by the direct effects on the generation or actions of somatomedins. The association of hypothyroidism and growth hormone deficiency is well recognized. Administration of exogenous growth hormone to hypothyroid rats did not restore the serum growth hormone levels of the latter to normal (95). But treatment with thyroid hormone brought a positive response to the stimulation test. Treatment of hypophysectomized rats with T_4 was shown to stimulate serum somatomedin activity in the absence of growth hormone (96). This further substantiates the fact that somatomedins are not totally growth hormone dependent, and that growth is a complex process involving several factors.

Plasma lactogen is a factor similar to growth hormone in both structure and biological properties (68). Apparently it has somatomedin

regulating effects similar to those of growth hormone (97, 98). Prolactin is a pituitary factor with some growth hormone-like properties. But there is less conclusive evidence that it also contributes to somatomedin regulation (99, 100).

The steroid hormones are associated with marked changes in growth, due however, only in part, to somatomedin modulation. While investigating hormonal effects on somatomedin generation, Phillips et al. (101) showed androgens to have little effect on somatomedin activities. High doses of testosterone had no significant effect on somatomedin generation. Estrogens in large doses, however, significantly inhibited growth hormone-induced somatomedin generation in vivo, but had no effect on somatomedin in vitro. These investigators thought that probably estrogens may antagonize the effect of growth hormone on skeletal growth by this inhibition of somatomedin generation. This antagonistic effect of estrogen provides a rational basis for therapy in some patients with acromegaly.

Administration of glucocorticoids universally causes inhibition of growth. Elders et al. (102) found that glucocorticoid administration (2.2 mg/kg of body weight of methyl prednisolone sodium succinate) decreased serum somatomedin activity ($P<0.001$) as assayed by radioactive sulphate uptake in embryonic chick cartilage. This provides a plausible explanation for the growth inhibition produced by these hormones. Glucocorticoid-induced retardation of growth may result from any four possible mechanisms: a decrease in growth hormone release, a decrease in growth hormone-induced somatomedin generation, a decrease in somatomedin action on cartilage, or a direct inhibitory effect of glucocorticoids on cartilage (101).

MATERIALS AND METHODS

Animals

Four-week old weanling, male rats of the Sprague-Dawley strain,[1] with weights ranging from 70-100 grams on arrival, were housed individually in mesh wire cages. They were maintained under constant environmental conditions (21-24°C, 40-42% relative humidity, and a 12-hour light cycle). During an initial acclimatization period of four days, rats received laboratory stock chow[2] and water ad libitum. Rats were assigned randomly to cages. The mean weight for each experimental group was tabulated and rat distribution was adjusted to account for unbalanced mean weights.

The experimental design is depicted in Fig. 1. There were 10 rats in each of the seven experimental groups. Rats were obtained in batches of 14/week and were assigned 2 rats/group over a five-week period, until all groups contained 10 animals/group. The seven experimental diets formulated were as follows: four control groups; A through D, fed an amino acid defined diet ad libitum, 15 g/day, 10 g/day and 5 g/day respectively, and three groups with modifications in the amino acid content of their diet; E through G, lysine deficient, methionine deficient and histidine deficient respectively. In addition, a control group was maintained on laboratory chow, fed ad libitum. Food intake and body weight were recorded for each animal on alternate days.

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[1] Obtained from Gibco Laboratories, Madison, Wisconsin.

[2] Rodent Laboratory Chow, #5001, obtained from the Ralston Purina Company, St. Louis, Mo.

EXPERIMENTAL DESIGN

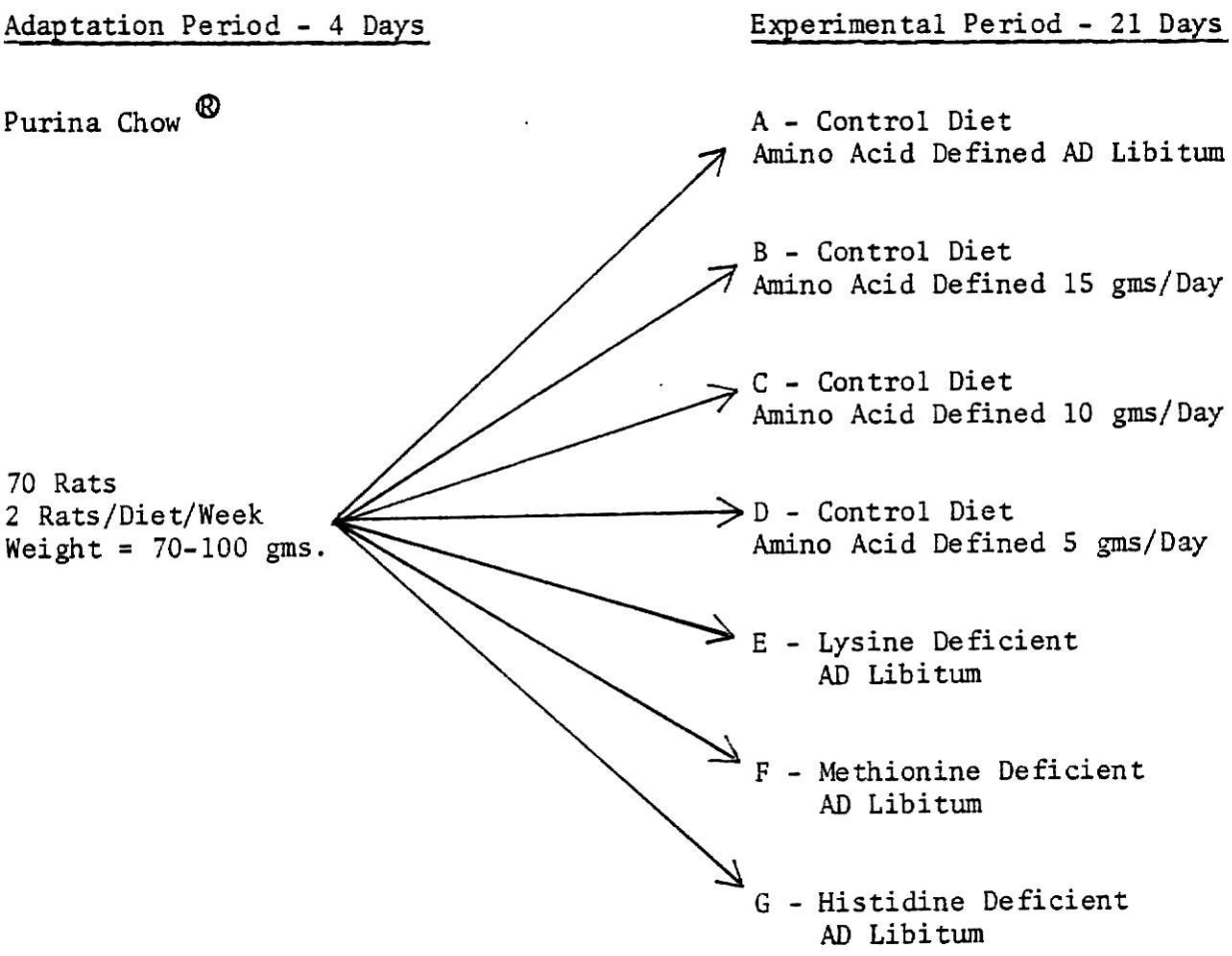

Fig. 1. The Experimental Design for 70 Rats Over a 21-Day Observation Period.

There were 10 rats in each of the 7 experimental diet groups. In addition, there was a control group maintained on laboratory chow fed ad libitum. All animals had ad libitum amounts of distilled water to drink.

All the rats were maintained on the experimental diets for a period of three weeks. Each animal was autopsied on the twenty-second day. At the time of sacrifice, blood and tissues were collected. Sixteen hours prior to autopsy, each rat was injected intra-peritoneally with 25 uCi of $^{35}S^{3}$ per 100 grams of body weight.

Diets

Synthetic amino acid test diets were prepared by Teklad Test Diet Corporation.[4] Composition of diets is shown in Tables 3-6.

Autopsy Procedure

All animals were sacrificed using a chloral hydrate-magnesium sulphate anesthesia[5] (L. A. Thesia ®, 0.5 c.c./100 grams of body weight), injected intra-peritoneally. Blood was obtained via abdominal aorta, centrifuged and serum used for assaying somatomedin activity and growth hormone concentration. The tissues removed at autopsy were rib cartilage, liver, brain, pituitary, parotid, kidneys and long bones. The 7th and 8th rib cartilage on the right side were removed and GAGS isolated as described below, for measuring sulphate uptake. All organ weights were recorded. Tissues were all frozen at -20°C until further assay.

Tissue Preparation

Plasma for somatomedin activity was separated, heated to 56°C, centrifuged and stored at -20°C until assay.

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[3] Isotope ^{35}S carrier-free obtained from ICN Pharmaceuticals, Chemical and Radioisotope division, Irvine, California.

[4] Obtained from the ARS/Sprague-Dawley Division of the Mogul Corporation, Madison, Wisconsin.

[5] Obtained from Haver-Lockhart Laboratories, Kansas.

TABLE 3

Composition of Amino Acid Defined Diet[1]

(Control)

Mineral Mix, Bernhart-Tomarelli (Cat. #170750)	4.0%
Vitamin Mix, Teklad (Cat. #40060)	1.0%
Fibre, non-nutritive cellulose	1.5%
Corn Oil	10.0%
Sucrose	56.5%
Corn Starch	10.0%
Amino Acid Mixture	20.0%

	gms/kg
L - Alanine	3.5
L - Arginine	11.2
L - Asparagine	6.0
L - Aspartic Acid	3.5
L - Cystine	3.5
L - Glutamic Acid	35.0
L - Histidine	4.46
L - Isoleucine	11.1
L - Lysine	14.4
L - Methionine	8.2
L - Phenylalanine	7.5
L - Proline	3.5
L - Serine	3.5
L - Threonine	8.2
L - Tryptophan	1.75
L - Tyrosine	5.0
L - Valine	8.2
Glycine	23.3

All amino acid defined diets were made isonitrogenous with non-essential amino acids (glycine) as the nitrogen source.

[1]Teklad Test Diets, TD 76471

TABLE 4

VITAMIN MIX[1]

	g/Kg of Vitamin Mix	Supplies in grams or Units per Kg of diet when added at 1% or 10 g/Kg of diet
P-Aminobenzoic Acid	11.0132	0.110132
Ascorbic Acid, Coated (97.5%)	101.6604	1.016604 = 0.9912 g Ascorbic Acid
Biotin	0.0441	0.000441
Vitamin B_{12} (0.1% trituration in mannitol)	2.9736	0.029736 = 0.0000297 g Vitamin B_{12}
Calcium Pantothenate	6.6079	0.066079
Choline Dihydrogen Citrate	349.6916	3.496916 = 1.4337 g Choline
Folic Acid	0.1982	0.001982
Inositol	11.0132	0.110132
Menadione (Vitamin K_3)	4.9559	0.049559
Niacin	9.9119	0.09119
Pyridoxine HCL	2.2026	0.022026
Riboflavin	2.2026	0.022026
Thiamine HCL	2.2026	0.022026
Dry Vitamin A Palmitate (500,000U/g)	3.9648	0.039648 = 19,824 Units
Dry Vitamin D_2 (500,000 U/g)	0.4405	0.004405 = 2,202.5 Units
Dry Vitamin E Acetate (500 U/g)	24.2291	0.242291 = 212.15 Units
Corn Starch	466.6878	4.666878

Designed for use at 1% or 10 g/Kg of diet.

[1]Teklad Test Diets, Catalog #40060

TABLE 5

Mineral Mix, Bernhart-Tomarelli[1,2]

		g/Kg
Calcium Carbonate	$CaCO_3$	21.0
Calcium Phosphate, dibasic	$CaHPO_4$	735.0
Magnesium Oxide	MgO	25.0
Potassium Phosphate	K_2HPO_4	81.0
Potassium Sulphate	K_2SO_4	68.0
Sodium Chloride	NaCL	30.6
Sodium Phosphate, dibasic	Na_2HPO_4	21.4
Cupric Citrate	$Cu_2C_6H_4O_7.2\ 1/2\ H_2O$	0.46
Ferric Citrate	(16.7% Fe)	5.58
Manganese Citrate	(.3.9% Mn)	8.35
Potassium Iodide	KI	0.0072
Zinc Citrate	$Zn_3(C_6H_5O_7)_2.2H_2O$	1.33
Citric Acid		2.2728

[1]Reference: Bernhart, F. W. and Tomarelli, R. M. (1966) J. Nutrition 89, 495-499. (Modified)

[2]Teklad Test Diets, Catalog #170750

TABLE 6

Composition of Amino Acid Deficient Diets

COMPOSITION OF AMINO ACID DEFINED DIET WITHOUT LYSINE[1]

Same formula as that used for control diet except:
1) L - Lysine was omitted.
2) Glycine was increased to 35.1355 g/kg to make diet isonitrogenous.
3) Sucrose adjusted to 567.5645 g/kg to balance the formula.

