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L-THREONINE: ITS ABILITY TO INCREASE
GLYCINE MEDIATED NEUROTRANSMISSION
AND SUPPRESS SPASTICITY

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Submitted to the Department of
Brain and Cognitive Sciences
in partial fulfillment of
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Doctor of Philosophy

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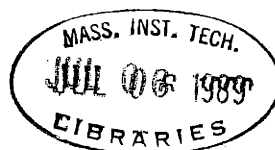


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ABSTRACT

Since Glycine has been accepted as a Central Nervous System inhibitory neurotransmitter, interest has arisen in its metabolism and pharmacology. Until Maher and Wurtman discovered that the administration of L-threonine led to an increase in glycine concentration in the spinal cord, there was no pharmacological tool (besides strychnine; a glycine antagonist) to study the effects of glycine.

Glycine has been postulated to be acting mainly in the spinal cord and the brain stem and has recently been shown to act on the NMDA receptor sites via different receptors than those mediating its direct neurotransmitter actions (i.e. the "strychnine receptors"). It is likely to be involved in modulating the reflex arc, in muscle tone and in central cardiovascular and respiratory regulation.

I examined the ability of L-threonine, and other amino acids which might influence threonine's uptake into the CNS, to increase glycine levels in the spinal cord. I then examined the biochemical and possible therapeutic effects of L-threonine in patients suffering from Familial Spasticity.

I confirmed previous findings that spinal cord threonine and glycine levels significantly increase in rats given L-threonine and extended it to show that this effect is inhibited by concurrent administration of both valine and serine. Serine, another glycine precursor, not only failed to increase cord glycine levels, but also significantly inhibited the uptake of threonine into the spinal cord and its ability to increase glycine levels.

The clinical studies had three phases:

-A preliminary open label study showing that threonine administration caused dose-related changes in clinical parameters of spasticity as well as in CSF levels of threonine and glycine.

A double blind study showing that L-threonine significantly improved some but not all of the manifestations of spasticity. The responders (77% of the patients), exhibited significant increases in CSF glycine levels. There was a significant increase in blood threonine in all the patients and in CSF threonine in 7 of the 10 patients from whom CSF was collected.

-A chronic study showing no significant side effects, no habituation or cumulative long term effects of L-threonine on spasticity.

These studies demonstrate the ability of exogenous L-threonine to increase glycine in the spinal cord and to improve spasticity.

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I. INTRODUCTION

Of all the classes of neurotransmitters known in the CNS, amino acids now appear to be quantitatively the most abundant. The amino acids can be divided into two general categories based on their neurophysiological actions: excitatory and inhibitory. Examples of excitatory amino acids include aspartic acid, glutamic acid, cysteic acid, and homocysteic acid (1, 2, 3, 4, 5). These excitatory amino acids, when applied iontophoretically to most cells of the mammalian central nervous system, cause the depolarization of the cells. Examples of inhibitory, amino acids include: gamma amino-butyric acid (GABA), taurine, beta-alanine and glycine (6, 7, 8, 9, 10). These substances usually cause a hyperpolarization of neurons.

Of the inhibitory amino acids, glycine and GABA are probably the most widely accepted candidates for chemical mediators of inhibition in the invertebrate and vertebrate central nervous system. In the mammalian spinal cord there appear to be approximately equal numbers of glycinergic and GABA-ergic nerve terminals (11, 12, 13). Throughout the central nervous system there are differences in the distribution of these two inhibitory neurotransmitters. GABA appears to be distributed throughout the central nervous system whereas glycine is mainly found in the spinal cord and brainstem.

Identification of glycine as an inhibitory neurotransmitter within the mammalian CNS system and its possible implication in a variety of functions, generated studies on factors that controlled its biosynthesis.

Glycine is a non-essential amino acid that can be synthesized from glucose and other substrates in all cells including those of the nervous system. Its synthesis from serine catalyzed by the enzyme Serine Transhydroxymethylase (STHM) is best documented (78, 85, 86) and, initial studies by Aprison et al. (122,93) found a highly significant correspondence between the levels of glycine in CNS regions and the activity of the enzyme STHM. It has thus, been generally assumed that serine is the specific precursor for glycine in the CNS.

However, glycine can also be synthesized from the amino acid L-threonine. Reports in the literature suggest that the enzymes catalyzing this reaction differ from those utilizing glycine formation from serine, and differ also in various tissues and species studied (104, 165, 166, 167). The cleavage of threonine into glycine has not, to my knowledge been investigated in the CNS.

The brain uptake index for threonine (i.e. the fraction of threonine in brain capillary taken up into the brain in a single pass), is about twice that for serine and four times that of glycine; 11.7, 7.5 and 2.53 respectively (79). This indicates that threonine enters the CNS more

readily. In contrast to threonine, glycine circulating in the plasma is largely excluded from the CNS by the blood-brain barrier (78): glycine administration increases plasma glycine levels but has little effect on the glycine levels in the CNS, unless massive doses are administered (145, 300, 301). In 1980 Maher and Wurtman conducted experiments in rats that eventually led to the discovery that threonine administration increased glycine levels in rats spinal cords (144). Seimers and Aprison (146) extended this finding by demonstrating a linear relationship between the concentrations of threonine and glycine in 10 sub-areas of the rat medulla, as well as in 8 major areas of the rat neuroaxis. Boyd et al. (147) studied the behavioral effects of threonine, and reported that it prevented the seizures in rats that usually follow intrathecally administered strychnine. Threonine's effect was mimicked by intrathecally administered glycine but not by serine. Taken together, these pre-clinical experiments indicate that administration of L-threonine increases CNS glycine levels, enhances glycinergic neurotransmission, and produces predicted behavioral effects in rats. If similar biochemical effects occurred in humans, L-threonine could be developed as a novel and effective treatment for CNS disorders in which there is a decrease in glycinergic neurotransmission. Spasticity is one clinical condition in which glycinergic neurotransmission is suspected to be

decreased (161, 162, 170).

The overall goals of this Thesis are: 1- to determine the circumstances under which, administration of the amino acid L-threonine increases in rat spinal cord glycine levels; 2- to determine whether threonine will increase CSF glycine in humans; and 3- to study threonine's safety and efficacy in treating patients with a disease which might involve glycinergic synapses, e.g. familial spasticity. Development of L-threonine as a novel treatment for familial spasticity would be a model of rational neuropharmacology. There is little information available regarding the biochemical changes that underlie specific aspects of familial spasticity in humans although inadequate GABA and glycine-related neurotransmission are highly suspected to be involved (131). The clinical study in this project addresses this gap in information, because changes in reflexes or other measures of spasticity induced by threonine would implicate glycinergic mechanisms.

II. LITERATURE REVIEW

A. Chemical neurotransmission.

Chemical neurotransmission was first suggested by Elliott in 1905 (155). Before this time most believed that neuronal transmission was an electrical phenomenon. Elliott noted the similarity between the effects elicited by the injection of adrenaline and the effects elicited by stimulation of the sympathetic division of the autonomic nervous system. Later, Dixon observed the similarities between injected muscarine and stimulation of parasympathetic division of the autonomic nervous system. Proof of chemical neurotransmission had however to await the work of Dale and Loewi.

In 1914, Dale (156) reinvestigated the pharmacology of acetylcholine and the similarities between acetylcholine, muscarine, and parasympathetic nervous system stimulation. He coined the term parasympathomimetic to describe the effects of these agents. The conclusive evidence for chemical neurotransmission came from Loewi in 1921 (157). He stimulated the vagus nerve of one isolated frog heart, noting the decrease in its rate, and allowed the effluent perfusate to come in contact with a second heart. The second heart's rate also slowed, indicating that some chemical was released by vagal stimulation of the first heart. This was the first demonstration that neural activity was mediated by chemical release at synapses.

B. CNS neurotransmitters.

At least three criteria must be satisfied before a substance can be considered a neurotransmitter. First, the putative transmitter should be found in the presynaptic neurons. Second, when the substance is applied iontophoretically to the postsynaptic membrane (pre-synaptic if this is how it acts), the changes in ionic permeabilities should mimic the natural neurotransmitter's action. And third, the substance in question should be released from the presynaptic terminal when the neuron is electrically stimulated. These three criteria are based on both neurochemical and neurophysiological data. The demonstration that a neuron possesses an uptake system for a particular substance may also be helpful in identifying neurotransmitters, but is not an absolute necessity.

Of all the classes of neurotransmitters known in the CNS, amino acids now appear to be quantitatively the most abundant.

The amino acids can be divided into two general categories based on their neurophysiological actions: excitatory and inhibitory. Examples of excitatory amino acids include aspartic acid, glutamic acid, cysteic acid, and homocysteic acid (1, 2, 3, 4, 5). These excitatory amino acids when applied iontophoretically to most cells of the mammalian central nervous system cause a depolarization of such cells. Examples of the other, inhibitory, category

of amino acids include: gamma amino-butyrlic acid (GABA), taurine, beta-alanine and glycine (6, 7, 8, 9, 10). These substances usually cause a hyperpolarization of neurons.

Of the inhibitory amino acids, glycine and GABA are probably the most widely accepted candidates for chemical mediators of inhibition in the invertebrate and vertebrate central nervous system. In the mammalian spinal cord there appears to be approximately an equal number of glycinergic and GABA-ergic nerve terminals. This was shown by Iversen and Bloom who incubated slices of spinal cord with [3H]-GABA or [3H]-glycine. Under the presumption that the nerve terminals which utilize these substances as neurotransmitter possess a specific uptake system (high affinity, sodium dependent) for that substance (11, 12), these researchers found that approximately 25% of nerve terminals were labelled with [3H]-GABA and 25% were labelled with [3H]-glycine (13). When tissues were incubated with both [3H]-GABA and [3H]-glycine, 50% of all terminals were labelled. Throughout the central nervous system there are differences in the distribution of these two inhibitory neurotransmitters. GABA appears to be active throughout the central nervous system whereas glycine is found in the spinal cord and brainstem. An average of 27% of the neurons in the cerebral cortex and 45% in the hippocampus will accumulate GABA, which

reinforces the importance of this substance as a central neurotransmitter (14).

C. Distribution of Glycine in the CNS

Dorsal root fibers enter the spinal cord and synapse on motoneurons or small interneurons with short axons found in the gray matter of the spinal cord. Since physiologically, inhibition in the spinal cord was demonstrated to be mediated through interneurons, it was reasoned that the highest concentrations, of inhibitory neurotransmitters should be found in the gray, especially ventral gray area of the spinal cord. Aprison and Werman (15, 16) found that in the cat's spinal cord, glycine concentrations were highest in the ventral gray and dorsal gray regions.

Lower concentrations of glycine were found in areas of white matter in the spinal cord. The distribution of GABA was similar to that of glycine and suggested that either substance could be the major inhibitory neurotransmitter of interneurons. These results were later confirmed in the cat (17) and in the rabbit (18) by other workers. It is interesting to note the distribution of the other two amino acids studied: glutamate, an excitatory amino acid, and glutamine, an amino acid with no apparent activity. These two amino acids were distributed quite differently from that of glycine and GABA.

To determine whether glycine or GABA was the major neurotransmitter of interneurons, Davidoff (19) took

advantage of the technique of aortic occlusion (20). Aortic occlusion produces a selective loss of the interneurons in the ventral gray matter. Rexed's lamina V-IX contain most of these interneurons and it was shown that aortic occlusion caused a decrease in the concentration of glycine but not of GABA. With a 50% reduction in the number of interneurons there was a 45% decrease in the concentration of glycine in that area. These studies indicated that glycine was the major inhibitory neurotransmitter in Rexed's lamina V-IX. More recent work with the localization of an enzyme responsible for GABA synthesis, glutamic acid decarboxylase, has reinforced the theory that GABA is primarily concerned with interneurons in the dorsal horn region and the area above the ventral gray (Rexed's lamina I-IV) (21) .