COMPOSITION OF AMINO ACID DEFINED DIET WITHOUT METHIONINE[2]

Same formula as that used for control diet except:
1) L - Methionine was omitted.
2) Glycine was increased to 27.4625 g/kg to make diet isonitrogenous.
3) Sucrose adjusted to 569.0735 g/kg to balance the formula.

COMPOSITION OF AMINO ACID DEFINED DIET WITHOUT HISTIDINE[3]

Same formula as that used for control diet except:
1) L - Histidine was omitted.
2) Glycine was increased to 28.091 g/kg to make diet isonitrogenous.
3) Sucrose adjusted to 564.669 g/kg to balance the formula.

[1]Teklad Test Diets, TD 79322 (Mod. TD 76471)

[2]Teklad Test Diets, TD 79323 (Mod. TD 76471)

[3]Teklad Test Diets, TD 79324 (Mod. TD 76471)

Tissues for GAGS (mucopolysaccharide) determination, i.e. rib cartilage, brain and liver, were prepared as follows: tissues were extracted with acetone:ether (1:1), 3 ml for cartilage and liver and 10 ml for brain, overnight at 5°C to remove neutral lipids. They were then dried in a 100°C oven for 4 hours and minced to powder. The powder was weighed to obtain lipid-free dry weight, and placed in flasks to which 0.1M sodium phosphate buffer pH 7.4 was added, approximately 1 ml/50 mg of tissue. The flasks were then placed in a boiling water bath for 10-20 minutes. After cooling to room temperature, the tissues were digested with pronase-B, 5 mg/g of tissue, and shaken in 65°C water bath for 24 hours. For removal of enzyme, contents of flasks were transferred to cooled graduated centrifuge tubes and precipitated with 0.1 ml of trichloroacetic acid. After standing 15-30 minutes at 4°C they were centrifuged at 2000 rpm for 15 minutes, following which precipitate was discarded. To the supernatants, 3 ml of cold 5% alcoholic potassium acetate was added, the mixture shaken well and allowed to stand at 4°C overnight. Next, they were centrifuged again at 2000 rpm and supernatants discarded. The precipitates were washed with 10 ml each of ethanol, ethanol:ether (1:1) and ether, and dried overnight. Finally, the samples were diluted in water for ^{35}S counts and uronic acid.

Sulphate uptake was expressed as the mean $\pm$ SEM counts per minute (CPM) per ug uronic acid. Measurement of sulphate uptake was calculated as follows:

$$\text{CPM/ug uronic acid} = \frac{\text{Total counts}}{\text{Total uronic acid}} \quad \text{(of fat-free dry weight)}$$

Bioassay for Somatomedin

Serum somatomedin activity was measured using a modification of the embryonic chick pelvic rudiment method described by Hall (103). Pelvic

rudiments were removed from 12-day old chick embryos, using sterile technique. Only rudiments from live embryos with a visible heartbeat were used. The rudiments were preincubated with shaking for four hours at 37°C in the nutrient mixture containing potassium penicillin G (Pfizerpen)[6] (315 units/ml) and streptomycin sulphate (31.2 ug/ml). Carrier-free sodium sulphate ^{35}S[7] (2 uCi) and serum were added to each flask containing two pelvic rudiments and the total volume adjusted to 2 ml by the addition of nutrient mixture. After incubating for 24 hours at 37°C, the rudiments were rinsed with water, boiled for one minute, and soaked in saturated sodium sulphate overnight to remove unbound ^{35}S. The cartilage was washed thoroughly in water, dissolved in 0.25 ml of 2.5N potassium hydroxide by boiling for 10 minutes, and the resultant solution diluted fivefold with distilled water. Aliquots were taken for radioactive counting and uronic acid.

Radioactivity was measured in a liquid scintillation spectrometer,[8] using the aqueous cellosolve-dioxane-xylene counting solution as described by Bruno and Christian (104). Somatomedin activity is expressed relative to pooled human reference serum which was assigned a somatomedin activity of 1.0.

Plasma Growth Hormone Assay

Plasma growth hormone[9] was measured using the double antibody radioimmunoassay method as described by Hervas and Escobar (105).

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[6]Park Davis and Company, Detroit, Michigan.

[7]Obtained from the same source as listed previously.

[8]Packard Autogamma Scintillation Spectrometer, Model 5230, Packard Instrument Company, Inc., Downers Grove, Illinois.

[9]Rat growth hormone preparations and the (monkey) anti-rat growth hormone serum was generously supplied by the NIAMDD rat pituitary hormone distribution program. The second antibody (rabbit anti-monkey gammaglobulin serum) was obtained from Rockland Laboratories, Gilbertsville, Pennsylvania. The non-immune monkey (Rhesus) serum was obtained from Pel-Freez Biologicals, Inc., Rogers, Arkansas.

Assay for Uronic Acid

Uronic acid level was determined by a modification of the carbozole method of Bitter and Muir (106). Reagents used were 0.025M sodium tetraborate, 0.125% carbazole in absolute ethanol and glucuronic standards of 4-40 ug/ml. Procedure used was as follows: 1 ml of the sample of standard was added to tubes fixed in a rack and cooled in an ice bath. Next, 5 ml of cold sulphuric acid was transferred to tubes with gentle shaking of the rack at first, followed by vigorous shaking. Cooling was kept constant. Tubes were then heated for 10 minutes in a vigorously boiling bath and cooled to room temperature. Carbazole reagent (0.2 ml) was then added and tubes shaken after each addition. They were then again heated in a boiling water bath for 15 minutes. Finally, optical density was read at 530 nm on a Spectrophotometer.[10]

Statistical Analysis

Data from all measurements were subjected to one-way analysis of variance (107). The means were separated by Fisher's Least Significant Difference (LSD), with probability at the 1% and 5% level as appropriate when the F-test rejected the hypothesis of equal means. Correlation coefficients were determined where appropriate to further delineate relationships among experimental variables and response variables.

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[10]Bausch & Lomb Colorimeter/Spectrophotometer, Standard Model 33-29-95-01, Rochester, New York.

RESULTS

Food Intake and Weight Change

The effect of dietary composition on weight change, food intake and food efficiency, is presented in Table 7. There were significant differences in each of these variables attributable to different dietary treatments.

Final body weights of rats fed the control diet ad libitum, 15 g, 10 g and 5 g per day were each higher ($P<0.01$) than those fed the amino acid deficient diets, except for the control 5 g group and the lysine deficient group between which there was no significant difference. Rats fed the control diet ad libitum had the highest body weights, their group mean being 157% higher than that of the rats fed the amino acid deficient diets. Within the amino acid deficient groups there were no significant differences. There was very high correlation between final body weights of all the rats and their weight change ($r = 0.99623$, $P<0.0001$) and between final body weights of the rats and their food and energy intake ($r = 0.9521$, $P<0.0001$).

Weight change (total, as well as daily weight gain or loss) was significantly different ($P<0.01$) for rats in each of the experimental groups, except between the groups of rats fed the methionine and histidine deficient diets. Weight gain was highest for rats fed the control diet ad libitum, whereas weight loss was greatest for rats fed the methionine and histidine diets. Correlation of weight change to food and energy intake of the rats was also very high ($r = 0.94$, $P<0.0001$).

Food and energy intake was higher ($P<0.01$) for rats in each of the control groups fed ad libitum, 15 g and 10 g of diet per day, as compared

TABLE 7

Effect of Dietary Composition on Weight Change, Food Intake and Food Efficiency[1]

Group	BODY WEIGHT				FOOD INTAKE		FOOD EFFICIENCY
	Initial	Final	Total Gain or Loss	Daily Gain or Loss			
			g		g/day	kcal/day	g gain/100 kcal
A Control Ad libitum	122.6[2,a] ±1.72	243.1[a] ±4.48	120.50[a] ±1.89	+5.48[a] ±0.086	14.19[a] ±0.243	64.13[a] ±1.099	8.56[a] ±0.17
B[3] Control 15 g	125.7[a] ±1.81	223.0[b] ±4.72	97.33[b] ±1.99	+4.42[b] ±0.090	12.60[b] ±0.256	56.97[b] ±1.159	7.77[b] ±0.18
C Control 10 g	124.8[a] ±1.72	173.5[c] ±4.48	48.7[c] ±1.89	+2.22[c] ±0.086	9.25[c] ±0.243	41.82[c] ±1.099	5.29[c] ±0.17
D Control 5 g	124.0[a] ±1.72	108.1[d] ±4.48	-15.9[d] ±1.89	-0.72[d] ±0.086	4.64[d] ±0.243	20.89[d] ±1.099	--[4]
E Lysine Deficient	124.2[a] ±1.72	100.5[de] ±4.48	-23.7[e] ±1.89	-1.08[e] ±0.086	7.33[e] ±0.243	33.14[e] ±1.10	--[4]
F Methionine Deficient	123.5[a] ±1.72	90.2[e] ±4.48	-33.3[fg] ±1.89	-1.51[fg] ±0.086	5.20[d] ±0.243	23.49[d] ±1.10	--[4]
G Histidine Deficient	125.1[a] ±1.72	93.0[e] ±7.08	-32.1[g] ±1.89	-1.46[g] ±0.086	5.25[d] ±0.243	23.73[d] ±1.10	--[4]

[1]Values shown are the mean ± SEM of 10 rats per group.

[2]Means in a column followed by the same superscript letter are not significantly different, $P<0.01$.

[3]Values given for this group are the mean ± SEM of 9 rats in the group.

[4]Food efficiency could not be calculated for these groups as there was net loss of weight.

with the other four groups. Within the latter four groups, i.e. rats fed 5 g of control diet, and the three amino acid deficient diets, there were no significant differences, with the exception of those animals fed the lysine deficient diet, which had a 45.7% higher food intake ($P<0.01$). Fig. 2 shows the relationship of food intake to weight change, further illustrating the differences due to the effect of different dietary treatments on the food intake of the animals and a corresponding weight change.

Relative to body weight, food intake was adequate only for three of the control groups; those fed ad libitum, 15 g and 10 g of diet per day. Food efficiency was influenced significantly ($P<0.01$) by the amount of control diet fed, being highest for rats fed the control diet ad libitum, and lowest for those fed 10 g of the control diet (Table 7).

Growth curves for each of the seven experimental groups are shown in Fig. 3. Major differences between the groups are readily apparent. Growth during the 21-day feeding period was influenced both by the amount of diet fed, as observed for different levels of the same control diet, as well as by the type of diet fed, as noticed for the modified composition of amino acid deficient diets fed ad libitum. Positive growth curves were obtained for the control groups fed ad libitum, 15 g and 10 g of diet per day. But for each of the remaining four groups, there is an initially sharp downward trend. In the case of the group of rats fed 5 g of control diet, this sudden loss of weight changes gradually, and in fact, is replaced by a slight gain by the 17th day, with a positive upward trend from then on. However, the three amino acid deficient groups do not show any weight gain throughout the 21-day experimental period. But their initial loss of weight gives way to a more gradual decline, levelling off somewhat toward the 19th day.

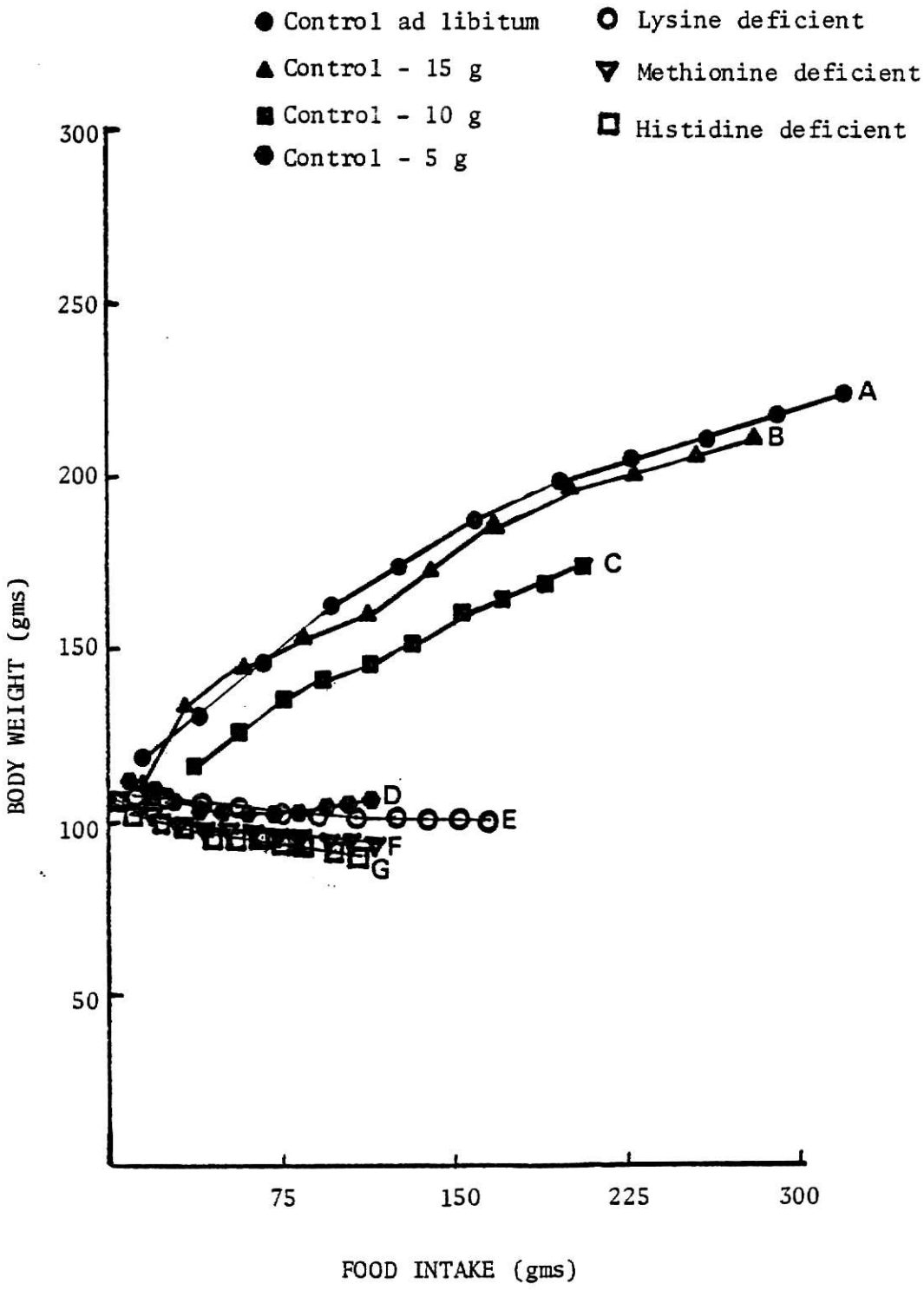


Fig. 2. Relationship of Food Intake to Weight Change during the 21-day feeding period.

Each curve represents the mean for 10 rats/group. Points on each curve represent the food intake and weight change recorded on a 2-day basis.

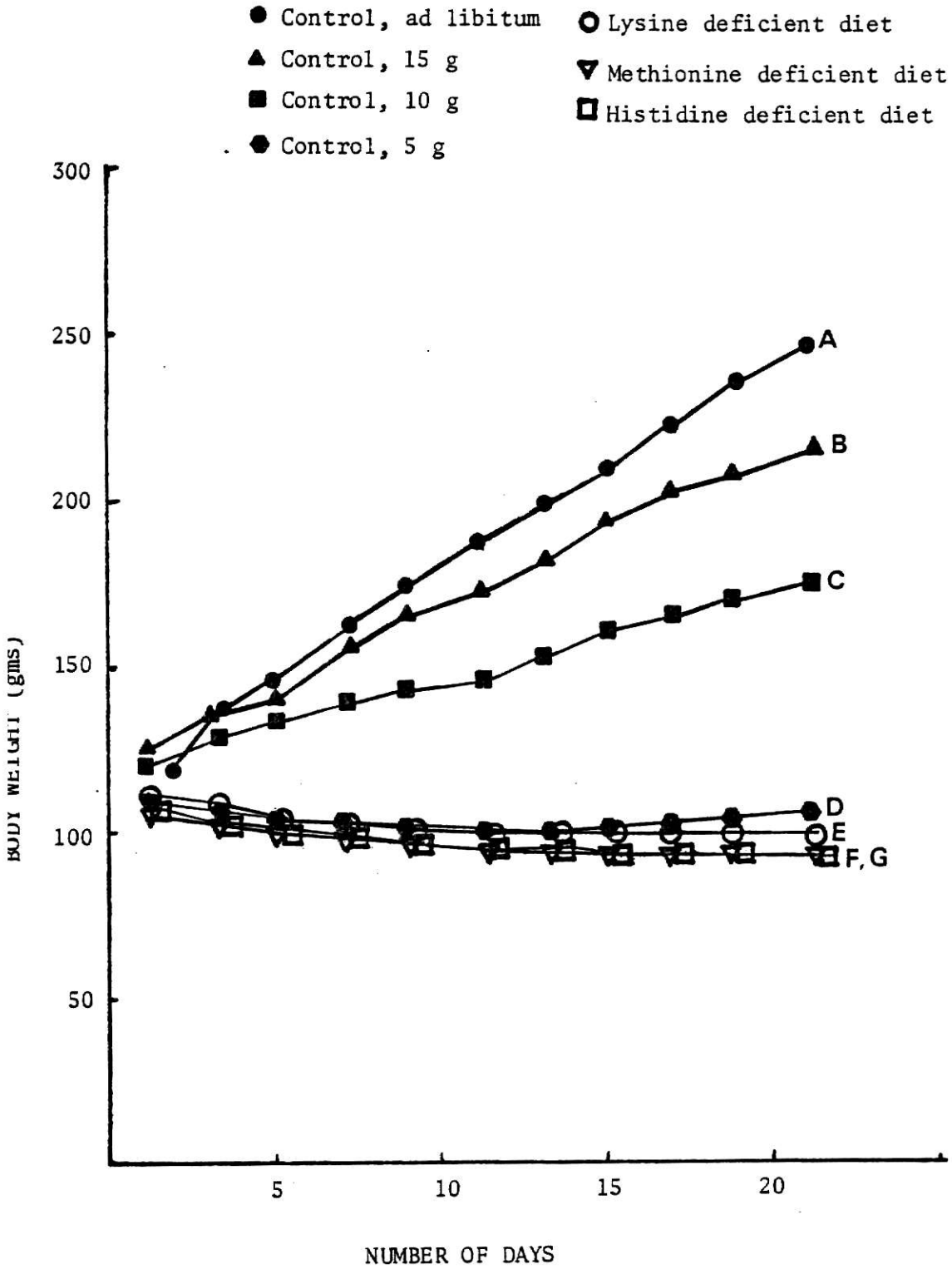


Fig. 3. Effect of Dietary Treatments on Body Weight Change of Rats during the 21-day Feeding Period. Body weights were recorded every alternate day, and each curve represents the mean for 10 rats/group. Significant differences are apparent between all groups except animals on the methionine and histidine deficient diets, $P<0.01$.

Organ Weights

The effect of dietary composition on organ weights is presented in Table 8. Different trends were observed for all the tissue weights: liver, brain, pituitary, parotid, kidneys and rib cartilage. However, apart from the pituitary, all the organ weights somewhat paralleled changes in body weights. The correlation between final body weights of all the rats and their liver weights was very high ($r = 0.9787$, $P<0.0001$). A high correlation was also found to exist between the final body weights of the rats and the weights of their parotid glands ($r = 0.9192$, $P<0.0001$). As the diet changed from amino acid deficient to control 10 g and higher, the tissues correspondingly increased in weight, reaching a maximum for the group of rats fed the Purina Chow diet, and in some cases for the group of rats fed the control diet ad libitum also.

There were no major effects of dietary treatments on the pituitary weights of the rats, and no significant differences were observed between any of the experimental groups, with the exception of the Purina Chow group which was 61.4% higher ($P<0.05$) than the other groups. In fact, the group of rats fed the Purina Chow diet was consistently higher ($P<0.05$) than the other groups in weights of all tissues, except for brain weights.

The liver and kidney weights of rats fed the Purina Chow diet and the control diet ad libitum, 15 g and 10 g were all higher ($P<0.05$) than those fed the control diet 5 g, and the amino acid deficient diets. Within the latter four groups there were no significant differences. The liver and kidney weights of the group of rats fed the Purina Chow diet were higher by 229% and 147% respectively, than the amino acid deficient groups.

Brain weights did not vary ($P<0.05$) for the three control groups fed either ad libitum, 15 g or 10 g of diet per day; also, no significant

TABLE 8

Effect of Dietary Composition on Organ Weights[1]

Group	ORGAN WEIGHTS					
	Liver	Brain	Pituitary	Parotid	Kidney	Rib Cartilage
	g					
A Control Ad libitum	11.05449[2,a] ±0.235	1.64853[ad] ±0.03	0.00775[a] ±0.003	0.20746[a] ±0.007	2.02746[a] ±0.036	1.81719[a] ±0.056
B[3] Control 15 g	9.44975[b] ±0.248	1.68755[ad] ±0.03	0.00760[a] ±0.003	0.20821[a] ±0.007	1.85336[b] ±0.036	1.68199[a] ±0.059
C Control 10 g	6.21043[c] ±0.235	1.58037[a] ±0.03	0.00643[a] ±0.003	0.17436[b] ±0.007	1.38864[c] ±0.036	1.35934[b] ±0.056
D Control 5 g	3.51052[d] ±0.235	1.50593[b] ±0.03	0.00390[a] ±0.003	0.12407[c] ±0.007	0.88916[d] ±0.036	0.84150[c] ±0.056
E Lysine Deficient	3.79179[d] ±0.235	1.52331[b] ±0.03	0.00416[a] ±0.003	0.09212[d] ±0.007	0.94777[d] ±0.036	0.78100[cd] ±0.056
F Methionine Deficient	3.54999[d] ±0.235	1.42656[b] ±0.03	0.00354[a] ±0.003	0.08968[d] ±0.007	0.85203[d] ±0.036	0.65016[d] ±0.056
G Histidine Deficient	3.42396[d] ±0.235	1.42090[b] ±0.03	0.00379[a] ±0.003	0.09210[d] ±0.007	0.86257[d] ±0.036	0.72889[cd] ±0.056
CONTROL R Purina Chow	11.81597[e] ±0.372	1.75063[d] ±0.06	---	0.32247[e] ±0.015	2.19111[e] ±0.057	2.19617[e] ±0.088

[1]Values shown are the mean ± SEM of 10 rats per group.

[2]Means in a column followed by the same superscript letter are not significantly different, $P<0.05$.

[3]Values given for this group are the mean ± SEM of 9 rats in the group.

difference was apparent between the former two groups and rats on the Purina Chow diet. However, all the four above mentioned groups showed markedly different means for brain weights as compared to rats fed either the control diet 5 g or the amino acid deficient diets.

More differences showed up between the various groups with regard to parotid and rib cartilage weights. Rats fed the control diet ad libitum and 15 g were 19% and 28.7% higher ($P<0.05$) in the weights of their parotid glands and rib cartilage tissues respectively, than the control group fed 10 g of diet, which in turn, was 40% and 61.5% respectively higher ($P<0.05$) than the group of rats fed 5 g of control diet. Rats fed the control diet 5 g had 37% higher ($P<0.05$) parotid weights than those fed amino acid deficient diets; however, the rib cartilage weights of rats fed 5 g of control diet did not differ significantly from those fed amino acid deficient diets, with the exception of the group of rats fed the methionine deficient diet, which was 23% lower than those on control diet 5 g ($P<0.05$).

Plasma Growth Hormone Concentration and Somatomedin Activity

Results for growth hormone (GH) concentration and somatomedin activity in the plasma are presented in Table 9. With regard to plasma GH concentration, analysis of data reveals no consistent trend in some of the differences observed among the experimental groups. Rats fed the control diet ad libitum and 15 g had higher ($P<0.05$) GH concentrations than those fed 10 g or 5 g of control diet, or those on the histidine deficient diet. No differences were apparent between rats consuming 15 g of control diet and those on lysine and methionine deficient diets. Similarly, feeding amino acid deficient diets or 5 g and 10 g of control diet produced no significant differences. However, all rats had sufficient GH.