Aprison and co-workers then studied the distribution of glycine in seven different vertebrate species (22). The brain and spinal cord were sectioned and assayed. In all species studied the concentration of glycine in the medulla oblongata was on the average four to seven times higher than the concentration in the higher brain regions. Moreover, the concentrations of glycine in the spinal cord were consistently higher than those in the medulla oblongata. Also the interneuronal inhibition is apparently involved in the coordination of antagonistic muscle groups, i.e. when a flexor muscle is excited, the tone of the

antagonistic extensor muscle must be decreased to allow the action of the flexor to be seen. In species with limbs, there is a great need for reciprocal inhibition and thus one sees an increased number of interneurons in those areas of the spinal cord innervating the limbs and a correspondingly increased concentration of glycine in these areas. Depending on which limbs are used the most in a given species, one would expect to find higher levels of glycine in the area of the spinal cord innervating that limb. In the pigeon, more glycine is found in the cervical enlargement than in the lumbar enlargement because of this animal's powerful wings. The bullfrog, on the other hand, with its powerful hind limbs, contains higher concentrations of glycine in the lumbar enlargement than in the cervical enlargement. This pattern of glycine distribution has also been reported in humans, with highest levels found in the ventral gray of the lumbar spinal cord (23).

The retina contains an interesting distribution of amino acids, especially glycine (24). When the retina from a Rhesus monkey was analyzed for amino acid content, glycine was found to be concentrated near the border of the inner nuclear and inner reticular layers. The adjacent ganglion cells and outer reticular layers contained less than 50% of the peak levels seen elsewhere. Peak levels of glycine

approached 40 umoles/g in the inner nuclear layers. This is an extremely high concentration when compared with 4.0 umoles/g found in the rat cervical enlargement of the spinal cord (22). This unique distribution in the retina suggests some possible transmitter role for glycine in this tissue.

D. Electrophysiological Studies

The inhibitory actions of glycine were well established by the early 1960's but most researchers disregarded glycine as a possible neurotransmitter by suggesting that its actions were nonspecific (25, 26, 27, 28, 29). Initial studies showed glycine to be less potent than GABA, beta-alanine and taurine (3). Subsequent studies have shown that the iontophoretic application of glycine to spinal motoneurons causes a hyperpolarization of the cell which is more potent and more prolonged when compared to the hyperpolarization caused by GABA (30, 31, 32).

When equimolar concentrations of GABA or glycine are applied iontophoretically to neurons in the cat, different potencies are seen. In the cerebral cortex GABA is greater than 12 times more potent than glycine in causing hyperpolarization, while in the lumbosacral region of the spinal cord, glycine is 5.6 times more potent than GABA. The response to glycine in the spinal cord can be maintained for up to one minute with constant application. On the other hand, the response to GABA fades much more quickly and plateaus at a lower membrane potential value: approximately equal to 29% of its initial peak potency. In the cerebral cortex this desensitization occurs to both GABA and glycine. Moreover, studies in the Mauthner cells of goldfish showed that glycine was 7.3 times as potent as

GABA in eliciting a depolarizing response (33).

The response to glycine is characterized by an increase in the conductance of chloride ion. The injection of bromide or iodide ions into the cell will invert the polarity of an evoked inhibitory postsynaptic potential (IPSP) and change the action of glycine to one of depolarization. This phenomenon may be explained by realizing that bromide and iodide enter or leave the cell by way of the same channel that chloride utilizes. When these channels open, as a result of glycine's action through interaction with a receptor located on the outside surface of the cell (the intracellular injection of glycine has no effect on the membrane potential of the cell), bromide and iodide are free to move out of the cell down their concentration gradients and thus cause a reversal of the evoked IPSP and change hyperpolarization to depolarization.

The glycine-induced hyperpolarization can be reversed by the intracellular injection of chloride ion as can the neurally-evoked IPSP, suggesting that chloride is the prominent ion in the production of IPSP's. The contribution of potassium ions in IPSP's is not fully known. Glycine, besides hyperpolarizing motoneurons, will also hyperpolarize interneurons in the spinal cord with a reversal potential identical to or very similar to that of the IPSP (34, 35). These results are in agreement with the

hypothesis that glycine acts as the natural inhibitory neurotransmitter at spinal cord interneurons and motoneurons.

Studies in lamprey reticulospinal neurons have shown that glycine is more potent than GABA in causing increases in the chloride conductance when these cells are bathed in a $3 \times 10^{-5}M$ solution of glycine or GABA (36). Another study (37) showed that lamprey giant reticulospinal neurons (Muller cells) did not respond to acetylcholine, carbamylcholine, norepinephrine, dopamine, histamine or serotonin but did respond to GABA and glycine. The reversal potential for both GABA and glycine averaged about -83 mV, which is about the same as that for the IPSP's produced by stimulation of the ipsilateral vestibular nerve.

The application of glycine to the V-3 visceral ganglion in Onchidium causes depolarization (38). All other neurons in the ganglion were not affected by glycine. This effect on V-3 is opposite to the effect on mammalian spinal motoneurons. Glycine has also been reported to cause depolarization in the isolated frog spinal cord (39).

E. Release-Uptake

Various preparations have been used to study glycine release and uptake (40-54) : synapsomal preparations, techniques including "push-pull" cannula, stimulation of intact cat spinal cord, medulla oblongata and medial lemniscus, cat retinal preparations and spinal cord slice electrical - or K^+ - evoked stimulation. Some of the results were inconsistent but the overall findings indicate that glycine is released from the spinal cord following electrical - or K^+ - evoked stimulation. There is a Ca^{++} dependent release of glycine from synaptosomes prepared from spinal cord and medulla oblongata but not from cerebro-cortical or hypothalamic synaptosomes (55, 56, 57, 58). The non-specific release blocker tetanus toxin has been reported not to change the levels of glycine in whole spinal cords (59) and also to significantly decrease glycine in gray matter, but not in white matter (60). Osborne (61) showed that rats treated with tetanus toxin had normal synaptosomal glycine contents and that there was a significant decrease in the release of glycine in these preparations following stimulation.

One of the problems in the study of glycine release is its possible rapid uptake. At the present time, it is not known whether glycine is taken up by the nerve cell following release; nonetheless, most researchers think that this could be the case because of the ratio of the

cerebrospinal fluid (CSF) glycine concentration to the blood glycine concentration. This ratio was one of the lowest for all amino acids (CSF = 15 nmole/ml, plasma = 348 nmole/ml, CSF/plasma ratio = 0.043), suggesting that glycine might have a functional role, which, in order to be properly regulated, must contain a very active uptake system to keep glycine at acceptable levels (40). This uptake system, if effective, could possibly prevent the detection of released glycine. Moreover, if an inhibitor of glycine uptake was known this would greatly facilitate the attempts to measure released glycine. To date no satisfactorily selective inhibitor is known.

Neal in 1969 (45) reported that rat spinal cord slices would accumulate [¹⁴C]-glycine rapidly. In forty minutes there was a tissue-to-medium ratio of 30:1. In order to determine if the glycine was being taken up to be utilized in protein synthesis, the amount of label in the protein precipitate was determined and found to be less than 2% of the [¹⁴C]-glycine. The uptake system had an apparent K_m for glycine of 31 μ M. Optimal uptake occurred at 37°C and was dependent on sodium (replacement of sodium chloride with choline chloride reduced glycine uptake to less than 1% of control). Strychnine, a postsynaptic glycine antagonist, did not affect uptake.

The uptake of glycine by the spinal cord showed

considerable specificity as incubations of tissue with GABA, DL-alpha-alanine, L-histidine, DL-aspartate, DL-valine, L-proline and L-glutamate at concentrations of 10^{-3} M did not significantly reduce the uptake of [14C]-glycine. In these studies glycine was taken up preferentially by gray matter (tissue: medium ratio of 1:5) after 30 minutes of incubation time. The ratio for white matter was similar to that obtained for the whole spinal cord at 0°C.

Johnston and Iversen (62), in 1971, studied the uptake of labelled glycine in various regions of the rat central nervous system. Areas generally not thought of as containing glycinergic synapses (cerebral cortex, cerebellum, midbrain) had high K_m values (50, 56, and 36 respectively). These results are consistent with the concept of low-, and high-affinity uptake systems. The K_m for glycine uptake in the cerebral cortex is similar to those for L-alpha-alanine, 2-aminoisobutyric acid and L-serine and therefore suggests that there is a distinct transport system for small neutral amino acids that is operating in the cerebral cortex. These amino acids did compete with glycine for uptake in the cerebral cortex but did not compete in the spinal cord. The low-affinity system for glycine uptake is present in the spinal cord but contributes only minimally to the total glycine taken up in this tissue. It has been estimated that less than 5% from

a 10⁻⁵M glycine concentration would be transported by this low-affinity uptake system in the spinal cord (63).

Logan and Snyder (12) studied the uptake of amino acids in synaptosomes prepared from rat cerebral and spinal cord. Synaptosomes from both areas exhibited the low-affinity uptake system. In the spinal cord the K_m for glycine uptake by the high-affinity uptake system was 26.5 μ M. The high-affinity systems were also found to be sodium-dependent. Bennett *et al.* (64) also used synaptosomal preparations from rat spinal cord and cerebral cortex. The high-affinity systems in the spinal cord for glutamic acid, aspartic acid and glycine were sodium-dependent and high-affinity systems for glutamic and aspartic acids in the cerebral cortex were also sodium-dependent. No high-affinity, sodium-dependent transport system was found in the cerebral cortex for glycine.

Using crude synaptosomal preparations (P2) from telencephalon and spinal cord, Aprison and McBride (65) reported that during the first three minutes of incubation with [U-14C]-glycine, these two preparations accumulated glycine at a rate of 0.100 and 0.0243 nmoles/mg. protein/minute respectively. The addition of ouabain or 2,4-dinitrophenol inhibited the accumulation of glycine in both preparations. Attempts to discover which subfraction of the P2 fraction contained the labelled glycine revealed

that the subfraction containing synaptosomes accounted for over 85% of the total label present. When the level of glycine was kept low (0.0375 mM) in the incubation medium, the P2 fraction from spinal cord had a higher rate of uptake than did the P2 fraction from telencephalon (approximately five times higher). At a higher level of glycine (0.150 mM) the spinal cord P2 fraction accumulated glycine approximately only twice as fast as did the P2 fraction from telencephalon.

Studies of glycine uptake in frog retina showed that this amino acid was concentrated in amacrine cells (66). The uptake process required sodium ions to be active and had a K_m for glycine of 16.7 μM . Moreover, light promoted the uptake of glycine by ganglion cells.

Most researchers believe that glycine is removed from the synaptic cleft following glycine release, and that the uptake system is a high-affinity, sodium-dependent system which is located only in areas of the central nervous system where glycine is suspected of having a functional (i.e., neurotransmitter) role.

F. Pharmacology

Neurophysiologically, the action of glycine is antagonized by strychnine (67, 68). Low doses of strychnine will block the response to iontophoretically applied glycine but not GABA, the other major inhibitory neurotransmitter in the spinal cord (34, 69). Strychnine-sensitive glycine inhibition is observed in the spinal cord and brainstem but decreases sharply in higher areas of the brain (70). This correlates well with the findings of lower glycine concentrations in the rostral brain areas where it is believed that glycine does not have a functional role. The actions of GABA are selectively blocked by low concentrations of bicuculline and picrotoxin (6). Strychnine, bicuculline and picrotoxin all cause convulsions which can be explained by the blocking effect of these agents on inhibitory processes.

[3]H - Strychnine with high specific activity was used to bind synaptic membranes. The dynamics of its displacement by glycine with scatchard analyses of binding revealed only one population of receptors (71).

Receptor distribution parallels closely that of glycine concentrations and neurophysiological activity. As expected, when the spinal cord of the monkey was separated into gray and white matter, highest binding occurred in the gray matter with almost no binding taking place in the

white matter (72). Protein-modifying agents were used to determine that strychnine bound to the same site on the receptor as did glycine (71). Diazonium tetrazole and acetic anhydride had very little effect on the total amount of strychnine bound to synaptic membranes but did interfere with the ability of glycine to displace bound strychnine. Tetranitromethane and dinitrofluorobenzene inhibited total strychnine binding but did not interfere with the ability of glycine to displace bound strychnine. Tetranitromethane and dinitrofluorobenzene inhibited total strychnine binding but did not interfere with the ability of glycine or strychnine to displace bound [3H]-strychnine. These data suggest that glycine and strychnine bind to separate sites on the receptor. Hill plots gave an n value of 1.0 for the displacement of [3H]-strychnine by strychnine and an n value of 1.7 for glycine. Glycine probably interacts with strychnine in a cooperative fashion, which appears to be a very important mechanism for controlling synaptic events. If a small amount of glycine were to leak into the synaptic cleft, the sigmoid characteristics of the dose-response curve should help to prevent premature firing of the neuron. This also eases the burden placed on the glycine-uptake system in that every last molecule of glycine does not have to be removed from the synaptic cleft to terminate an action.

The ability of various anions to modify strychnine

binding has suggested that strychnine binds to some portion of the receptor that is intimately involved with the chloride channel (71). Anions can be injected into neurons, the neuron then is given inhibitory stimuli and if the anion is capable of traversing the chloride channel (depends on the hydration radii) a reversal of the IPSP will occur (i.e., depolarization). The anions that were capable of reversing the IPSP (bromide, iodide, nitrate) were also active in inhibiting strychnine binding. Anions that were not capable of reversing the IPSP (acetate, bicarbonate, fluoride) did not affect strychnine binding.

When a variety of pharmacological agents was tested for their ability to displace bound strychnine, it was found that diazepam inhibits binding by 50% of the strychnine binding at 200uM (72). To determine if the pharmacological activity of the benzodiazepines was a result of an interaction with the glycine receptor, Young and Snyder then compared the activity of 20 different benzodiazepines on strychnine binding inhibition, with their clinical and other pharmacological potencies. The "human bioassay", defined as the minimal dose at which 50% of the subjects experience subjective effects, correlated closely with the binding inhibitory activity (correlation coefficient $r = 0.74$). Seven other tests in laboratory animals revealed similarly high correlation coefficients. Other drugs, notably centrally acting muscle relaxants, were tested and

none showed any ability to inhibit strychnine binding. The authors then tested the ability of the benzodiazepines to modify [3H]-dihydromorphine binding to opiate receptors in rat brain to rule out the possibility that the lipid solubility, and therefore accessibility to the brain, might explain the close correlation seen with the clinical potencies of the benzodiazepines and their ability to displace bound strychnine. No correlation was seen. These data, when one considers that benzodiazepines have muscle-relaxant properties and that glycine is probably involved in reciprocal inhibition in the spinal cord, suggested at the time that the benzodiazepines might act by way of the glycine receptor.

Studies were then performed in the intact animal to determine if the benzodiazepines acted in a manner suggested by the above in vitro determinations. Curtis et al. (73), using mice, calculated the dose of strychnine and bicuculline needed to cause convulsions in 50% of the animals (CD50). They then calculated this same CD50 for these two antagonists for diazepam (2.5 mg/kg) in pretreated mice. The CD50 for strychnine was 1.54 mg/kg in untreated mice and 1.46 mg/kg in diazepam-pretreated mice. The CD50 for bicuculline was 4.2 mg/kg in untreated mice and 8.5 mg/kg in diazepam-pretreated mice, thus suggesting that the anticonvulsant properties of the benzodiazepines

are mediated by way of a GABA-ergic mechanism and not a glycinergic mechanism. Studies involving the GABA antagonist, picrotoxin, also suggested that benzodiazepines act through a GABA-ergic mechanism (74).

An investigation into the action of benzodiazepines on single neurons in the rat medulla oblongata showed that bicuculline could block the depressant actions of flurazepam and GABA but not of glycine (75). Strychnine, on the other hand, did not affect the action of flurazepam or GABA but did antagonize the action of glycine.

Glycine has also been found to have activity at N-Methyl D-Aspartate (NMDA) receptor sites. It greatly increases the frequency of channel openings evoked by NMDA but does not open the channel when applied alone (164). This effect is not antagonized by strychnine but has recently been shown to be antagonized by Indole-2-Carboxylic acid (313).

G. Metabolism

Glycine, aminoacetic acid ($\text{NH}_2\text{CH}_2\text{COOH}$), is a nonessential amino acid with a molecular weight of 75.07 daltons and is the only naturally occurring amino acid to lack an asymmetric carbon atom. When compared with other generally accepted neurotransmitters such as norepinephrine, serotonin, and acetylcholine, glycine was considered to be too "simple" to be a serious neurotransmitter candidate despite its potent neurophysiological actions. Following the discovery of the unique distribution of glycine in the mammalian central nervous system and the discovery of a specific receptor antagonist, strychnine, the metabolism of glycine was reinvestigated.

Since glycine is a nonessential amino acid, glycine levels in the central nervous system could be maintained by: (A) the transport of glycine from the periphery, (B) the transport of precursors from the periphery, (C) resynthesis from catabolic products, or (D) a combination of these possibilities.

The ability of glycine to enter the brain from the periphery is limited by the blood-brain barrier. Another route of entry between the periphery and the central nervous system is by way of the choroid plexus which forms the blood-cerebrospinal fluid barrier (76). The surface area of the blood-brain barrier is at least 5,000 times that of the choroid plexus and therefore is probably the

most important limiting factor for the entry of circulating substances into the central nervous system (77).

A method to study the ability of a substance to enter the brain was developed by Oldendorf (79). It measures the loss of the ^{14}C -labelled test substance to brain during a single capillary passage following the rapid injection into the rat carotid artery. The test substance is mixed with ^3H -water and allowed one circulatory pass following injection. The $^{14}\text{C}/^3\text{H}$ ratio is determined in the tissue and is divided by the same ratio in the injected mixture and the result is multiplied by 100 to provide the uptake of the test substance relative to the uptake of water. This value is known as the brain uptake index (BUI) and is thus calculated:

$$\text{BUI} = \frac{\text{tissue} - ^{14}\text{C}/\text{tissue} - ^3\text{H}}{\text{mixture} - ^{14}\text{C}/\text{mixture} - ^3\text{H}} \times 100$$

Water is used as a reference because of its very high brain uptake and, as a reference, (by definition), the BUI for water is equal to 100. The BUI for tritiated HOH with respect to butanol, for example, varies between 60 and 70% depending on the CNS area studied (312).

The BUI's for threonine, alanine, serine, glycine and GABA were 11.7, 7.5, 7.5, 2.53 and 2.2 respectively (79). Shank and Aprison (78) investigated the ability of glycine to penetrate the blood brain barrier following intraperitoneally administered, labelled glycine. Their

results indicated that although glycine can pass from blood to brain and spinal cord of rat, most of the glycine in the central nervous system is synthesized de novo from serine. Intraperitoneally administered, labelled serine is capable of entering the brain but ultimately glucose acts as the major source of carbon units for both glycine and serine.

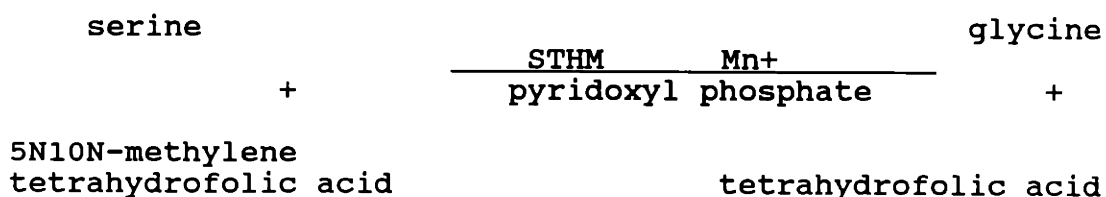
The ability of glucose to enter the brain is dependent upon the hexose carrier and has a BUI of 33 (79). Glucose can then be metabolized via four pathways leading to glycine. In the first two pathways glucose is metabolized through glycolysis to glycerate-3-phosphate. Glycerate-3-phosphate then can be converted to either hydroxypyruvate-3-phosphate or glycerate-2-phosphate. Hydroxypyruvate-3-phosphate can be converted to serine-3-phosphate and finally to glycine via serine, while glycerate-2-phosphate can be converted to glycerate and finally to glycine via serine. The former pathway is referred to as the phosphorylated pathway and the latter as the nonphosphorylated pathway. The two other pathways from glucose to glycine are via glyoxylate. Hydroxypyruvate can be converted to glycoaldehyde, then to glyoxylate and finally to glycine. Glycerate-2-phosphate can be converted to oxalacetate, then to isocitrate, glyoxylate and finally to glycine.

Bridgers (80) has demonstrated that the phosphorylated pathway of serine synthesis is most abundant in mouse brain

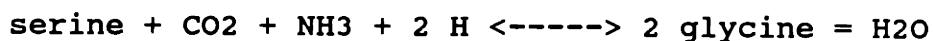
extracts of the two serine pathways and has suggested that the control point in serine synthesis is the phosphoserine phosphohydrolase reaction which converts serine-3-phosphate to serine (81). Other workers have shown that the nonphosphorylated pathway of serine synthesis is operative in the central nervous system of the rat (82) and cat (83). These studies showed that glycine appeared to inhibit D-glycerate dehydrogenase of rat cortex in a manner non-competitive with respect to D-glycerate, and to inhibit 3-phosphoglycerate dehydrogenase to a much smaller degree. These findings suggested that glycine might act as an end-product inhibitor of the nonphosphorylated pathway. Major criticisms of this study have dealt with the poorly purified extracts employed. When the activities of D-glycerate dehydrogenase in various areas of the cat central nervous system (cortex, caudate, thalamus, mesencephalon, cerebellum, pons, medulla and spinal cord) were compared with the levels of glycine in these areas, correlation coefficients of 0.77 and 0.85, respectively, were obtained. When the activities of 3-phosphoglycerate dehydrogenase or lactate dehydrogenase were compared with glycine levels, no correlation was seen. In opposition to these findings, Feld and Sallach (84) have challenged the importance of the nonphosphorylated pathway in the porcine central nervous system.

The above two pathways have a common immediate precursor

to glycine: serine. The conversion of serine to glycine takes place by way of the enzyme serine transhydroxy-methylase (STHM) in both the periphery (85) and the central nervous system (78, 86). This reaction is diagrammed below:



This reaction yields one-carbon units in the form of 5N, 10N-methylene tetrahydrofolic acid which is needed for purine and thymine biosynthesis. Methionine is regenerated from homocysteine with the aid of the one carbon unit donated by serine (87). These findings suggest another important role for STHM. Serine can be converted to glycine via another reversible pathway in the periphery (88):



In the liver this reaction is catalyzed by a complex of four proteins known as the glycine cleavage system (GCS) which is located within the inner-membrane fraction of mitochondria (89). The GCS has been found to be present in the central nervous system of the rat, cat and sheep with an activity and distribution similar to that found in liver (90). Because the GCS reaction accounts for a large

proportion of the degradation of glycine in the periphery, it was unexpected to find low levels of the GCS in the medulla and spinal cord while high levels were found in the cerebellum. These puzzling findings were confirmed by others (91) and suggested that the GCS does not contribute to central nervous system glycine synthesis to any great extent but may be involved with the degradation of glycine. To what extent the GCS contributes to the inactivation of neurotransmitter glycine is not known but most feel that the potent reuptake system for glycine is probably the most important mechanism (45).