TABLE 9

Effect of Dietary Composition on Plasma Growth Hormone Concentration and Relative Plasma Somatomedin Activity[1]

Group	Plasma Growth Hormone Concentration	Plasma Somatomedin Activity
	ng/ml	u/ml
A Control, ad libitum	250.66[2a] ±43.29	1.62838[a] ±0.19
B[3] Control 15 g	224.68[ab] ±48.40	0.96505[b] ±0.21
C Control 10 g	65.88[c] ±43.29	0.66504[bc] ±0.19
D Control 5 g	21.32[c] ±45.63	0.81102[bc] ±0.20
E Lysine Deficient	112.79[bc] ±43.29	0.54869[bc] ±0.19
F Methionine Deficient	121.58[bc] ±43.29	0.32420[c] ±0.19
G Histidine Deficient	47.70[c] ±48.40	0.37823[c] ±0.20

[1]Values shown are the mean ± SEM of 10 rats per group.

[2]Means in a column followed by the same superscript letter are not significantly different, $P<0.05$.

[3]Values given for this group are the mean ± SEM of 9 rats in the group.

Plasma somatomedin activity increased as the diet changed from amino acid deficient to control, being highest for the group of rats fed the control diet ad libitum, the latter being significantly different ($P<0.05$) from all the other groups. In addition, rats fed the methionine and histidine deficient diets had a 64% lower plasma somatomedin level than those rats fed the control diet at a level of 15 g per day ($P<0.05$).

The effect of different dietary treatments on plasma somatomedin activity is illustrated in Fig. 4. Differences are readily apparent in rats fed control diets and those fed amino acid deficient diets. Rats which consumed ad libitum amounts of control diet had a 118% higher plasma somatomedin level than those rats fed the control diet at levels of 15 g, 10 g or 5 g per day or those who consumed the lysine deficient diet. The control ad libitum fed rats had plasma somatomedin levels 364% higher than those rats consuming methionine and histidine deficient diets. Variations are also apparent within the different amino acid deficient groups; rats fed the lysine deficient diet showing a 56% higher somatomedin activity level than those fed the methionine and histidine deficient diets.

Weight change plotted against relative somatomedin activity, with the means coded for the different experimental diets, is depicted in Fig. 5. Although a very high correlation was not obtained ($r = 0.5269$, $P<0.0001$), the increase in weight gain, with a corresponding increase in somatomedin activity is nevertheless apparent for the rats fed the control diet ad libitum, 15 g and 10 g. Similarly, the loss of weight observed for rats consuming the amino acid deficient diets corresponds to decreased plasma somatomedin levels.

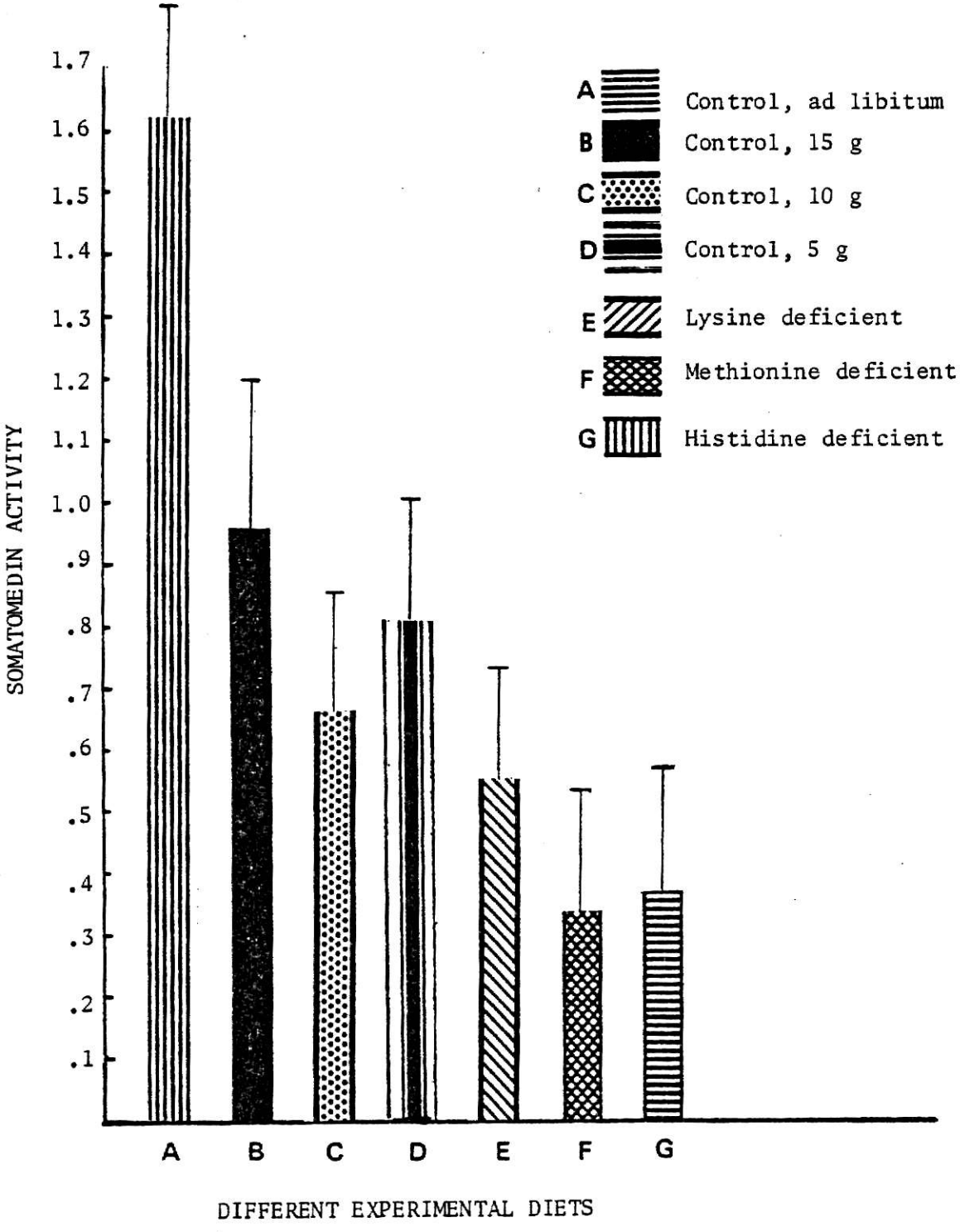


Fig. 4. Effect of Different Dietary Treatments on Somatomedin Activity in Rats. Each bar represents the mean ± SEM for the somatomedin activity of the 10 rats in each of the seven experimental groups. Somatomedin activity in rats fed the control diet ad libitum was significantly greater than the other groups ($P<0.05$).

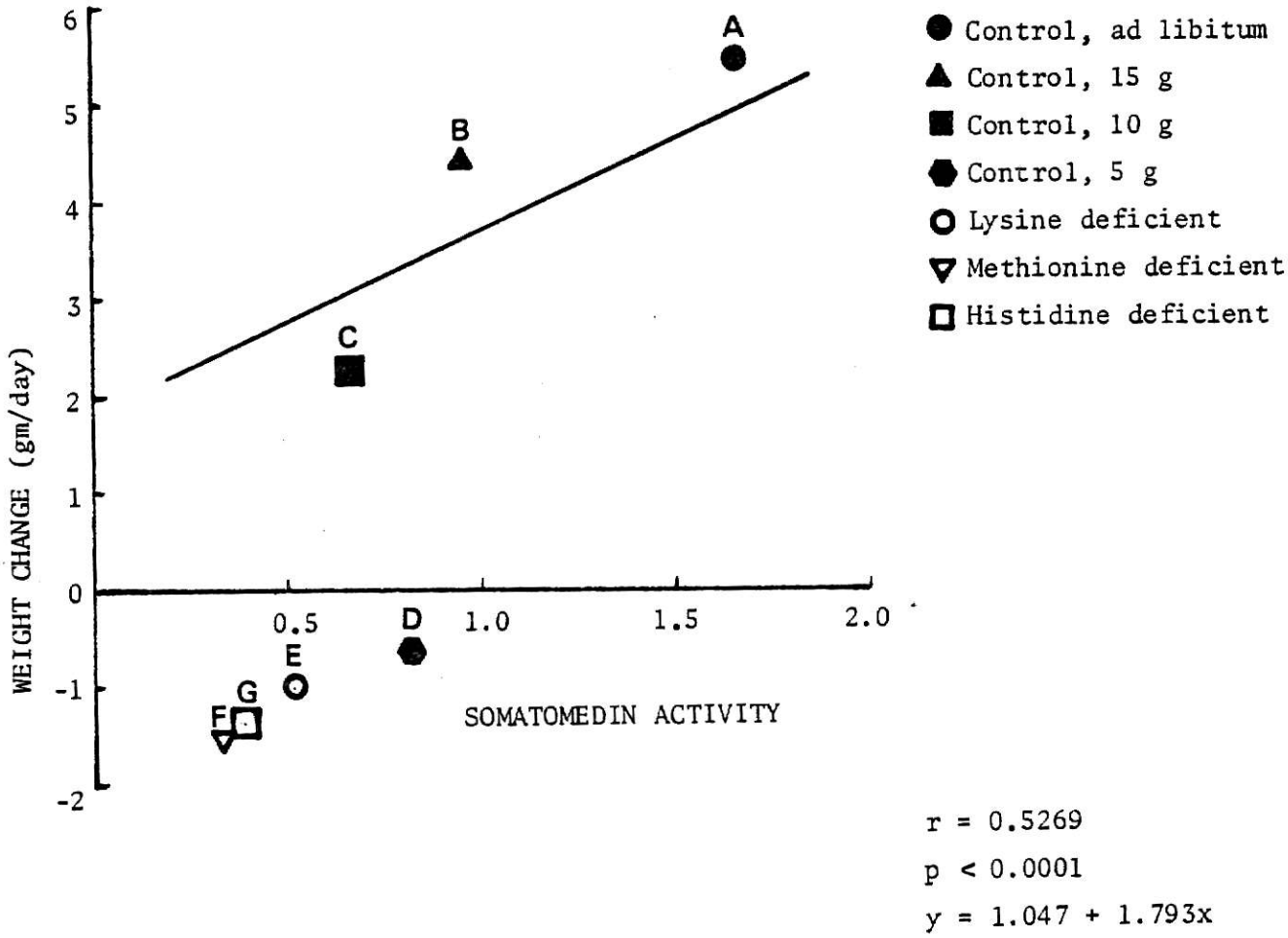


Fig. 5. Correlation of Relative Plasma Somatomedin Activity to Body Weight Change in Rats Fed Seven Experimental Diets. Each point represents the mean of 10 rats/group over a 22-day observation period.
Shown is the least-square regression line.

Glycosaminoglycan Determination

Results for the glycosaminoglycan (GAGS) determination on rib cartilage, brain and liver are presented in Table 10. Values are expressed as CPM ^{35}S/ug uronic acid. Analysis of data reveals that the values obtained for uptake of $^{35}SO_4$ by GAGS of rib cartilage vary significantly and more consistently within the experimental groups, when compared to other data, than did the values obtained for brain or liver. The two latter organs were more random in variance, no definite trend being evident for the differences observed.

With regard to GAGS determination on rib cartilage, rats fed the control diet ad libitum and 15 g had higher ($P<0.05$) uptake of $^{35}SO_4$ than those rats fed control diet at levels of 10 g and 5 g, as well as those fed amino acid deficient diets, but were not significantly different from the group of rats fed the Purina Chow diet. In addition, animals fed the histidine deficient diet were much lower ($P<0.05$) than those receiving 10 g of control diet, lysine deficient or Purina Chow diets.

Further effects of dietary composition on uptake of $^{35}SO_4$ by GAGS of rib cartilage are illustrated in Fig. 6. GAGS values decrease as the amount of control diet decreases from ad libitum to a level of 5 g per day, with a further decrease observed as the composition of the diet changes from control to methionine and histidine deficient diets. Lysine shows an exception here. Finally a significant increase is observed in the uptake of $^{35}SO_4$ as the diet changes once again from amino acid deficient to Purina Chow.

The relationship between weight change and $^{35}SO_4$ uptake by GAGS of rib cartilage is shown in Fig. 7, the means being coded for the different experimental diets. A moderate correlation ($r = 0.61747$, $P<0.0001$), was

TABLE 10

Effect of Dietary Composition on GAGS Determination in Rib Cartilage, Liver and Brain[1]

Group	Uptake of ^{35}S-sulphate by GAGS		
	Rib Cartilage	Liver	Brain
	CPM ^{35}S x 10^3/ug uronic acid		
A Control, ad libitum	3.08[2,a] ±0.22	0.02[ab] ±0.04	2.64[a] ±0.31
B Control 15 g	2.68[a] ±0.23	0.04[ab] ±0.05	2.44[ad] ±0.31
C Control 10 g	1.95[b] ±0.22	0.39[ab] ±0.04	2.34[b] ±0.28
D Control 5 g	1.70[bc] ±0.22	0.19[a] ±0.05	2.99[c] ±0.28
E Lysine Deficient	1.84[b] ±0.22	0.05[a] ±0.04	2.97[ad] ±0.28
F Methionine Deficient	1.70[bc] ±0.22	0.16[c] ±0.05	4.26[cd] ±0.31
G Histidine Deficient	1.08[c] ±0.22	0.07[b] ±0.04	1.96[acd] ±0.28
CONTROLS[4] Purina Chow R	2.33[ab] ±0.40	0.03[ab] ±0.07	2.46[acd] ±0.44

[1]Values shown are the mean ± SEM of 10 rats per group.

[2]Means in a column followed by the same superscript letter are not significantly different, $P<0.05$.

[3]Values given for this group are the mean ± SEM of 9 rats in the group.

[4]Values shown for this control group are the mean ± SEM of 4 rats in the group.

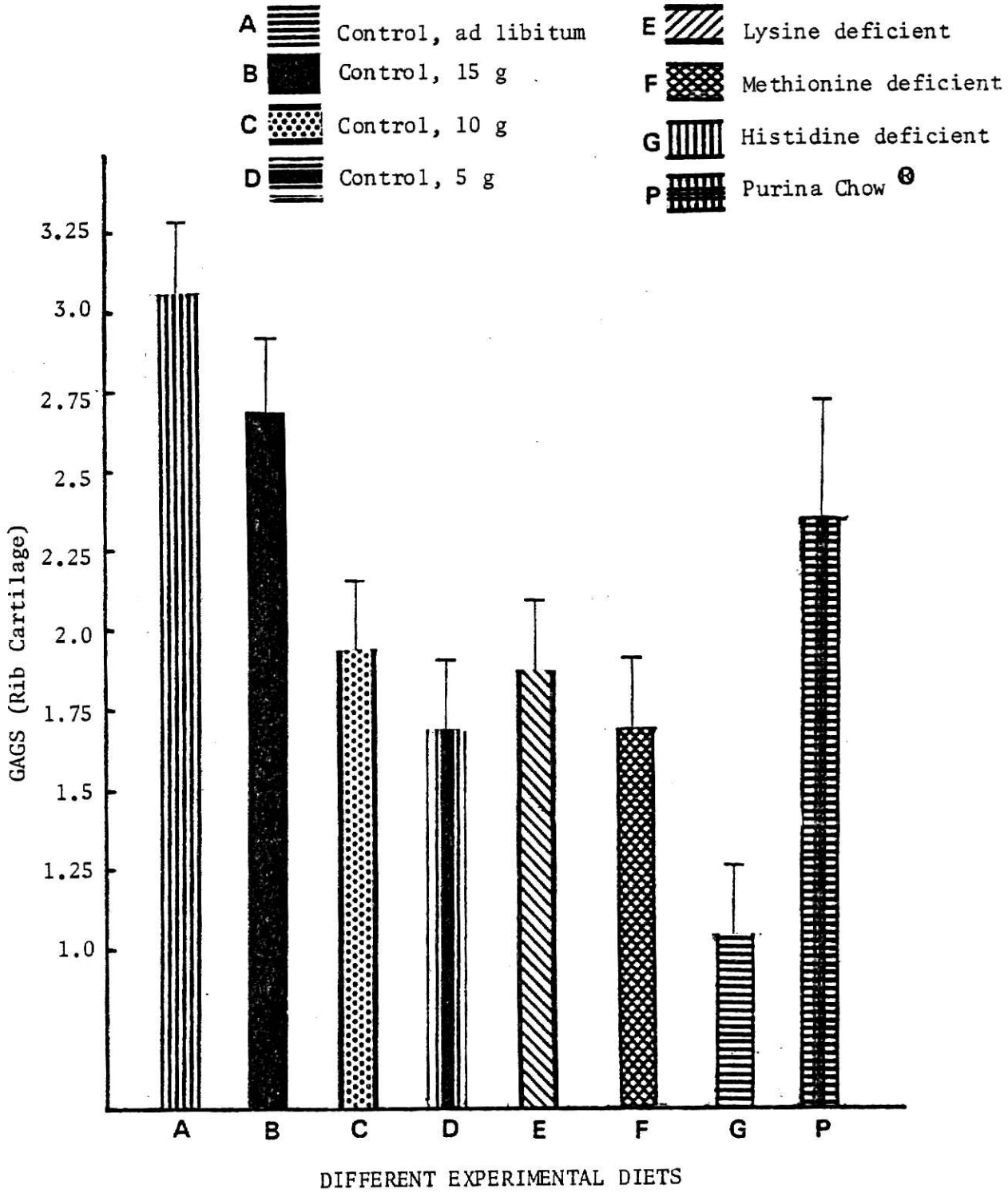
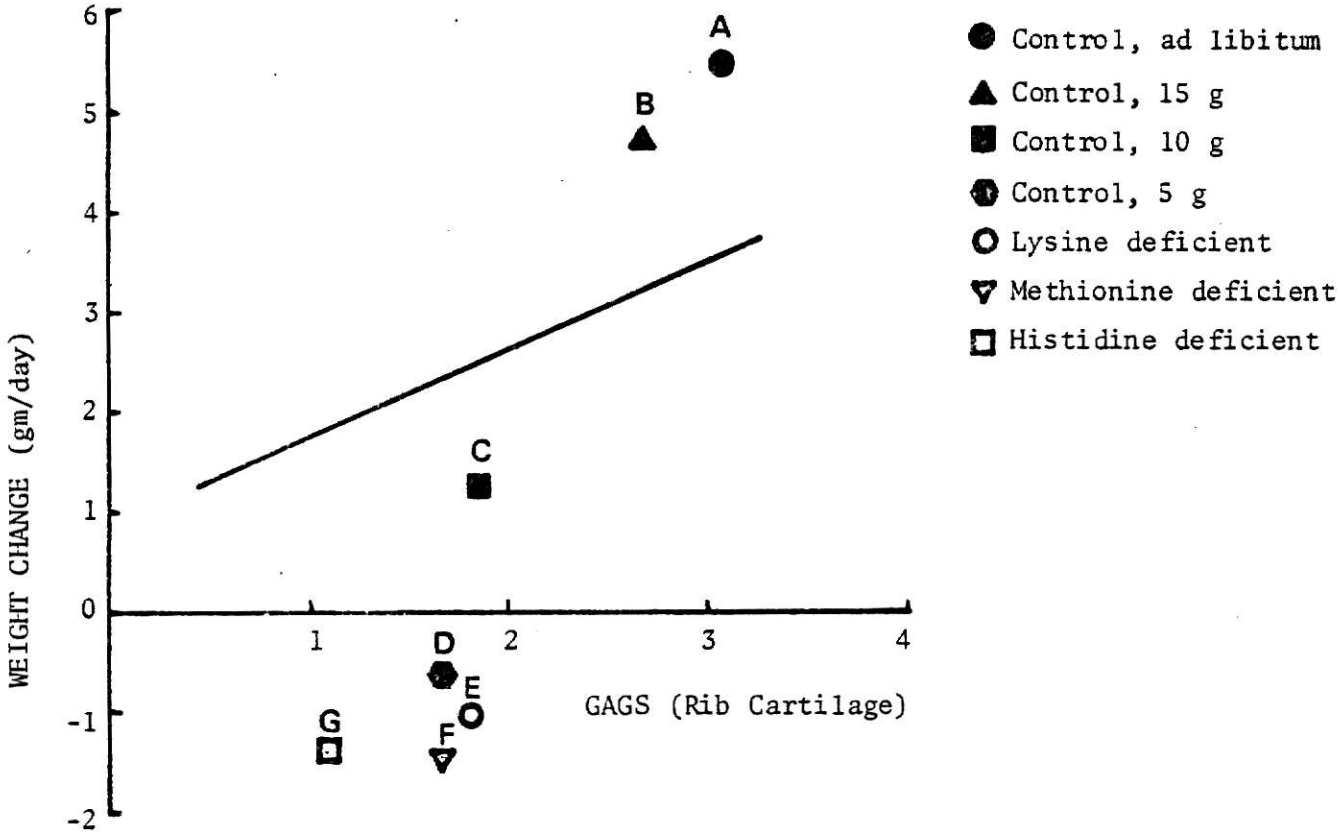


Fig. 6. Effect of Different Dietary Treatments on Uptake of ^{35}S-sulphate by GAGS of Rib Cartilage. ^{35}S-sulphate uptake is expressed as CPM $^{35}SO_4$/ug uronic acid. Each bar represents mean ± SEM for the 10 rats in each of the eight diet groups.



$r = 0.61747$

$p < 0.0001$

$y = 0.8095 + 0.7985x$

Fig. 7. Correlation of ^{35}S-sulphate uptake by GAGS of Rib Cartilage to Body Weight Change in Rats Fed Seven Experimental Diets. Each point represents the mean of 10 rats/group over a 22-day observation period.
Shown is the least-square regression line.

found to exist between uptake of $^{35}SO_4$ and weight change. As the weight increased for rats consuming the control diet ad libitum, 15 g and 10 g, $^{35}SO_4$ uptake was correspondingly greater. On the other hand, as rats fed 5 g of control diet, as well as those fed amino acid deficient diets, lost weight, their uptake of $^{35}SO_4$ correspondingly decreased.

DISCUSSION

Data has been presented on the effect of quantity and composition of diet on growth rate, food intake, body and organ weights, plasma growth hormone concentration and plasma somatomedin activity, and GAGS metabolism in rats. These data indicate that feeding amino acid deficient diets as well as low-calorie diets, results in a decrease in plasma somatomedin activity and growth cartilage activity, in addition to loss of body weight in rats.

Dietary deficiencies of the three amino acids studied, lysine, methionine and histidine, caused a marked decrease in food intake, and as a consequence reduced the caloric intake in the animals as well. This observation agrees with the generally accepted fact that feeding amino acid deficient diets leads to a decrease in the total caloric intake. However, the four different levels of control diet (ad libitum, 15 g, 10 g and 5 g), containing a defined mixture of amino acids as their only nitrogen source, were used as "pair-fed" controls for the different amino acid deficient diets. Rats fed the methionine and histidine deficient diets had an average caloric intake of 5.2 g per day, which was comparable to the control group of animals provided with 5 g of control diet. This quantity of food was not adequate enough to permit a gain in weight. Average caloric intake for rats fed the lysine deficient diet was 7.3 g per day, which was comparable to a level of intake that lay between 5-10 g of control diet. These results are somewhat in agreement with a previous report by Yoshiaki et al. (108), who showed that rats fed lysine deficient diets consumed approximately

10 grams/day, leucine and tryptophan deficient 7.5 grams/day, and the other essential amino acid deficiencies approximately 5.0 grams/day.

Somatomedin levels of the "pair-fed" controls were not significantly different from those produced by amino acid deficient diets. Therefore, the decrease in somatomedin activity as well as growth cartilage activity, observed in rats fed amino acid deficient diets, cannot be attributed directly to the absence of these particular amino acids. The decreased activity could be equally related to the quantity of calories consumed.

The above observation is in direct agreement with the study performed by Phillips et al. (109) who, in order to evaluate a dietary regulation of somatomedin activity and growth cartilage activity, developed a model in which growth-related responses could be measured in animals consuming selected quantities of food over a 24-hour period. Within their system of acute refeeding of fasted rats, somatomedin activity and growth cartilage activity appeared to be linked more closely to the quantity of calories consumed.

But despite the above observations, some findings in this study do indicate that growth impairment is correlated to amino acid deficiency, and not just to caloric deficiency alone. In spite of a food intake greater than that of rats consuming 5 g of control diet, the group of rats fed the lysine deficient diet had a lower body weight (Fig. 2). This may be attributed to the deficiency of lysine, in addition to inadequate calories.