In order to study the importance of STHM upon the regulation of central nervous system glycine concentrations two laboratories have studied the regional distribution of this enzyme within the central nervous system. Davies and Johnston (92) found levels of STHM activity that ranged from 2.32 to 2.64 $\mu\text{mole/hr/g}$ wet weight in rat cerebral cortex, cerebellum, medulla-pons, diencephalon, mesencephalon and spinal cord. There was no correlation between glycine content and STHM activity but higher levels of STHM were found in the cat spinal gray matter as compared with white matter and ventral gray was higher than dorsal gray. Later Daly and Aprison studied the distribution of STHM in five areas of the rat central nervous system (93). They found an excellent correlation between the level of STHM and glycine in the spinal cord,

medulla-pons, midbrain and telencephalon whether $\mu\text{mole/hr/g}$ tissue or $\mu\text{mole/hr/mg}$ protein were used to express the STHM activities (correlation co-efficient = 0.997). There was a poor correlation between glycine content and STHM activity for cerebellar data. When the levels of succinate dehydrogenase were used to estimate the density of mitochondria per tissue (94) and used to calculate the STHM activity per density of mitochondria, an excellent correlation coefficient was obtained for all areas including the cerebellum (correlation coefficient = 0.952). The cerebellum was found to have a high density of mitochondria and five to ten times the amount of DNA when compared with the other regions studied. High STHM levels may indicate a greater need for one-carbon units in this tissue. The excellent correlation of STHM activity with the concentrations of glycine in the central nervous system suggests that STHM may play an important role in the regulation of neuronal glycine levels.

Around the same time that serine was being given serious consideration as the precursor for neuronal glycine, some researchers suggested that glyoxylate may serve as a precursor for glycine. Johnston and Vitali (95) noted the presence of a transaminating activity between glutamate and glyoxylate in rat spinal cord extracts. The enzyme responsible for the conversion of glyoxylate to glycine is glycine-2-oxoglutarate transaminase, which is one of the

glycine transaminases (GT) which utilizes glutamate as an amino group donor. Other ninhydrin-positive substances can also donate amino groups for the production of glycine. The reverse reaction may occur to a very small extent in the mammalian central nervous system by way of the enzyme D-amino acid oxidase but does not appear to be an important pathway for glycine metabolism (96). When the levels of GT were studied in cat spinal cords, higher levels were found in the gray matter than in the white matter (97). Another study showed no correlation between glycine content and levels of GT activities in the rat central nervous system (93).

Shank et al. (98) studied the incorporation of various precursors of glycine in vivo in the rat to determine the relative contributions of the various biosynthetic pathways discussed previously. Following the intraperitoneal administration of [U - 14C]-serine, label accumulated in the glycine within 20 minutes. The highest glycine-to-serine ratio (G/S) was found in the spinal cord, followed by the medulla. Low G/S ratios were found in the telencephalon and cerebellum where glycine probably does not act as a neurotransmitter. Following the administration of [U - 14C]-glucose intraperitoneally, label accumulated in both serine and glycine with a similar G/S pattern as seen with the [U - 14C]-serine injection. The authors concluded that the label found in the central nervous

system was primarily the result of metabolism within the central nervous system rather than metabolic conversion in other tissues prior to passage into the brain. Because the [U - ¹⁴C]-glucose G/S's were similar to the [U - ¹⁴C]-serine G/S's in each of the four regions studied, the authors also concluded that the flow of carbons from glucose to glycine occurs predominantly through serine.

In order to determine what amount of glycine was being synthesized from glucose via serine and glyoxylate, the authors then administered intracisternally [3,4 - ¹⁴C]-glucose and [1 - ¹⁴C]-glucose to rats and measured the label present in the medulla-pons. Because the number 3 and 4 carbon atoms of glucose end up in the carboxyl position of serine and remain as the carboxyl carbon in glycine, one would expect [3,4 - ¹⁴C]-glucose to label glycine via the serine pathway. When [1 - ¹⁴C]-glucose is converted to glycine via serine, the number one carbon is not incorporated into glycine, as it is lost as N⁵, N¹⁰-methylenetetrahydrofolate (the label could be incorporated into glycine via the GCS reaction but this pathway does not seem to contribute to central nervous system glycine to any great extent). Because the carbon atoms in the 3 and 4 positions are oxidized to CO₂ prior to the reaction in which glyoxylate is made via isocitrate or glycoaldehyde, one would not expect to find label present in glycine if [3,4 - ¹⁴C]-glucose goes through the

glyoxylate pathways. Carbon one is incorporated into glyoxylate via either pathway and would be expected to label glycine. When the medulla-pons was analyzed it was found that five times more glycine was labelled by [3,4 - 14C]-glucose than by [1-14C]- glucose.

Factors that argue against the role of glyoxylate as a major source of glycine in the central nervous system include (A) low levels of glyoxylate in the central nervous system, (B) toxicity of glyoxylate to nerve cells (99), and (C) a lack of readily available precursor for glyoxylate in the central nervous system (100).

In order to determine which pool (metabolic, presynaptic, glial) was being altered by the administration of labelled serine or glucose, the central nervous system must be subfractionated and examined. When the brain or spinal cord is homogenized with a moderate shear force (clearance of mortar and pestle equal to 0.25mm) while suspended in iso-osmotic aqueous sucrose (0.32 M), the presynaptic boutons, with a portion of the immediately opposed postsynaptic membrane, are torn away from the axon and seal up to form detached particles known as synaptosomes (101). Synaptosomes can then be separated from other subcellular particles by ultracentrifugation on a sucrose density gradient. One finds that myelin will float on 0.8 M sucrose, mitochondria are denser than the 1.2 M sucrose layer and therefore precipitate, and the synaptosomes are

found at the interface of the 0.8 M and 1.2 M layers (102). These isolated synaptosomal particles retain the morphological features and, to a great extent, the chemical composition (neurotransmitters, enzymes) of the intact presynaptic terminal.

When P2 fractions (crude synaptosomes - not separated on sucrose gradient) were examined for their ability to metabolize labelled serine and glucose, an interesting finding was obtained (103). The rate of conversion of [14C]-serine to glycine was highest in the cerebellum and lowest in the medulla. The spinal cord had an intermediate rate. The reverse reaction (glycine ----> serine) was lowest in the cerebellum. These findings show that the P2 fractions maintain at least some of the enzymatic activity necessary for the conversion of serine to glycine. The high rates of glycine synthesis in the cerebellum probably reflect the need for one-carbon units by this tissue.

Most of the data published to date points to serine, derived from glucose, as the major precursor for central nervous system glycine. Shank and Aprison (78) have approximated the rate at which glycine and serine are inter-converted in the central nervous system of the rat. The fluxes of glycine and serine from blood into the central nervous system are 0.03 - 0.15 and 0.15 - 0.25 umole/hr/g, respectively. The rate of biosynthesis of glycine from serine located within the central nervous

system is 1.0 - 3.0 umole/hr/g, while the rate of biosynthesis of serine from glucose under the same conditions is approximately 1.0 umole/hr/g. These data suggested that serine is the immediate precursor of neuronal glycine.

Another precursor of glycine is threonine. Threonine is converted into glycine and acetaldehyde by the enzyme threonine aldolase which was identified by Schirch and Gross in 1968 as being identical to the enzyme STHM (104) in the rabbit liver. Other investigators studying the cleavage enzymes of threonine and serine leading to glycine have identified these enzymes to be different in Clostridium pasteurianum (165), rat liver (166), and in cat hepatocytes (167). Nasuda et al. (166) demonstrated that in the rat liver, STHM was capable of forming a schiff base with L-threonine, but could not cleave L-threonine into glycine and acetaldehyde. L-threonine did not compete with serine for metabolism into glycine in cat hepatocytes (167). Two other pathways are currently known for the catabolism of threonine in rat liver: threonine dehydratase converts threonine into ammonia and 2-ketobutyrate; the latter compound, in turn is metabolized to propionyl CoA. The third pathway involves the conversion of threonine into 2-amino-3-oxobutyrate (precursor of aminoacetone) which itself undergoes a CoA-dependent cleavage to glycine and

third pathway involves the conversion of threonine into 2-amino-3-oxobutyrate (precursor of aminoacetone) which itself undergoes a CoA-dependent cleavage to glycine and CoASAc by 2-amino-3-oxobutyrate-CoA ligase (168).

Glycine catabolism occurs primarily through glycine synthase, the glycine cleavage enzyme (314). This enzyme system consists of four enzyme proteins: a pyridoxal-containing protein, glycine decarboxylase, a lipoic acid containing protein, aminomethyl transferase, a methylene tetrahydrofolate synthesizing protein and a flavoprotein, dihydrolipoyl dehydrogenase. The four components appear to be present as an enzyme complex located in the inner mitochondrial compartment (316).

H. The Glycine Receptors

In the past several years, Snyder and colleagues have conducted a detailed investigation of the glycine receptor by using (3H)strychnine as a marker for the receptor. They were able to show that a) there is higher (3H)strychnine binding in the spinal cord and medulla than in other brain areas (169); b) the binding of (3H)strychnine in the spinal cord and medulla is associated with the synaptic membrane portion (71); c) glycine and strychnine appear to bind to two separate sites of the same receptor, which are capable of mutually influencing each other (171); d) the high affinity uptake system of glycine and the (3H)strychnine binding develop between days 14 and 21 in the chick embryo (172), and these data are consistent with the time of emergence of electrophysiologically observable inhibition in the chick embryo spinal cord (173, 174); e) the (3H)strychnine binding site and the competition of different anions with this site suggest a close association with the ionic conductance mechanisms of the receptor (171).

More recently, glycine has also been found to have activity at N-Methyl D-Aspartate (NMDA) receptor sites (164). The potentiating effect of glycine on the NMDA receptor is not antagonized by strychnine (164).

J. Precursor Control

Wurtman and coworkers recognized that rapid and specific changes in brain composition normally occur in response to circulatory neurotransmitter precursors (80).

Fernstrom and Wurtman (81) realizing that brain tryptophan levels were usually below the level needed to saturate the enzyme responsible for serotonin synthesis: tryptophan hydroxylase, studied the effects of tryptophan administration on plasma and brain tryptophan levels and brain serotonin levels. Brain serotonin levels at 1 PM were significantly increased one hour following an intraperitoneal dose of tryptophan which was smaller than one-twentieth of the normal daily dietary intake. Following this injection of 12.5 mg/kg of tryptophan, plasma and brain tryptophan levels were significantly increased within one minute. The results of this experiment suggested to the authors that changes in plasma tryptophan, are capable of influencing brain serotonin levels. A new field had been opened with respect to the control of central neurotransmitters.

The authors then studied the dynamics of how precursors compete to enter the brain and found that the ratio of the precursor amino-acid to the other amino-acids sharing the same transport system, determines the rate of entry of that precursor. In the case of tryptophan, the competing amino-acids were the large neutrals (LNAA), and the ratio is

TRP/LNAA. They found for example that protein meals increased the denominator more than the numerator of this ratio; hence the TRP/LNAA decreased, thereby diminishing tryptophan entry into the brain (82).

The administration of choline intraperitoneally or by way of the diet causes an increase in plasma and brain choline levels along with an increase in the level of acetylcholine (83, 84). Further studies have indicated that plasma choline normally does fluctuate postprandially depending on what has been eaten (293) and that when acetylcholine levels are elevated by precursor administration (choline or lecithin), there is a parallel increase in the amount of acetylcholine released into synapses, which enhances cholinergic transmission (294). Utilizing this information, Davis, et al. (295) found choline to be of use in the treatment of tardive dyskinesia. These findings were later confirmed by Growdon et al. (296).

Wurtman et al. (297) have suggested that nutritional and endocrine factors might normally influence brain catecholamine synthesis by controlling the availability of the precursor: tyrosine. The administration of tyrosine (50 mg/kg intraperitoneally) increased brain tyrosine and dopa within one hour in animals pretreated with R04-4602 (a dopa decarboxylase inhibitor). Large doses of tryptophan, leucine or parachlorophenylalanine lowered brain tyrosine and dopa contents while a low dose of phenylalanine raised

brain tyrosine probably through the conversion of phenylalanine to tyrosine in liver. Larger doses of phenylalanine lowered brain tyrosine. This finding can be explained by realizing that phenylalanine is a LNAA and would be expected to compete with tyrosine for uptake at the blood-brain barrier.

When catecholamine synthesis is altered by administration of precursor, parallel changes in the release of dopamine and norepinephrine are seen (298). These changes are detected by measuring changes in brain levels of the major metabolites, homovanillic acid and methoxyhydroxyphenylethylglycol (MOPEG) sulfate. Sved et al. (299) have reported that the administration of tyrosine reduces blood pressure in spontaneously hypertensive (SHR) rats. A dose of 50 mg/kg intraperitoneally lowers blood pressure by 12 mm Hg while 200 mg/kg lowers blood pressure by 40 mm Hg. Tryptophan also lowered blood pressure but was only half as effective as an equivalent dose of tyrosine and the hypotensive response could be blocked with pretreatment with metergoline, a serotonergic antagonist. There was a significant correlation between the effect of those doses of tyrosine that were able to lower blood pressure and their effect upon increasing brain MOPEG-sulfate. Other LNAA alone did not alter blood pressure but when combined with tyrosine decreased the hypotensive effectiveness of tyrosine, indicating that competition at

the blood-brain barrier does occur.

Wurtman and coworkers traced the effect of precursor control one step further by showing that it not only enhanced transmitter release but also neurotransmission giving rise to a chemical change in the postsynaptic cells (294). Furthermore, they demonstrated a relationship between the firing frequency of a neuron and the extent to which it responds to an increased supply of its transmitter's precursor. Treatments which accelerated splanchnic nerve firing, (including putting animals in cold environment, giving them very large doses of insulin and administering drugs that cause prolonged depression of blood pressure), potentiated the ability of choline to increase the amount of acetylcholine released (294). The relationship of firing rate and precursor control was further demonstrated by Hefti et al. (170) in the striatum. They damaged the striatum on one side of the brain causing it to increase its firing rate on that side. Tyrosine led to an increase in the dopamine metabolite, homovanilic acid in the side with the elevated firing rate but not on the normal side.

Wurtman summarized the general laws governing the relationships between the synthesis of neurotransmitters and the plasma levels of their precursors as follow:

1. The limiting step in the biosynthesis of the

transmitter must be catalyzed by a low-affinity enzyme which, at normal substrate concentrations, is unsaturated with the precursor.

2. This enzyme must not be subject to significant end-product feedback control when the neuron containing it is firing frequently.

3. The amount of the enzyme's substrate (the neurotransmitter precursor) present within the neuron must depend on its concentration in the plasma, either because the nerve terminal is unable to make the precursor (e.g., tryptophan) and obtains it solely by influx from the plasma, or because, even though the neuron can synthesize the precursor (e.g., choline), it tends to lose it by efflux into the plasma at a rate that varies inversely with the precursor's plasma concentration.

4. A mechanism must exist which facilitates the precursor's passage from the bloodstream to the brain, and vice versa (i.e., a blood-brain barrier transport system); moreover the affinity of this mechanism for the circulating precursor must, like that of the rate-limiting enzyme, be relatively low. A physiologic increase in, for example, plasma tyrosine levels, must increase the transport system's saturation with tyrosine and must thereby facilitate tyrosine's entry into the brain.

Unfortunately, since the kinetics of the enzyme responsible for the cleavage of threonine into glycine and

acetaldehyde in the CNS are not known, it is not possible to relate these laws to the relationship of plasma threonine and CNS glycine. As previously discussed however (section II G), a mechanism for transport of threonine into the CNS exists and threonine is capable of increasing CNS glycine.

J. Anatomy, Physiology, and Biochemistry of Familial Spasticity.

Spasticity has been defined as: "a motor disorder of spinal proprioceptive reflexes, characterized by a velocity dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex as one component of the upper motor neuron syndrome" (105). It is a common but not inevitable consequence of lesions that damage corticofugal pathways, including the pyramidal tract, at any level--cortex, internal capsule, brainstem, or spinal cord. The release of proprioceptive reflexes that can occur with lesions at any of these levels has essentially the same functional result, even though the precise pathophysiological mechanisms may vary from lesions to lesion. This does not imply that the clinical presentation of patients with lesions at different levels need be the same. Spasticity occurs in a wide variety of conditions with different etiologies: trauma to the spinal cord, multiple sclerosis, and stroke are common examples.

Selective sections of the pyramidal tract at the medullary pyramids or cerebral peduncles have been performed on nonhuman primates or on man (undertaken to control hemiballism or Parkinsonian tremor). They produce discrete and minor permanent sequelae. they certainly do

not produce a well-developed "pyramidal" syndrome with prominent spasticity (175). In nonhuman primates, the only permanent defect in function is the inability to control the digits of the affected upper limb independently (176). In one human patient, the permanent effects of loss of 83% of the fibers in one pyramidal tract were mild weakness of hand and foot, no increase in muscle tone, tendon jerks that were a little more active, and an extensor plantar response (268). However, it is possible that such ablation experiments do not reveal the normal function of the pyramidal system, only those aspects for which compensation cannot be made adequately.

The "pyramidal", upper motoneuron syndrome has both "negative" and "positive" features. The negative features are weakness and loss of dexterity, particularly for fine manual manipulation. The positive features include abnormal posture, exaggeration of proprioceptive reflexes "spasticity," and exaggeration of some exteroceptive (cutaneous) reflexes of the limbs, producing flexion withdrawal spasms, extensor spasms, and the Babinski response. The extent to which the positive features represent release phenomena or result from plastic changes in spinal circuits to compensate for the removal of descending inputs cannot be settled conclusively, but both are likely to occur to varying degrees in different

patients.

The acute response to destruction of pyramidal tract fibers in man is paralysis and profound hyporeflexia for the innervated musculature, a condition referred to as "shock." When severe, the clinical findings will be those of a dense flaccid paralysis involving all muscle groups in the involved limb and complete areflexia. When of lesser severity, cutaneous reflexes such as the Babinski response may be elicited, tendon jerks may be retained even though depressed, and there may be some residual power in "postural" muscles, particularly in the upper limb, in proximal muscles (271). The emergence from this state may be very gradual.

With traumatic spinal cord lesions, the onset of spinal shock is said to be immediate or virtually so [though decapitated criminals apparently retain knee jerks for up to 90 sec., according to Sternberg (1893), quoted in reference 177]. It lasts typically some 1 to 6 weeks (177, 243). There is some unpredictability of the outcome of more rostral upper motoneuron lesions due, for example, to stroke.

The less abrupt the onset of pathology, the more likely is the patient to bypass the shock phase. Patients with slowly progressive compressive lesions of the spinal cord and degenerative diseases such as motoneuron disease, particularly amyotrophic lateral sclerosis, Marie's spastic

ataxia, and familial spastic paraplegia, do not experience a phase of hyporeflexic hypotonia. Instead, positive features of the upper motoneuron syndrome tend to develop in parallel with the negative (271).

Weakness in the upper motoneuron syndrome may be extensive, involving all muscles in the affected limb, particularly soon after a lesion of abrupt onset. With less abrupt pathology, or in the recovery phase following an acute lesions, the degree of weakness differs for different conditions and for different muscle groups in the involved limb (244,245).

Positive Features

The resistance to passive stretch felt when an examiner tests muscle tone in a spastic patient depends on the velocity of the imposed movement. When at rest, even in a stretched position, "spastic" muscles are flaccid (246, 247). The adequate stimulus to demonstrate spasticity is movement--in particular, rapid movement (105-108, 247, 248). The receptor responsible for detecting the imposed stretch is the primary ending of the muscle spindle. The rapidly conducting group Ia afferent fibers from the primary spindle endings excite the homonymous and synergistic motoneuron pools through monosynaptic, oligosynaptic, and possibly polysynaptic reflex circuits

within the spinal cord (Fig. 33) (109-113, 251-256), producing a reflex that opposes the stretch.

When a spastic patient is examined, a phenomenon (247), of "catch-and-give" is experienced. This is called the clasp-knife response. Classical texts assumed that the inhibition responsible for the clasp-knife phenomenon was due to mobilization of Golgi tendon organs as an overload protection mechanism when muscle tension exceeds a given level. This explanation does not fit with current views on the sensitivity of tendon organs (249, 250, 257). Other candidate receptors are secondary spindle endings in quadriceps (248); nonencapsulated mechanoreceptors in quadriceps with group II, III and IV afferents--i.e., neither spindle nor tendon organ; and knee-joint mechanoreceptors (269). These afferents share a common feature: together with cutaneous afferents, they have been classified by Lundberg and colleagues as "afferents which may evoke the flexion reflex" in spinalized animals [hence "Flexor Reflex Afferents"]. Their reflex pathways are subject to supraspinal control by a number of pathways, but particularly by a bulbospinal pathway (the "dorsal reticulospinal system") which projects bilaterally from the pontomedullary reticular formation down the dorsolateral funiculus, inhibiting the first-order interneuron of the FRA reflex pathway (251, 258-261). The clasp-knife phenomenon is a "flexor reflex afferent" phenomenon,

resulting from disinhibition of interneurons in the FRA pathway (271).

The exaggerated tendon jerk of spasticity can be viewed as a synchronized reflex response generated by an abrupt mechanical disturbance, utilizing the same reflex pathways as are activated by the less abrupt manual stretch used to test muscle tone (245, 262).

Clonus can be produced by either abrupt passive stretch or tendon percussion when the responding muscle is under a critical degree of stretch. The first contraction of clonus is analogous to the tendon jerk. As this reflexly induced contraction relaxes, primary spindle endings are again stretched, and, provided the stretch is sufficiently abrupt to produce a synchronized group Ia afferent volley, another reflex contraction will ensue, and so on (112, 264). It is not possible to have clonus without tendon jerk hyperreflexia, but the reverse is certainly possible.

The clasp-knife phenomenon, group II-IV muscle afferents, joint afferents, and cutaneous afferents may evoke a flexion withdrawal response of the lower limb, with "triple flexion"--dorsiflexion of the ankle, flexion of the knee, and flexion of the hip. These afferents [the "flexor reflex afferents" (FRA)] share a common polysynaptic reflex pathway, exciting flexor motoneurons and inhibiting extensor motoneurons (fig.). It was shown by Holmqvist and Lundberg (261) that these are not the sole actions of

the FRA. There are alternative reflex pathways from these afferents, including extensor facilitation and flexor inhibition (251).

The FRAs have complex long-latency reflex actions that are also suppressed by the dorsal reticulospinal system but are revealed in the acutely spinalized cat following administration of monoaminergic precursors (251). These reflex actions are believed to be involved in the generation of rhythmic locomotion (251). According to Kugelberg: "A function of the pyramidal tract may be to choose the synaptic actions of the FRA appropriate to a particular task. In spinal preparations and in spastic man, the active pathways are those that tend to produce the flexion reflex--extensor inhibition and flexor" facilitation (265).

Transmission in short-latency FRA pathways can be controlled by a number of descending systems, including the nonmonoaminergic dorsal reticulospinal system and two monoaminergic reticulospinal pathways (noradrenergic and serotonergic), all of which suppress transmission in the FRA circuit; and the corticospinal (pyramidal) tract and the rubrospinal tract, both of which facilitate FRA transmission (271).

Paraplegic patients may pass through a phase of alternating flexor and extensor spasms at about 4 to 6 months and into a final phase of predominant extensor spasm

6 to 12 months after injury (177, 243). Extensor spasm can turn the lower limb into a rigid pillar sufficient to support the body. They may be provoked by nonnociceptive cutaneous stimuli to specific skin regions, such as the groin, and are probably manifestations of the cutaneous extensor reflexes studied by Hagbarth in the cat (266) and in man (265, 267).

The exact mechanisms causing spasticity are still not elucidated. It is now accepted that the local reflex arcs (including alpha and gamma motoneurons and Ia, Ib and FRA afferents) remain intact but function under abnormal suprasegmental influences that normally maintain appropriate balance between excitatory and inhibitory signals (111-115, 247, 271).