Although not statistically significant, some of the data presented here indicate certain trends. A definite trend is observed in relative somatomedin activity. All rats fed the three amino acid deficient diets showed considerably lower somatomedin activity as compared with the

"pair-fed" controls. In fact, rats fed the methionine and histidine deficient diets had 57% less somatomedin activity than those rats fed 5 g of the control diet. The absence of statistical significance could be attributed to the bioassay methods for somatomedin which estimate only the relative excess of somatomedin stimulatory and inhibitory activity (110). The sensitivity of the assay lessens as somatomedin activity falls to very low levels, as were present in the amino acid deficient rats in this study. The resulting error is consequently larger, influencing statistical analysis.

Previous studies by Salmon (99) have shown that essential amino acids act synergistically with either somatomedin or insulin to stimulate in vitro sulphate uptake by cartilage from rats which have undergone hypophysectomy. Other investigators have shown that amino acid imbalance can impair sulphation, and limit the effect of somatomedin as a sulphation factor (111). This is understandable from the fact that sulphate incorporation reflects synthesis of protein polysaccharide complexes, and amino acids obviously are required for protein synthesis.

Despite the importance of a balanced mixture of amino acids, this study brings to light some different effects observed, with regard to the different single amino acids depleted from the diets fed to rats. Changes brought about by lysine deficiency were somewhat different to those produced by either methionine or histidine deficiencies. Feeding methionine and histidine deficient diets resulted in greater depletion effects, with a subsequently more severe growth impairment in the rats, than feeding a lysine deficient diet. This suggests a greater importance for methionine and histidine in the diet of weanling animals. The significantly lower uptake of ^{35}S by the histidine deficient group of animals suggests a

connection between this amino acid and GAGS biosynthesis, although no facts can be stated without further investigation. Histidine is known to be an essential amino acid in the diet of young children and growing animals, and could have relative importance in long bone growth through its influence on GAGS biosynthesis.

Since significant differences in plasma somatomedin activity were not observed among rats fed either the amino acid deficient diets or similar amounts of the control diet, the concept of Salmon (99) appears to be confirmed, that the stimulation of cartilage uptake by somatomedin is dependent on the amino acid pool of the tissue, rather than on a single amino acid. On the other hand, the possibility exists that, with improved precision techniques, the amino acids studied in this experiment, might be shown to have significant influence in regulating plasma somatomedin activity.

Although the nature of all the dialyzable sulphation-promoting activity of serum remains unknown, non-essential amino acids may be involved, in addition to the essential amino acids (99). Studies by Koumans and Daughaday (112) indicate that serine is important in stimulating sulphation activity. The significance of the requirement for serine, however, is unclear. Non-essential amino acids have been found to stimulate growth of cells in tissue culture in the presence of essential amino acids and dialyzed serum (113).

The importance of protein depletion is further illustrated in a study of Pimstone et al. (114). The degree of elevation of human growth hormone levels in nutritional marasmus and kwashiorkor, were shown by these investigators to correlate with the severity of protein depletion. Their

finding, that adequate caloric intake failed to depress plasma human growth hormone until protein was added, suggests that caloric depletion is relatively less important than that of protein in stimulating growth hormone secretion.

Data from this study confirms reports that somatomedin activity is reduced in conditions of protein-calorie malnutrition, both in humans (8, 81, 87) and in animals (10-12, 94, 109), despite normal to elevated plasma levels of growth hormone. The low somatomedin activity decreased growth cartilage activity and decreased rate of growth observed in this study appears to be related directly to nutritional status and dietary amino acid intake rather than a lack of growth hormone, since plasma concentrations of the latter were normal to high, and little affected by dietary manipulation. This concept was stated previously by Grant et al. (87) who suggested that factors other than growth hormone, such as serum insulin and nutrition, may be equally important in regulating somatomedin activity. The inverse relationship between somatomedin and growth hormone is thought by Hintz et al. (81) to fit the hypothesis that somatomedin is involved in the feedback inhibition of growth hormone release.

Although not specifically investigated in this study, the possibility exists that the reduced somatomedin activity observed may be due to the presence of somatomedin inhibitors. Hintz et al. (81), studying malnourished children, and Salmon (115), studying fasted rats, reported that the decrease in plasma somatomedin activity was associated with a somatomedin inhibitor. However, Phillips and Young (10) and Grant and associates (87) were unable to demonstrate inhibition of somatomedin. The nature of inhibitors of the somatomedin bioassay is not well defined (83). Inhibitors may play a

physiologic role in preventing somatomedin action on tissues. Whether the action of the inhibitor is specific for somatomedin activity, remains to be seen. However, inhibitors are an important pitfall in somatomedin bioassay studies. In order to control the influence of inhibitors, all sera used in the somatomedin assay was heated to 56°C prior to use. The inhibitors are thought to be heat labile and destroyed at this temperature, whereas somatomedin is heat stable.

Another possibility which exists in animals fed amino acid deficient diets, is that the decreased somatomedin activity may be due to a limitation of substrate at the cartilage level (111, 112). If the cellular environment of a chondrocyte contains the essential amino acids as well as the necessary non-essential amino acids, somatomedin is capable of stimulating the cell to synthesize increased amounts of chondromucoprotein as determined by sulphate uptake (109). Results from a recent study by Kilgore et al. (116) suggest that the primary action of somatomedin on GAGS biosynthesis is at the level of synthesis of the acceptor protein, requirements of which are the presence of all the essential amino acids.

Although a very high correlation between somatomedin activity and organ weights was not apparent, the significant decrease in liver weights of animals fed amino acid deficient diets suggests certain possibilities. The liver is considered to be major site of somatomedin formation (52). Shapiro et al. (59) demonstrated the reduction in the generation of somatomedin activity by the liver during protein-energy malnutrition, in spite of raised or normal levels of growth hormone. They suggest that this is not simply a reflection of differences in liver or body weight, as weight-matched, well-nourished controls generated more somatomedin activity. The reduced

somatomedin activity may be a specific homeostatic mechanism by which the growth stimulating action of growth hormone might be modulated at a time when growth is inappropriate for survival. On the other hand, it may be a response to alterations in liver function and the general synthesis of proteins by the liver (114).

In view of the fact that somatomedin mediates the growth-promoting effect of growth hormone, the low plasma somatomedin levels found in rats fed amino acid deficient diets could be advantageous, directing available resources away from long-term requirements and non-essential processes, such as growth, toward more urgent needs. The decreased somatomedin activity would appear to be an important protective mechanism conserving vital amino acids for essential metabolic needs.

CONCLUSIONS

Based on the results obtained in this study, amino acid deficient diets, as well as low-calorie complete diets decrease plasma somatomedin activity and growth cartilage activity, and consequently lead to growth impairment. In addition, feeding amino acid deficient diets decreases total caloric intake of the animals, resulting in loss of weight greater than that observed for animals fed similar amounts of control diet.

Within the amino acid deficient groups, greater depletion effects were noticed when methionine and histidine were eliminated from the diet, than the effects observed as a result of lysine deficiency, suggesting a significant importance for the two amino acids, methionine and histidine, for the preservation of growth in animals.

This study confirms the widely-held belief that adequate nutrition is required for the maintenance of normal levels of somatomedin, and demonstrates that nutrition plays an important role in modulating the generation of somatomedin with or without growth hormone. However, the mechanisms by which nutrition modulates plasma somatomedin activity remain unclear. The future unravelling of these mechanisms will aid in our understanding of the growth retardation associated with malnutrition.

SUMMARY

The effects of specific amino acid deficiencies on plasma somatomedin activity and glycosaminoglycan (GAGS) metabolism were investigated. Four-week old weanling male Sprague-Dawley rats were placed on each diet in one of seven experimental groups, 10 rats per group. Four control groups were fed the amino acid defined (control) diet ad libitum, 15 g, 10 g and 5 g per day. Three groups with modifications in the amino acid content of their diet were fed lysine deficient, methionine deficient and histidine deficient diets. In addition, a control group of animals was maintained on Purina Chow ® fed ad libitum.

Animals were maintained on their diets for 21 days. Food intake and body weight were recorded at regular intervals. Sixteen hours prior to sacrifice rats were injected with ^{35}S (carrier-free). At the time of sacrifice, blood was collected for measuring plasma growth hormone concentration and somatomedin activity. Tissues removed during autopsy were liver, brain, pituitary, parotid, kidney and rib cartilage. All organ weights were recorded. To determine the effect of diet on cartilage GAGS, the uptake of ^{35}S into GAGS isolated from rib cartilage, brain and liver was measured.

Results obtained in this study show significant differences in food intake and weight change, attributable to different dietary treatments. Dietary deficiencies of these three amino acids, lysine, methionine and histidine, caused a significant ($P<0.01$) decrease in food intake and consequent reduced caloric intake in the animals, leading to greater ($P<0.01$) loss of weight, as compared to rats fed the control diet.

Average caloric intake for rats fed the methionine and histidine deficient diets was comparable to the 5 g level of control diet. Lysine deficient animals had a caloric intake that lay between 5-10 g of control diet. These levels of control diet served as "pair-fed" controls for the amino acid deficient animals when comparing data for somatomedin activity and growth cartilage activity, to help in differentiating between protein deficiency and caloric deficiency.

Plasma somatomedin levels of the amino acid deficient rats were significantly lower ($P<0.01$) than those of the group of rats fed the control diet ad libitum, but were not significantly different to the somatomedin levels of the "pair-fed" controls, although a definite trend was apparent. Somatomedin activity decreased as the diet changed from control to amino acid deficient. A similar trend was observed for cartilage GAGS biosynthesis. Methionine and histidine deficient animals had a significantly lower ($P<0.05$) uptake of ^{35}S than the lysine deficient group, suggesting a greater importance for these two amino acids, methionine and histidine, in the diet of growing animals.

Despite the low somatomedin levels obtained in most of the groups, all animals had adequate plasma growth hormone concentrations, confirming previous reports that nutrition is an important modulator of somatomedin activity with or without growth hormone.

Change in organ weights also showed a definite trend. Apart from the pituitary weights which did not differ significantly except for the animals fed the Purina Chow diet ($P<0.05$), all tissues decreased in weight as the diet changed from control to amino acid deficient. Within the amino acid deficient groups there were no significant differences in any of the organ weights.

The results of this study confirm previous reports that somatomedin activity and growth cartilage activity are reduced in conditions of protein-calorie malnutrition, despite normal to elevated plasma concentrations of growth hormone. However, adequate evidence could not be obtained to attribute the decreased somatomedin activity and growth cartilage activity directly to the specific amino acids investigated in this study. The decreased activity could be equally related to the quantity of calories consumed. Nevertheless, the conclusion can be drawn, that adequate nutrition is required for the maintenance of normal levels of somatomedin and growth cartilage activity.

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APPENDIX

TABLE 11A

Initial Weight, Final Weight, Total Weight Gain/Loss, Daily Weight Gain/Loss, Food Intake (g/day and kcal/day) and Food Efficiency for Individual Rats Over a 21-Day Feeding Period

Group	BODY WEIGHT				FOOD INTAKE		FOOD EFFICIENCY
	Initial	Final[1]	Total Gain/ Loss	Daily Gain/ Loss			
	g				g/day	kcal/day	g/gain 100 kcal
A							
Control Ad Libitum							
RAT # 1	123	251	128	5.82	15.64	70.69	8.23
2	128	242	114	5.18	13.55	61.25	8.46
3	110	245	135	6.14	14.16	64.00	9.59
4	132	244	112	5.09	13.18	59.57	8.54
5	111	236	125	5.68	13.61	61.52	9.23
6	128	261	133	6.05	16.93	76.52	7.90
7	124	238	114	5.18	12.99	58.71	8.82
8	125	236	111	5.05	12.95	58.53	8.63
9	123	248	125	5.68	14.84	67.08	8.47
10	122	230	108	4.91	14.03	63.42	7.74
Mean ± SEM[2]	122.6 ±2.23	243.1 ±2.81	120.5 ±3.10	5.48 ±0.14	14.19 ±0.46	64.13 ±1.83	8.56 ±0.18
B							
Control 15 g							
RAT # 1	128	226	98	4.45	12.56	56.77	7.84
2	125	230	105	4.77	12.18	55.05	8.66
3	130	224	94	4.27	12.73	57.54	7.42
4	--[3]	--	--	--	--	--	--
5	123	223	100	4.55	12.45	56.27	8.09
6	122	230	108	4.91	13.45	60.79	8.08
7	122	217	95	4.32	12.18	55.05	7.85
8	130	217	87	3.95	11.64	52.61	7.51
9	119	215	96	4.36	12.95	58.53	7.45
10	132	225	93	4.23	13.30	60.12	7.04
Mean ± SEM	125.7 ±1.50	223.0 ±1.86	97.33 ±2.12	4.42 ±0.10	12.60 ±0.19	56.97 ±0.87	7.77 ±0.16