Lundberg and others, studying the supraspinal control of transmission in reflex paths in the cat, found that the corticospinal cortex has a complex input on the motoneurons; monosynaptic EPSPs, dysynaptic and trisynaptic EPSPs and IPSPs on to the flexors and extensors (272-274).

Lundberg also showed that the facilitation/inhibition of the motoneuron by the pyramidal tract can be mediated through propriospinal neurons capable of evoking monosynaptic EPSPs and oligosynaptic EPSP and/or IPSP in many flexor and extensor motoneurons (275-277). The pyramidal tract can also produce primary afferent depolarization with facilitatory action on the excitatory and inhibitory paths

from Ia, Ib and FRA afferents (272) For example, in one experiment with intracellular recording from a gastrocnemius motoneuron, a Ia volley from the antagonist pretibial flexors did not evoke any IPSP. When the same Ia volley was preceded by stimulation of the sensorimotor cortex, a large Ia IPSP was evoked. He notes that: "There is a powerful facilitation of the Ia inhibitory pathway at a strength of cortical stimulation that in itself does not evoke any synaptic actions in the motoneurons" (272).

Presumably the effect is due to excitatory action from the sensorimotor cortex on the inhibitory interneuron known to exist in the Ia inhibitory pathway (272).

Beside the pyramidal tract, the Ia inhibitory interneuron can be excited by volleys in rubrospinal, and vestibulospinal pathways; by propriospinal pathways; by homonymous group Ia afferents; and by the flexion reflex afferents. It inhibits not only the antagonistic motoneuron pool but also the Ia inhibitory interneurons of that pool. It is inhibited by the Renshaw cell (and also by the Ia inhibitory interneurons of the antagonist). Reciprocal inhibition has been studied extensively by Tanaka and his colleagues in human subjects (262, 268); a voluntary contraction of tibialis anterior not only directly activates the motoneuron pool of tibialis anterior but also potentiates transmission across the Ia inhibitory interneuron from tibialis anterior to soleus. In

hemiparetic patients with spasticity, there was prominent reciprocal inhibition from extensors (triceps surae) to flexors (tibialis anterior) but not vice versa. Alcohol injection into the motor points of triceps surae relieved the intense spasticity without significantly affecting muscle power, but it also produced an increase in voluntary power of tibialis anterior. The latter effect was attributed to removal of tonic reciprocal inhibition (263).

In summary, supraspinal control of various reflex circuits is impaired in patients with upper motoneuron lesions. There is evidence that a loss of presynaptic inhibition of the monosynaptic Ia pathway and a disturbance of reciprocal Ia inhibition may contribute to the hyperreflexia seen in spastic patients at rest, but there is no evidence that loss of recurrent inhibition does so. It should not be assumed that these abnormalities are adequate to produce spasticity or that they are present in all forms of spasticity. However, they are examples of how hyperreflexia can result from disturbed supraspinal control of spinal circuitry. Studies are yet to be undertaken to quantify possible changes in other reflex circuits in spasticity: polysynaptic Ia pathways, Ib inhibition, and effects from spindle group II afferents and other FRA.

Finally, it is important to distinguish between

experimentally produced hypertonus, such as decerebrate or decerebellate rigidity, and clinically observed motor impairment, such as spasticity and rigidity. Spasticity has three distinguishing characteristics: (1) it is unidirectional. This term is used because the resistance is usually much greater in antigravity muscles. Therefore, extending the arm meets with more resistance than flexing it because of increased tone in the biceps. (The biceps of the arm are an example of physiological extensors, as flexing the elbow normally opposes gravity). (2) As discussed earlier, the resistance of the spastic muscle to passive extension largely depends upon the velocity of the movement; in mild spasticity little resistance is offered to slow extensions, whereas increased resistance is clearly present with rapid extension. (3) Finally, spastic patients show a hyperactive tendon jerk.

Rigidity is one of the major signs of parkinsonism. In contrast to spasticity, rigidity has the following characteristics: (1) The increased resistance to passive movement is bidirectional; that is, the resistance is seen more or less equally in flexors and extensors. (2) Rigidity is relatively independent of the velocity of movement. (3) Finally, parkinsonian patients do not show a hyperactive tendon jerk.

Familial spastic paraparesis constitutes the most pure example of spasticity because there is a single lesion affecting the corticospinal tract in the spinal cord (116-117, see section II, M); there are no associated lesions to complicate the basis of spasticity. Patients with familial spastic paraparesis, therefore, constitute an ideal group for the proposed studies on testing the safety and efficacy of L-threonine as a treatment for spasticity.

Electrophysiologic Techniques for Assessing Spasticity in the Legs.

The earliest test of spinal circuitry was the tendon reflex. To obtain results which can be qualified and compared, it was necessary to standardize the procedures of percussion and recording. Alternatively, this can be accomplished by recording the reflex elicited by low-intensity stimulation of a mixed nerve (287). This is the classical Hoffmann reflex which was originally applied to the soleus muscle but can also be applied to other muscles such as quadriceps, extensor digitorum brevis in the lower limb, and flexor carpi ulnaris in the upper (288).

The Hoffmann technique was undertaken again in 1952 by Magladery and his collaborators at Johns Hopkins. They established that the Hoffmann reflex is monosynaptic and associated with activation of large afferent IA fibers

(292).

The consequences of identifying the H-reflex as a monosynaptic reflex are interesting. For example, variations in reflex amplitude when the stimulus is constant must reflect variations in excitability of the motoneuron pool (289). When the maximum amplitude of the H-reflex is expressed against the amplitude of the motor response, the resulting ratio H_{\max}/M_{\max} is a measure of the excitability of the motoneuron pool (290).

Tanaka (291) demonstrated in man the activity of the IA inhibitory interneurons, connecting spindle afferents of one muscle to the motoneurons of its antagonist. The technique which he employed is simple: "the Hoffmann reflex is conditioned by a just liminal stimulus to the lateral popliteal nerve. At a very brief interval after the stimulus, inhibition of the Hoffmann reflex is observed, which can be attributed to activity of the IA interneuron. At rest, the observed inhibition is moderate, but becomes markedly more intense if the subject voluntarily contracts tibialis anterior. Descending pathways which activate tibialis anterior motoneurons simultaneously reinforce the inhibitory activity exerted on antagonist motoneurons". Microiontophoretic, electrophysiologic and pharmacological studies in experimental animals show that glycine is the transmitter involved in reciprocal inhibition (27-36, 93, 122, 126, 127). As discussed above, (section II, I), the IA

inhibitory interneurons might be involved in the pathogenesis of spasticity in man. This technique seemed a logical electrophysiological approach to assessing the response of patients with FSP to threonine. We have attempted to obtain stable baselines from individual patients and healthy volunteers using the Tanaka technique at the Neurophysiology Laboratory at the Massachusetts General Hospital with no success. In a review discussing the electrophysiological testing of spastic patients, Delwaide recognizes the high variability in the results obtained. He writes: "the contribution to the total picture of each physiopathological disorder varies in each case, and the same degree of spasticity can result from a mixture in different proportions of the various contributory mechanisms. If patients with similar clinical features are chosen (age, etiology, site of lesion, etc.) and submitted to the same tests, the results vary widely from one patient to another" (289).

Other techniques including the tonic vibration reflex presumably related to presynaptic inhibition in the cat and responsive to GABA are available (289) and have been attempted in the preliminary phases of the present research. Unfortunately again, the results were not reproducible and these techniques could not be used reliably in this project.

The body of knowledge describing the biochemical basis of spasticity is less systematized than information regarding anatomic and physiologic aspects of spasticity. Glycine and gamma-aminobutyric acid (GABA) have been identified as the major inhibitory transmitters in the spinal cord (118-120). Glycine is more plentiful in the cord than GABA, and is the major postsynaptic inhibitory transmitter (121-124); GABA is believed to mediate presynaptic inhibition (125). There is convincing evidence that glycine is the transmitter released by inhibitory interneurons located in the grey matter of the spinal cord and by Renshaw cells responsible for recurrent inhibition (27-36, 93, 122, 126, 127).

In two studies by Hall, Aprisson and coworkers (161, 162) experimental spasticity was induced in dogs following high thoracic spinal cord transections. The onset and degree of spasticity were assessed by observing the relative reflex activity of the hindlimbs and tail and by observing the relative monosynaptic and polysynaptic reflex responses. The tendon reflexes were used to assess monosynaptic activity and the response to noxious cutaneous stimuli to determine polysynaptic activity. The amino acids were measured in several areas of the spinal cord. Significant decreases in the levels of glycine and aspartate in ventral central gray occurred following spinal cord transection, and these changes followed the onset of spasticity. It is

possible that descending inputs are important in sustaining the levels of glycine.

Gundlach and coworkers, recently reported a deficit of spinal cord Glycine/strychnine receptors in inherited myoclonus of Poll Hereford calves (270). This disease is characterized by hyperesthesia and myoclonic jerks of the skeletal musculature, which occur spontaneously and in response to sensory stimuli.

L-glutamate and L-aspartate have been proposed as excitatory transmitters released from terminals of large diameter myelinated primary afferent fibers and from excitatory interneurons, respectively (128-129). Acetylcholine is the neurotransmitter released at the neuromuscular junction from axons whose cell bodies originate in the anterior horn of the spinal cord. An increase in serotonin neurotransmission produces extensor postures in rats, whereas an increase in norepinephrine transmission facilitates flexor reflexes (130). Although the anatomic location of neurons that release these various neurotransmitters is relatively known, there is little information available regarding the biochemical changes that underlie specific aspects of spasticity in humans (131). The clinical study in this project will address this gap in information, because changes in reflexes or other measures of spasticity induced by threonine would implicate glycinergic mechanisms.

I. Pharmacology of Spasticity

Drugs used in the treatment of spasticity (132-133) have aimed at either enhancing presynaptic inhibition (benzodiazepines, such as diazepam; baclofen) or at diminishing muscular contraction to excitatory stimulation (dantrolene). Benzodiazepines increase presynaptic inhibition by increasing the affinity of GABA receptor sites for the endogenous ligand; at therapeutic doses, they do not normally affect synaptic events mediated by other transmitters such as glycine (134,135). By increasing presynaptic inhibition, benzodiazepines decrease release of excitatory transmitters from afferent fibers with a resultant decrease in the gain of the stretch reflex. Baclofen was originally thought to function as a GABA agonist (acting on the GABA a receptor) because it is a lipophylic derivative of GABA that crossed the blood-brain barrier and entered the CNS (133). Although baclofen does show synaptic transmission in the cord, its electrophysiological and pharmacological profile is quite different from GABA (136-140). Baclofen reduces excitatory transmitter release, apparently by activating GABA b receptors which restricts calcium influx into presynaptic terminals (141,142). It is this restriction that presumably reduces evoked excitatory transmitter release and is responsible for the diminished reflex activity. As with benzodiazepines, the net effect of reducing excitatory

transmitter release by afferent fibers (and presumably by interneurons) is to suppress reflex activity. Dantrolene's therapeutic effect occurs directly on contractile mechanisms within muscle, without any specific action on reflex pathways (132,133). It reduces the depolarization induced calcium efflux into the sarcoplasm caused by conducted muscle action potentials. Because sarcoplasmic calcium is necessary in order to activate myosin-ATPase and cause actin and myosin filaments to slide past one another, the force produced by muscle in response to its electrical activation is diminished by dantrolene, although the electromyogram is unchanged. These current modalities for treating spasticity are only partially effective in altering muscle tone and reflexes, and have significant side effects. Diazepam and baclofen do suppress flexor spasms in many patients, but neither improves walking (132,133,136). Side effects of these drugs include drowsiness, light-headedness, and confusion; furthermore, additive CNS depression may occur when other agents, such as alcohol, are taken concomitantly (132). Dantrolene administration lessens muscle tone and hyperreflexia to the extent that it produces muscular paralysis. Thus, its use is counterproductive in those patients whose spasticity masks limb weakness; these individuals benefit from increased extensor tone that enables them to stand and walk on otherwise weak legs. Hepatic toxicity is a serious

potential side effect that tempers physicians' inclination to prescribe dantrolene (143).