TABLE 11A (Continued)

Group	BODY WEIGHT				FOOD INTAKE		FOOD EFFICIENCY
	Initial	Final	Total Gain/ Loss	Daily Gain/ Loss			
	g				g/day	kcal/day	g gain/ 100 kcal
C							
Control 10 g							
RAT # 1	117	172	55	2.50	9.58	43.30	5.77
2	133	191	58	2.64	9.47	42.80	6.17
3	126	168	42	1.91	8.55	38.65	4.94
4	124	174	50	2.27	9.34	42.22	5.38
5	122	171	49	2.23	9.38	42.40	5.26
6	123	170	47	2.14	9.48	42.85	4.99
7	132	172	40	1.82	8.75	39.55	4.60
8	121	174	53	2.41	9.14	41.31	5.83
9	128	170	42	1.91	9.34	42.22	4.52
10	122	173	51	2.32	9.49	42.89	5.41
Mean ± SEM	124.8	173.5	48.7	2.22	9.25	41.82	5.29
	±1.58	±2.03	±1.89	±0.09	±0.11	±0.49	±0.17
D							
Control 5 g							
RAT # 1	124	104	-20	-0.91	4.66	21.06	
2	126	98	-28	-1.27	4.61	20.84	
3	121	104	-17	-0.77	4.76	21.52	
4	126	113	-13	-0.59	4.84	21.88	
5	118	100	-18	-0.82	4.75	21.47	
6	123	111	-12	-0.55	4.75	21.47	
7	129	115	-14	-0.64	4.74	21.42	
8	122	100	-22	-1.00	4.73	21.38	
9	124	115	- 9	-0.41	4.26	19.26	
10	127	121	- 6	-0.27	4.32	19.53	
Mean ± SEM	124	108.1	-15.9	-0.72	4.64	20.98	
	±1.01	±2.50	±2.05	±0.09	±0.06	±0.28	

TABLE 11A (Continued)

Group	BODY WEIGHT				FOOD INTAKE		FOOD EFFICIENCY
	Initial	Final	Total Gain/ Loss	Daily Gain/ Loss			
	g				g/day	kcal/day	g gain/ 100 kcal
E							
Lysine Deficient							
RAT # 1	122	101	-21	-0.95	6.16	27.84	--
2	127	105	-22	-1.00	8.77	39.64	--
3	120	98	-22	-1.00	5.67	25.63	--
4	131	106	-25	-1.14	7.14	32.27	--
5	123	94	-29	-1.32	8.18	36.97	--
6	120	100	-20	-0.91	6.52	29.47	--
7	122	100	-22	-1.00	7.50	33.90	--
8	126	104	-22	-1.00	7.73	34.94	--
9	131	101	-30	-1.36	8.89	40.18	--
10	120	96	-24	-1.09	6.77	30.60	--
Mean ± SEM	124.2	100.5	-23.7	-1.08	7.33	33.14	
	±1.36	±1.21	±1.07	±0.05	±0.34	±1.55	
F							
Methionine Deficient							
RAT # 1	125	90	-35	-1.59	5.98	27.03	--
2	125	93	-32	-1.45	5.93	26.80	--
3	128	91	-37	-1.68	5.41	24.45	--
4	119	87	-32	-1.45	4.16	18.80	--
5	114	89	-25	-1.14	5.00	22.60	--
6	125	93	-32	-1.45	5.73	25.90	--
7	121	92	-29	-1.32	4.41	19.93	--
8	129	88	-41	-1.86	5.59	25.27	--
9	120	87	-33	-1.50	5.02	22.69	--
10	129	92	-37	-1.68	4.75	21.47	--
Mean ± SEM	123.5	90.2	-33.3	-1.51	5.20	23.49	
	±1.55	±0.74	±1.42	±0.06	±0.20	±0.90	

TABLE 11A (Continued)

Group	BODY WEIGHT				FOOD INTAKE		FOOD EFFICIENCY
	Initial	Final	Total Gain/ Loss	Daily Gain/ Loss			
	g				g/day	kcal/day	g gain/ 100 kcal
G							
Histidine Deficient							
RAT # 1	129	97	-32	-1.45	5.61	25.36	--
2	120	89	-31	-1.41	5.27	23.82	--
3	127	94	-33	-1.50	4.32	19.53	--
4	124	94	-30	-1.36	4.80	21.70	--
5	130	99	-31	-1.41	5.68	25.67	--
6	110	81	-29	-1.32	4.32	19.53	--
7	140	104	-36	-1.64	6.43	29.06	--
8	122	90	-32	-1.45	5.32	24.05	--
9	125	92	-33	-1.50	5.45	24.63	--
10	124	90	-34	-1.55	5.30	23.96	--
Mean ± SEM	125.1 ±2.43	93.0 ±1.98	-32.1 ±0.64	-1.46 ±0.03	5.25 ±0.20	23.73 ±0.91	

[1]Values for rats at the end of the 21-day feeding period.

[2]These means ± SEM were calculated for each individual group, and were not part of the analysis of variance calculations.

[3]Rat #4 in this group was sick; data obtained for him was therefore deleted.

TABLE 12A

Organ Weights (Liver, Brain, Pituitary, Parotid, Kidney and Rib Cartilage) for Individual Rats at the End of the 21-Day Feeding Period

Group	ORGAN WEIGHTS (grams)					
	Liver	Brain	Pituitary	Parotid	Kidney	Rib Cartilage
A						
Control Ad Libitum						
RAT # 1	12.97918	1.68347	0.00749	0.19744	2.24737	1.53823
2	10.43212	1.77200	0.00870	0.21244	1.89632	1.51316
3	10.25323	1.65640	0.00630	0.15019	1.99048	1.80406
4	10.54336	1.80705	0.00842[2]	0.21661	2.01267	1.78858
5	11.44268	1.16620	0.05790[2]	0.18603	2.20919	1.58144
6	12.92148	1.77600	0.08310[2]	0.21317	2.16071	2.00616
7	9.65727	1.68035	--	0.23892	1.90378	1.98172
8	10.46254	1.69087	0.00760	0.21533	1.75256	1.92778
9	11.91216	1.58200	0.00956	0.23429	2.10724	2.18404
10	9.94088	1.67100	0.00619	0.21016	1.99425	1.84676
Mean ± SEM[1]	11.05449	1.64853	0.00775	0.20746	2.02746	1.817193
	±0.38	±0.06	±0.0005	±0.008	±0.05	±0.07
B						
Control 15 g						
RAT # 1	9.73529	1.74650	0.00867	0.20270	2.18566	1.67108
2	9.27703	1.74217	0.00709	0.20430	1.81362	1.64855
3	9.00140	1.57547	0.00694	0.20062	1.98187	1.28513
4	--[3]	--	--	--	--	--
5	10.21528	1.67707	0.00776	0.19426	1.71436	1.59511
6	9.71896	1.65436	0.00775	0.20875	1.82379	1.76009
7	8.81573	1.73658	0.06820[2]	0.27424	1.80278	1.89648
8	9.46242	1.66969	0.00766	0.20790	1.70771	1.67346
9	9.53122	1.68102	0.00733	0.15643	1.70890	1.69602
10	9.29014	1.70507	0.00759	0.22471	1.95156	1.81200
Mean ± SEM	9.44975	1.68755	0.00760	0.20821	1.85336	1.68199
	±0.14	±0.02	±0.0005	±0.01	±0.05	±0.05

TABLE 12A (Continued)

Group	ORGAN WEIGHTS (grams)					
	Liver	Brain	Pituitary	Parotid	Kidney	Rib Cartilage
C						
Control 10 g						
RAT # 1	6.20902	1.54613	0.00630	0.15125	1.34305	1.40840
2	5.85070	1.53850	0.00630	0.19654	1.59445	1.92211
3	5.62745	1.69932	0.00638	0.13889	1.41133	1.01206
4	6.00340	1.64246	0.00679	0.16162	1.36506	1.22570
5	6.66785	1.58570	0.00615	0.20008	1.30147	1.29891
6	6.06342	1.54472	0.00683	0.19830	1.37287	1.26642
7	6.42553	--	0.00589	0.17383	1.35886	1.15808
8	6.59015	1.58254	0.00625	0.16363	1.34385	1.58405
9	6.16134	1.56985	0.00700	0.16989	1.43339	1.42033
10	6.50541	1.51407	0.00639	0.18957	1.36203	1.29736
Mean $\pm$ SEM	6.21043	1.58037	0.00643	0.17436	1.38864	1.35934
	$\pm$0.11	$\pm$0.02	$\pm$0.0001	$\pm$0.007	$\pm$0.025	$\pm$0.08
D						
Control 5 g						
RAT # 1	3.58116	1.22857	0.00250	0.11566	0.91964	0.91780
2	3.07325	1.45452	--	0.10222	0.79109	0.59478
3	3.30025	1.53036	0.00358	0.12815	0.81900	0.73316
4	3.53305	1.53825	0.00485	0.12275	0.89610	0.83015
5	3.42424	1.64263	0.00431	0.10829	0.90328	0.90704
6	3.53127	1.53373	0.00264	0.12280	0.91078	0.73621
7	3.98354	1.52260	0.00489	0.13741	0.86910	0.86016
8	3.10060	1.46120	0.00357	0.11375	0.83950	0.78154
9	3.80165	1.53140	0.00407	0.13152	0.91661	0.86208
10	3.77619	1.61601	0.00432	0.15812	1.02651	1.19213
Mean $\pm$ SEM	3.51052	1.50593	0.00390	0.12407	0.88916	0.84150
	$\pm$0.09	$\pm$0.04	$\pm$0.0003	$\pm$0.005	$\pm$0.02	$\pm$0.05

TABLE 12A (Continued)

Group	ORGAN WEIGHTS (grams)					
	Liver	Brain	Pituitary	Parotid	Kidney	Rib Cartilage
E						
Lysine Deficient						
RAT # 1	4.18736	1.44805	0.00389	0.09922	0.93674	0.74520
2	4.11320	1.48834	0.00390	0.07200	0.95766	0.67110
3	3.55725	1.55639	0.00366	0.07976	0.92309	0.87711
4	3.96753	1.46143	0.00514	0.08121	1.00938	0.93712
5	3.51676	1.52842	0.04450[2]	0.15768	1.08743	0.64408
6	3.68045	1.49783	0.00329	0.07841	0.96782	0.81286
7	3.36615	1.61766	0.00503	0.08357	0.80621	0.74106
8	3.88216	1.53614	0.00439	0.08544	0.87204	0.74573
9	3.55100	1.52176	0.00448	0.08657	0.98223	0.85904
10	4.09603	1.57705	0.00364	0.09734	0.93505	0.77709
Mean ± SEM	0.379179	1.52331	0.00416	0.09212	0.94777	0.78100
	±0.09	±0.02	±0.0002	±0.008	±0.02	±0.03
F						
Methionine Deficient						
RAT # 1	3.44334	1.48188	0.00346	0.12000	0.77348	0.65196
2	3.57238	1.44688	0.00396	0.06543	0.87190	0.43849
3	3.50258	1.42982	0.00369	0.08757	0.88652	0.69188
4	3.6522	1.40504	0.00307	0.06535	0.79331	0.70132
5	4.03170	1.42164	0.00343	0.08927	0.94853	0.64630
6	3.6911	1.52986	0.00341	0.09880	0.83655	0.56470
7	3.33838	1.49688	0.00385	0.08536	0.78617	0.70014
8	3.36450	1.45719	0.00342	0.09554	0.85109	0.68111
9	3.36504	1.21433	0.00329	0.07125	0.86415	0.69732
10	3.53868	1.38206	0.00382	0.09821	0.90863	0.72840
Mean ± SEM	3.54999	1.42656	0.00354	0.08768	0.85203	0.65016
	±0.07	±0.03	±0.0009	±0.005	±0.02	±0.03

TABLE 12A (Continued)

Group	ORGAN WEIGHTS (grams)					
	Liver	Brain	Pituitary	Parotid	Kidney	Rib Cartilage
G						
Histidine Deficient						
RAT #1	3.53665	1.46674	0.00431	0.07432	0.94022	0.81354
2	3.04810	1.44802	0.00324	0.09208	0.78300	0.80673
3	3.21027	1.58455	0.00325	0.09006	0.85335	0.72362
4	3.34940	1.47966	0.00353	0.08242	0.88956	0.73491
5	3.86760	1.37388	0.00400	0.09087	0.88390	0.75530
6	3.02380	1.25414	0.00326	0.09878	0.78440	0.59520
7	4.23924	1.47829	0.00464	0.09196	0.94155	0.74526
8	3.54568	1.39817	0.00399	0.09484	0.82823	0.73959
9	3.27136	1.38002	0.00400	0.11301	0.82146	0.71273
10	3.14748	1.34554	0.00369	0.09268	0.90000	0.66203
Mean ± SEM	3.42396	1.42090	0.00379	0.09210	0.86257	0.72889
	±0.12	±0.03	±0.0002	±0.003	±0.02	±0.02
CONTROLS						
Purina Chow						
RAT # 1	11.28185	--	--	--	2.17575	2.20890
2	14.91829	1.80592	0.1160[2]	0.33812	2.53481	2.73128
3	9.08740	1.59666	--	--	1.87087	1.89603
4	11.97635	1.84930	0.10757[2]	0.30681	2.18300	1.94845

[1]These means ± SEM were calculated for each individual group and were not part of the analysis of variance calculations.

[2]These figures seemed to indicate an error of one decimal point; hence they were deleted from statistical calculation of mean ± SEM.

[3]Rat #4 in this group was sick; data obtained for him was therefore deleted.

TABLE 13A

Plasma Growth Hormone and Relative Somatomedin Activity in Individual Rats at the End of the 21-Day Feeding Period

Group	Plasma Growth Hormone Concentration	Plasma Somatomedin Activity[1]
	ng/ml	U/ml
A		
Control Ad Libitum		
RAT # 1	48.5	0.6890
2	29.0	0.7206
3	400	3.4081
4	400	2.1184
5	19.5	0.6448
6	400	0.5649
7	9.6	0.4251
8	400	0.6505
9	400	4.2141
10	400	1.8483
Mean ± SEM	250.7 ± 61	1.6284 ± 0.41
B		
Control 15g		
RAT # 1	152.5	1.1450
2	--	--
3	400	2.3736
4	--[3]	--
5	400	0.7304
6	400	0.4327
7	29.6	0.5506
8	121.5	1.1830
9	286.6	0.7483
10	7.2	0.5568
Mean ± SEM	224.7 ± 59.3	0.9651 ± 0.22
C		
Control 10 g		
RAT # 1	4.1	0.5305
2	45.1	0.4680
3	400	0.8522
4	6.0	1.2306
5	83.2	0.4917
6	27.6	0.3667
7	6.5	0.4054
8	18.1	0.7394
9	25.6	0.6236
10	42.6	0.9423
Mean ± SEM	65.88 ± 37.9	0.6650 ± 0.1

TABLE 13A (Continued)

Group	Plasma Growth Hormone Concentration	Plasma Somatomedin Activity[1]
	ng/ml	U/ml
D		
Control 5 g		
RAT # 1	39.1	0.5383
2	20.5	0.4046
3	14.8	1.2464
4	35.0	1.0587
5	3.0	0.4669
6	15.8	0.7203
7	--	--
8	4.9	0.2639
9	42.8	1.0904
10	16.0	1.0592
Mean ± SEM	21.32 ± 4.7	0.8110 ± 0.1
E		
Lysine Deficient		
RAT # 1	400	0.2746
2	86.9	0.0579
3	136.4	0.6933
4	5.9	0.8484
5	129.7	0.3235
6	42.2	0.9422
7	254.6	0.3780
8	39.5	0.4868
9	4.8	0.5973
10	27.9	0.8849
Mean ± SEM	112.8 ± 40.2	0.5487 ± 0.1
F		
Methionine Deficient		
RAT # 1	66.5	0.3248
2	400	0.2562
3	29.9	0.6038
4	5.5	0.5103
5	31.6	0.5292
6	12.1	0.1506
7	37.3	0.1397
8	28.9	0.2153
9	246.2	0.3134
10	357.8	0.1987
Mean ± SEM	121.6 ± 48.4	0.3242 ± 0.1

TABLE 13A (Continued)

Group	Plasma Growth Hormone Concentration	Plasma Somatomedin Activity
	ng/ml	U/ml
G		
Histidine Deficient		
RAT # 1	198.5	0.2028
2	30.3	0.4410
3	14.9	0.4508
4	26.8	0.4552
5	--	--
6	--	0.2320
7	5.3	0.4570
8	5.9	0.8754
9	8.7	0.0948
10	91.2	0.1951
Mean ± SEM	47.7 ± 23.7	0.3782 ± 0.1

[1]Somatomedin activity is expressed relative to pooled human reference serum which was assigned a somatomedin activity of 1.0.

[2]These means ± SEM were calculated for each individual group and were not part of the analysis of variance calculations.

[3]Rat #4 in this group was sick; all data obtained for him was therefore deleted.

TABLE 14A

Uptake of ^{35}S by Glycosaminoglycan (GAGS) of Rib Cartilage, Liver and Brain in Individual Rats

Group	UPTAKE OF ^{35}S BY GAGS		
	Rib Cartilage	Liver	Brain
	CPM ^{35}S x 10^3/ug uronic acid		
A			
Control Ad Libitum			
RAT #1	2.7	0.0063	--
2	1.9	0.0040	--
3	2.9	0.1600	2.40
4	3.6	0.0111	2.92
5	2.7	0.0084	3.22
6	3.9	0.0079	2.89
7	3.7	0.0057	2.52
8	2.6	0.0034	1.58
9	3.1	0.0133	3.00
10	3.7	0.0077	2.59
Mean ± SEM[1]	3.08 ± 0.2	0.0228 ± 0.02	2.64 ± 0.2
B			
Control 15 g			
RAT #1	2.7	0.0177	2.87
2	1.6	0.0656	--
3	2.2	0.1289	3.70
4	--[2]	--	--
5	3.4	0.0040	1.12
6	3.5	0.0062	3.86
7	2.1	0.0005	1.44
8	2.8	0.0064	2.13
9	3.1	0.0099	2.86
10	2.7	0.1437	1.56
Mean ± SEM	2.67 ± 0.2	0.0472 ± 0.02	2.41 ± 0.3

TABLE 14A (Continued)

Group	UPTAKE OF ^{35}S BY GAGS		
	Rib Cartilage	Liver	Brain
	CPM ^{35}S x 10^3/ug uronic acid		
C			
Control 10 g			
RAT #1	1.6	0.7663	3.20
2	1.8	0.8615	2.74
3	1.7	0.2133	3.21
4	2.5	0.6983	4.42
5	1.4	0.0133	1.72
6	1.7	0.0919	0.86
7	2.3	0.0731	1.69
8	2.2	0.520	1.80
9	1.7	--	1.48
10	2.6	0.2396	2.24
Mean ± SEM	1.95 ± 0.1	0.3975 ± 0.1	2.34 ± 0.3
D			
Control 5 g			
RAT #1	1.6	0.2031	2.0
2	1.3	0.1205	2.66
3	1.8	0.1690	3.64
4	1.1	0.1411	3.06
5	1.2	0.0102	2.10
6	2.0	0.3205	4.0
7	1.2	--	3.70
8	2.1	0.3025	3.16
9	1.9	--	2.81
10	2.8	0.2317	2.79
Mean ± SEM	1.7 ± 0.2	0.1873 ± 0.04	2.99 ± 0.2

TABLE 14A (Continued)

Group	UPTAKE OF ^{35}S BY GAGS		
	Rib Cartilage	Liver	Brain
	CPM ^{35}S x 10^3/ug uronic acid		
E			
Lysine Deficient			
RAT #1	0.9	0.0050	1.69
2	1.1	0.0079	3.25
3	1.0	0.0053	2.23
4	1.0	0.0059	5.11
5	0.8	0.1293	2.48
6	1.5	0.1130	2.97
7	2.3	0.1009	2.65
8	2.1	0.0949	2.89
9	3.0	0.0115	3.05
10	4.7	0.0144	3.34
Mean ± SEM	1.84 ± 0.4	0.0488 ± 0.01	2.97 ± 0.3
F			
Methionine Deficient			
RAT #1	2.5	0.1848	7.23
2	1.7	0.1128	3.53
3	1.2	--	2.95
4	0.9	0.2365	4.12
5	1.3	0.0078	4.52
6	1.4	0.3905	--
7	1.8	0.1652	--
8	3.0	0.2127	3.41
9	1.1	0.1068	4.24
10	2.1	0.0161	4.1
Mean ± SEM	1.7 ± 0.2	0.1592 ± 0.04	4.26 ± 0.5

TABLE 14A (Continued)

Group	UPTAKE OF ^{35}S BY GAGS		
	Rib Cartilage	Liver	Brain
	CPM ^{35}S x 10^3/ug uronic acid		
G			
Histidine Deficient			
RAT #1	0.7	0.0635	1.28
2	0.7	0.0155	1.36
3	0.8	0.0945	1.08
4	0.9	0.1352	1.79
5	1.1	0.0700	2.09
6	1.2	0.1061	2.68
7	0.9	0.0078	2.0
8	1.1	0.0157	2.47
9	0.9	0.0167	2.80
10	2.5	0.1497	2.09
Mean $\pm$ SEM	1.08 $\pm$ 0.2	0.0675 $\pm$ 0.02	1.96 $\pm$ 0.2
CONTROL			
Purina Chow			
RAT # 1	2.0	0.0499	2.29
2	2.4	0.0070	2.0
3	--	0.0617	3.42
4	2.6	0.0100	2.14
	2.3 $\pm$ 0.2	0.03215 $\pm$ 0.01	2.46 $\pm$ 0.3

[1]These means $\pm$ SEM were calculated for each individual group and were not part of the analysis of variance calculations.

[2]Rat #4 in this group was sick; data obtained for him was therefore deleted.

INFLUENCE OF SELECTED AMINO ACID DEFICIENCIES ON SOMATOMEDIN AND GLYCOSAMINOGLYCAN METABOLISM

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The effects of specific amino acid deficiencies on plasma somatomedin activity and glycosaminoglycan (GAGS) metabolism were investigated. Four-week old male weanling Sprague-Dawley rats were placed on each diet in one of seven experimental groups. Four control groups were fed the amino acid defined (control) diet ad libitum, 15 g, 10 g and 5 g per day. Three groups with modifications in the amino acid content of their diet were fed lysine deficient, methionine deficient and histidine deficient diets. In addition, a control group was maintained on laboratory chow fed ad libitum. Animals were maintained on their diets for 21 days. At the time of sacrifice, blood and tissues were collected for assays.

Results obtained show significant differences in food intake and weight change attributable to dietary treatments. Dietary deficiencies of the three amino acids caused a significant ($P<0.01$) decrease in food intake and consequently reduced caloric intake in the animals, leading to greater loss of weight as compared to rats fed the control diet. Caloric intake for rats fed the methionine and histidine deficient diets was comparable to the 5 g level of control diet. Lysine deficient animals had a caloric intake that lay at a level between 5-10 g of control diet. These levels of control diet were used as "pair-fed" controls for the amino acid deficient group of animals.

Plasma somatomedin levels of the amino acid group of rats were significantly lower than those of the group of rats fed the control diet ad libitum but were not different to the somatomedin levels of the "pair-fed" controls ($P<0.01$) although a definite trend was apparent. Somatomedin activity decreased as the diet changed from control to amino acid deficient.

A similar trend was observed for cartilage GAGS biosynthesis. In addition, methionine and histidine deficient animals had a significantly lower ($P<0.05$) uptake of ^{35}S than the lysine deficient group, suggesting a greater importance for methionine and histidine in the diet of growing animals.

Despite the low levels of somatomedin obtained in some groups, all animals had adequate plasma concentrations of growth hormone, confirming previous reports that nutrition is an important modulator of somatomedin activity with or without growth hormone.

Apart from the weight of the pituitary, changes in organ weights paralleled body weight changes, decreasing as the diet changed from control to amino acid deficient. Within the amino acid deficient groups there were no significant differences.

These results confirm previous reports that somatomedin activity and growth cartilage activity is reduced in conditions of protein-calorie malnutrition, despite normal to elevated plasma concentrations of growth hormone. However, adequate evidence could not be obtained to attribute the decreased somatomedin activity and growth cartilage activity to the specific amino acids investigated in this study. The decreased activity could be equally related to the quantity of calories consumed. Nevertheless, the conclusion can be drawn that adequate nutrition is required for the maintenance of normal levels of somatomedin and GAGS metabolism.