An alternate approach to the treatment of spasticity is to increase inhibitory (glycinergic) tone in spinal cord interneurons. This is a rational neuropharmacological strategy, but was impossible to implement prior to the discovery that threonine increased glycine levels in the cord (144). The studies undertaken in this thesis are based upon this discovery and offer a novel approach to treating spasticity based upon enhancing glycinergic neurotransmission.

L. Why L-threonine?

As described above, amino acid transmitters can be divided into two general categories based on their neurophysiological actions: excitatory and inhibitory. Examples of excitatory amino acids include aspartic acid, glutamic acid and cysteic acid; examples of the inhibitory amino acids include glycine, GABA and taurine. Although difficulties distinguishing the metabolic vs. neurotransmitter roles of these amino acids hamper definitive conclusions, the evidence that glycine is a major neurotransmitter has been discussed above and is based on the following observations (see significance): non-homogeneous distribution throughout the CNS; active when applied iontophoretically; released when nerves are stimulated; and specific postsynaptic receptors which are activated by glycine and antagonized by strychnine.

Attempts at increasing brain or spinal cord levels of glycine by administering glycine were generally unsuccessful due to the limited passage of glycine across the blood-brain barrier (145). Glycine can be synthesized from serine in a reaction catalyzed by STHM; however, exogenous administration of serine did not increase brain glycine levels either (300, 301). Maher and Wurtman (144) found that threonine (the other glycine precursor) administration to rats caused dose-dependent increases in

spinal cord threonine and glycine levels. Seimers and Aprison (146) extended this finding by demonstrating a linear relationship between the concentrations of threonine and glycine in 10 sub-areas of the rat medulla, as well as in 8 major areas of the rat neuroaxis. Boyd et al. (147) studied the behavioral effects of threonine, and reported that it prevented the seizures in rats that usually follow intrathecally administered strychnine. Threonine's effect was mimicked by intrathecally administered glycine but not by serine. Taken together, these pre-clinical experiments indicate that administration of L-threonine increases CNS glycine levels, enhances glycinergic neurotransmission, and produces predicted behavioral effects in rats. If similar biochemical effects occurred in humans, L-threonine could be developed as a novel and effective treatment for CNS disorders, such as spasticity, in which increases in inhibitory tone are desired. Shortly after Maher and Wurtman's (144) original discovery, Barbeau et al. (148) reported that L-threonine suppressed spasticity in 6 of 6 patients with spino-cerebellar degeneration; all showed improvement with lessening of tendon reflexes and muscle spasms. A placebo-controlled double-blind drug study with more patients than Barbeau et al. tested is required however before L-threonine can be developed as treatment for spasticity.

For this thesis, patients with familial spastic paraparesis were selected. They constitute an ideal population for studying the effects of L-threonine on spasticity for at least two reasons: first, the neuropathological substrate of spasticity in this condition is uncontaminated by other CNS lesions, and second, there is preservation of interneurons whose inhibitory actions are mediated by glycine.

M. Familial Spastic Paraplegia: Inheritance and Pathology.

The first descriptions of Familial Spastic Paraplegia (FSP), were those of Stumpell in 1880, 1886 and 1904 (179, 180). Stumpell reported two brothers in whom spastic paraplegia developed at the ages of 37 and 56 years and another family in which the age of onset was in the third and fourth decade. All had almost identical clinical features with abnormal findings mostly confined to the legs. Inheritance was probably autosomal dominant.

Until the work of Harding in 1981 (278), there had been no large surveys of patients with familial spastic paraplegia. Bell and Carmichael (283), Oszvath (284), Holmes and Shaywitz (285) have all reviewed previously published reports of families with the disease; these studies have yielded rather conflicting results with respect to the clinical and genetic features of the disorder. It has been suggested, on the basis of variation in age of onset, that the autosomal dominant form of FSP is genetically heterogeneous (180, 279, 280) but this hypothesis had not been tested on a large series of patients with a carefully defined clinical syndrome. Harding in 1981 (278), studied 22 families with the pure form of FSP. They were investigated as part of a clinical and genetic study of 200 families with progressive cerebellar and spinocerebellar degenerations. Minimal

criteria for diagnosis were: Hyperactive tendon jerks, babinski sign and spastic gait. He found inheritance to be dominant in 19 families and recessive in three. Examination of intrafamilial correlation of age of onset in the dominant cases suggested that the disorder is genetically heterogeneous. In the three families demonstrating autosomal recessive inheritance, the clinical features were similar to those of dominant cases. In a recent report on 275 family members from six generations, Boustany et al. (150) reported the inheritance to be autosomal dominant with the suggestion that the GC locus on chromosome 4 might be linked with FSP.

Pathology.

Schwarz in 1952 (178) provided an extensive review of the pathological literature. He stated that he considered all neuropathological knowledge of the condition resting on the descriptions of seven cases--six described before 1912 and one in 1937. The cases were Strumpell's original two cases in 1886 and 1904 (179, 180), Newmark's three in 1904, 1906 and 1911 (181, 182, 183), and the single reports of Jakob in 1909 (184) and Kahlstorf in 1937 (185). In addition, he thought that possibly four others--two cases of Bischoff in 1902 (186) and the one described by Raymond and Rose in 1909 (187) and the case of Farago in 1947 (188)--might have been of the same condition. Reducing the descriptions of

the disease to this small number, he found a uniformity in the lesions noted. He added the pathological description of a typical case from a family which had been studied by Bayley in 1897 and Spiller in 1902. Schwarz and Liu in 1956 (189) then reported the clinical findings in one more family with the necropsy study from a case in a different family, thus bringing the number of necropsies on acceptable cases of Stumpell Familial Spastic Paraplegia (FSP) recorded in the literature to a total of nine.

In his first paper, Schwarz summarized the pathological features (as revealed by Weil myelin stain) of all the seven previously reported cases as follows : first, in all there was bilateral degeneration of the crossed corticospinal tracts, with involvement of the uncrossed tracts in four. The Betz cells were judged to be atrophic in three cases and the motor horn cells were reduced in number in one of these three specimens. With regard to the ascending fibres, there was invariably symmetrical degeneration of the fasciculus gracilis, visible at thoracic level and increasing at cervical regions up to the medullary nuclei. The spinocerebellar tracts were involved in four cases, with bilateral loss of fibres. No lipid degeneration products were found. Atrophy of the basal ganglia was mentioned in one case.

Having reviewed the literature up to 1950, Schwarz and Liu (190) went on to discuss the 16 families which were reported between 1950 and 1955, with five neuropathological reports. Most of the reports dealt with patients showing Strumpell's FSP among other neurological deficits. Of the cases that came to necropsy, the two described by Appel and Van Bogaert in 1952 (191) were most similar to those previously characterized (the other three cases were of quadriplegic idiots). The first case was reasonably typical clinically. Pathologically, there was degeneration and gliosis of crossed pyramidal tracts in the spinal cord, with minor involvement of the uncrossed tracts. In the cervical levels of cord only there was slight degeneration of the gracile and cuneate fasciculi. There was, however, degeneration throughout the cord of anterior horn cells: an atypical finding in FSP. On clinical examination the second case had a spastic quadriparesis, slight atrophy of the muscles of the calves, thighs, thenar eminences, and the backs of the hands, but the pathological examination was more characteristic of FSP as previously described, with degeneration and gliosis of pyramidal tracts extending from the cerebral peduncle to the sacral levels, and similar changes in the posterior columns from the second thoracic level to the medulla. There were no anterior horn cell changes. It has to be admitted that neither of these cases is typical of uncomplicated FSP.

The two new cases that Schwarz reported showed findings similar to those expected. In the first case (178), only the brain-stem and cord were examined. The major findings were of bilateral fiber loss in both crossed and uncrossed corticospinal tracts, worse at lower levels, with possible thinning of the medullary pyramids. The fasciculus gracilis showed slight loss of fibers at lower cervical level. Possible degeneration in the spinocerebellar tracts was commented on. The second case (190) was interesting in that all changes were so much more severe at thoracic level that the authors postulated that this was the original site of damage. Again, bilateral loss of fibers in the lateral corticospinal tracts was found, increasing caudally and most severe at thoracic level, and mild myelin pallor was recorded at the level of the pyramidal tract decussation. There was degeneration of the fasciculus gracilis from the lowest thoracic level upwards with a loss of neurones from Clarke's columns, but no involvement of the fasciculus cuneatus. The dorsal spinocerebellar tracts showed a fibre loss which could be traced into the medulla. Schwarz and Liu concluded also in this case, on the basis of counting procedures, that there was a reduction in the Betz cell population, and a loss of fibres in the medullary pyramids.

Two cases were examined histologically by Wilhelmina, et al., in 1974 (192) and they show the same pattern, with

clear-cut lesions: corticospinal tract degeneration from the medullary pyramids downwards, increasing caudally, and posterior column degeneration, without loss of posterior root fibres, increasing rostrally. These findings are almost identical with those in Strumpell's original descriptions and those of Schwarz (178) and Schwarz and Liu (190). Thus a strong case can be made for regarding FSP, as originally described by Strumpell, as a well-defined clinical and pathological entity. Inheritance is autosomal, and usually dominant. Including, as Schwarz does, Newmark's three cases and the single cases of Jakob and Kahlstorf in addition to those of Strumpell, Schwarz, and Liu, and the two cases reported by Wilhelmina, brings the total up to 11.

The nature of the disease, as with other primary neuronal degenerations, is obscure. At first sight, it appears to show, in common with other degenerations, the phenomenon of 'dying-back'--that is, a progressive withering of axons beginning at their terminations and proceeding towards the cell body, which eventually disappears. This 'dying-back' is a recognized nature of the corticospinal tracts in motor neurone disease, of the pontocerebellar fibres in olivopontocerebellar atrophy, and of the posterior spinocerebellar tracts in Friedreich's ataxia (192). On the other hand, there is no evidence, in SFSP, of the

phenomenon of trans-synaptic, or 'chain' degeneration, seen in olivopontocerebellar degeneration and Friedreich's ataxia. It is worth noting, too, that the two tracts which are constantly affected in FSP contain the longest fibres in the central nervous system (190-192); and that, in spite of the long duration of the disease, the cells of origin of these tracts--in the cerebral cortex and posterior root ganglia respectively--show little, if any, depletion at the time of death. It may well be that the mechanism of distal fibre degeneration in FSP is different from that in the so-called 'system' degenerations, in which collections of neurones are affected regardless of the length of their axons. The findings, in fact, could be explained as the result of a generalized failure of nerve cells to maintain the vitality of axons of more than a certain length, rather than as a specific disease of particular types, or systems, of neurones. This conjectured difference may or may not be real; in any case, it cannot be assumed that the basic cause is the same in all kinds of primary neuronal degeneration.

Greenfield (193) commented on the pathogenesis of the spinocerebellar degenerations and suggested that the lesion would ultimately be found to be an enzyme deficiency. It has been shown that accidental organic phosphate poisoning in man has resulted in degeneration of long tract fibres--

corticospinal and gracile--especially at the distal ends (194); and experimentally Cavanagh (195), using tri-ortho-cresyl phosphate, and Fenton (196) with di-iso-propyl-fluorophosphonate, were able to reproduce similar but more acute changes in chickens. The organic phosphates destroy pseudocholinesterase, an enzyme which has no known function in the central nervous system; but effects on other enzymes as well is a possibility. A premature aging process has been suggested as the pathogenesis of various spinocerebellar disorders, a concept proposed by Raymond (197) and then expanded to include a wide variety of familial or hereditary disorders which show distal axonal atrophy. In FSP, no excess of the signs commonly associated with aging in the central nervous system has been reported in the literature.

A feature of these disorders relatively common in CNS disease is the age of onset (e.g. Huntington disease, Alzheimer and Amyotrophic Lateral Sclerosis). Due to a not fully understood genetic program, in combination with possible environmental factors, there is cell death or tissue degeneration that start later in life. In the case of metachromatic leukodystrophy one can understand why the disorder is not seen in infants before 2 1/2 or 3 years of age, because it is known that aryl sulphatase-A deficiency will not produce an effect until myelin formation has occurred (198). There is no explanation, however, for

similar cases of this disorder starting in adulthood. Whether or not what is inherited in FSP is a tendency to 'switch-over' enzyme production from normal to abnormal at a certain age, cannot be determined. Since the flow of nutrients is from perikaryon to axonal termination, it is understandable that the distal parts of the long axons would be affected first if there were any derangement in cell nourishment.

The number of associated neurological symptoms that have been added to the relatively specific entity described by Strumpell continues to grow and make analysis of the cases difficult. Variants of familial spastic paraplegia have included familial spastic paraplegia with spinocerebellar atrophies such as Friedreich's ataxia and Charcot-Marie-Tooth disease (199), ataxia (200), dementia (201, 191), visual defects (202), mental deficiency (201, 203), central retinal degeneration (204, 205), ichthyosis (206), sensory impairment (207), extrapyramidal features (208), optic atrophy (209), epilepsy (210), retrobulbar neuritis (211), amyotrophy (212, 213), leucodystrophy (214), and Huntington's chorea (215). Of 267 families with familial spastic paraplegia, Ozsvath noted that 57% were associated with other defects: cerebellar symptoms in 23%, amyotrophic lateral sclerosis in 17%, extrapyramidal symptoms in 7% and optic atrophy in 8%.

Rhein (1916) (216) was the first to point out that forms

exist between pure spastic paraplegia, familial amyotrophic lateral sclerosis, familial cerebral diplegia, and familial disseminated sclerosis. Since Rhein, many other authors including Ross (199) and Landau and Gitt (217) have emphasized the interweaving of patterns in certain of the heredodegenerative diseases notably the Friedreich's complex, Charcot-Marie-Tooth disease, Roussy-Levy syndrome, familial spastic paraplegia, and feel that in some families there may be genetic vulnerability of certain tracts in the CNS to degeneration in either a simple or complex fashion producing mild or complicated neurological disturbances.

Although it is interesting to speculate about possible interrelationships among the disease groups mentioned above, there is a consensus, among the authors who studied them (178, 190-192), that there is indeed a separate, although rare, clinical entity of pure Strumpell's familial spastic paraplegia with its unique modes of inheritance, clinical course and pathology.

More recently, cases of spastic paraplegia have been documented with an epidemiology strongly indicating an infectious transmission. Most noted is Tropical Spastic Paraplegia.

Tropical Spastic Paraplegia.

In 1982 (239), 83 cases of Tropical Spastic Paraparesis (TSP) were reported in the Seychelles Islands of the Indian Ocean in addition cases were observed in Jamaica,

Martinique and Tumaco. The clinical picture of this endemic disease is that of a chronic spastic myelopathy of slow onset and progression (240-242), accompanied initially by lumbalgias and foot dysesthesias, increased urinary frequency, and constipation. Weakness and spasticity of the legs result in a slow shuffling gait that eventually requires support from a cane or crutches. One-third of the cases have a relatively acute onset and rapid progression, incapacitating the patient in 1 to 2 years. Neurologic examination reveals signs of pyramidal tract involvement in the legs, and in one-half the patients also in the arms, along with minimal evidence of dorsal column and peripheral nerve involvement.

In Jamaica (218, 219) and Tumaco (221) TSP affects both sexes equally, although Vernant et al (220) found that the majority of TSP cases in Martinique occurred in women. Most TSP patients are of predominantly black African ancestry although cases were observed in predominantly Caucasian and Indian patients in the Seychelles as well as in a South American aborigine in Tumaco (221). Conjugal cases were identified in Colombia (221), but not in the Seychelles. In Mahe, TSP occurred in blood relatives in a previous generation. A genetic pattern of transmission was absent.

Neuropathologic examination of TSP in the Seychelles (223-224) show that the basic lesion is a bilateral degeneration of the corticospinal tracts and dorsal funiculi, with preservation of Betz cells and dorsal root ganglia neurons and, in addition, an inflammatory process characterized by proliferative vasculopathy, perivascular cuffing, and chronic leptomeningitis. The peripheral nerves also show evidence of axonal and myelin involvement, which could be due in part to an ischemic or compressive component. The neuropathologic changes in the spinal cord are similar to those observed in Jamaican tropical myeloneuropathy (218, 225) and the vacuolar myelin changes resemble those of subacute combined degeneration due to vitamin B12 deficiency, and those of the myelopathy recently described in AIDS (226-228).

The cause of endemic TSP seem to be due to a retrovirus (238). An important finding regarding the etiology of TSP in the Seychelles was the demonstration of HTLV-1 antibodies in 85% of the patients, and in only 12.5% of controls (236).

In summary, TSP shares with FSP some of the manifestations of spasticity; its pathology is however different. The corticospinal degeneration in TSP is wider; the dorsal funiculi are also involved and there is an inflammatory process with proliferative vasculopathy, perivascular cuffing, and chronic leptomeningitis in

addition to an evidence of peripheral nerve axonal and myelin involvement. These changes have not been observed in FSP. The clinical courses of FSP and TSP also differ: In TSP, one-third of the cases have a relatively acute onset and rapid progression, incapacitating the patient in 1 to 2 years and in one-half the patients signs of pyramidal tract involvement are also seen in the arms. This is not the case in TSP. The mode of transmission for TSP indicates an infectious route while FSP is genetically transmitted.

In conclusion, most authors (178, 190-192) consider that uncomplicated FSP is a well-defined clinical and pathological entity, and recommend the use of the eponymic term 'Strumpell's familial spastic paraplegia' to distinguish it from the bewildering array of familial system degenerations in which spastic weakness of the legs is only one feature in a complex or variable neurological picture.

III. EXPERIMENTAL METHODS

A. PRECLINICAL

As discussed above, it has been shown that intraperitoneal administration of threonine leads to an increase in both threonine and glycine levels in blood and spinal cords of experimental animals. The simultaneous administration of large neutral amino acids inhibited the CNS increase in threonine and glycine according to Maher and Wurtman (144). Tews found that the small neutral amino acids particularly serine and also GABA (158) had a greater competitive effect on the transport of threonine across the blood brain barrier and argues that they might be sharing a common transport system different from the LNAA one.

This part of the thesis aimed at :

- confirming that L-threonine administration increases spinal cord and brain threonine, and spinal cord glycine concentrations in rats.

- examining the effects of other amino acids given alone or with L-threonine on brain and spinal cord glycine and threonine.

In this experiment, male Sprague-Dawley rats weighing 150-200 gms were utilized. They were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. They were housed one per cage and allowed a one week acclimation period before experimentation. They were provided with food (Charles River Rat, Mouse and Hamster Maintenance Formula) and water ad lib and exposed to light 12 hours each day. The diet contained 24-26% protein with a threonine content of about 1%. The rat consumes about 12 gms/day and was thus ingesting about 120 mgs of threonine.

Effects of threonine and threonine plus large or small neutral amino acids on central nervous system amino acid concentrations.

Six groups of 6 rats received, respectively, threonine (400 mg/kg IP), threonine (400 mg/kg IP) plus a large neutral amino acid (400 mg/kg valine IP), threonine (400 mg/kg IP) plus a small neutral amino acid (400 mg/kg serine IP), valine (400 mg/kg IP), serine (400 mg/kg IP), or 0.9% saline IP. Solutions were prepared the day of the experiment with 0.9% saline to a concentration such that animals receive a volume in mls equivalent to their weight divided by 200. One hour after injection the animals were killed by decapitation and the brains and spinal cords

removed and quickly frozen on dry ice. Tissues were stored overnight at -40 C.

The tissues were later prepared for analysis of amino acid content by the following method (144):

1. Weigh frozen tissues and place in two volumes of ice cold H₂O.
2. Homogenize tissues for 30 seconds with a Polytron Homogenizer (Brinkman Instr., N.Y.).
3. Deproteinize homogenates with 1/2 volume 50% trichloroacetic acid.
4. Centrifuge at 15,000 RPM (Sorval RC 2-B) for 15 minutes.
5. Extract supernate 4 times with 5 volumes of diethyl ether.
6. Lyophilize to dryness overnight .
7. Reconstitute samples with 2 ml distilled deionized H₂O.
8. Analyze aliquots of 20 ul for amino acid content (threonine and glycine) with a Beckman Amino Acid Analyzer.

Data were analyzed by a one-way analysis of variance and Newman-keuls test.

B. CLINICAL STUDIES.

Single Drug Open Label Study of L-threonine in Treating Patients with Familial Spastic Paraparesis

Six patients with Familial Spastic Paraparesis (FSP) participated in a preliminary study to investigate whether L-threonine administration would increase CSF glycine levels and improve spasticity. The study was conducted according to a single drug open label protocol approved by the Human Studies Committee at MGH and the FDA (IND# 24,812). Patients with documented familial spastic paraparesis were admitted to the General Clinical Research Center (GCRC) at the Massachusetts General Hospital for 5 days. They related a complete history and underwent physical examinations and laboratory tests as described in the protocol. Special emphasis was placed upon the detailed neurological examination, in which various features of spasticity were scored according to the research protocol. Forms used in rating spasticity are in Appendix A. Lumbar punctures (LP) were performed on the morning of the second hospital day after admission; blood samples were obtained at the same time, and plasma and CSF were frozen at -70°C until subsequent amino acid analysis. Patients were then started on L-threonine by mouth in three daily divided doses and examined daily. Blood and CSF collections were

repeated on the 4th admission day two hours after the morning L-threonine dose. All samples were stored at -70°C until submitted to amino acid analysis. Complete physical examination and laboratory tests were repeated on the 5th admission day.

Patients were discharged taking L-threonine in three divided daily doses; they returned as outpatients two and again four weeks later for neurological examination and assessment of drug safety and efficacy.

Double Blind Cross-Over Design Study of L-threonine in Treating Patients with Familial Spastic Paraparesis.

1. Subjects

Twenty men and women with familial spastic paraparesis were invited to participate in this research project. They were drawn from a larger number of patients with familial spastic paraparesis being seen in the MGH-Shriver Center Neurogenetics Unit where there has been a long-standing interest in the genetics of familial spastic paraparesis. Several representative family pedigrees are illustrated in figures:11-14; geneticists and neurologists continue to refer other families with affected members.

Although familial spastic paraparesis is relatively rare, we have obtained a sufficient number of subjects interested in the research to conduct the study.

a. Definition of familial spastic paraparesis

Familial spastic paraparesis is a slowly progressive neurological disorder that is probably genetically heterogeneous (117,149). There is evidence for autosomal dominant genetic transmission in the majority of cases (116,150) but in a few families an indistinguishable disorder apparently occurs as a result of autosomal recessive inheritance. The illness generally begins in the 3rd or 4th decade of life; the course of illness is not inconsistent with a full life span. Clinically, the lower limbs are earliest and most severely affected, and the dominant cause of disability is spasticity rather than weakness. Difficulty walking is the major symptom reported by most patients; this is generally progressive and after many years may lead to use of a wheelchair. Additional symptoms that occur later in the course of the illness include urinary urgency and incontinence, and sometime fecal incontinence; sexual potency tends to be preserved. Deep tendon reflexes are heightened and there are other signs of damage to suprasegmental descending projections including clonus and bilateral Babinski signs.

Neuropathologically, the principal lesion is degeneration of the corticospinal (pyramidal) tracts (117). Other descending and ascending long tracts are generally spared,

as are the interneurons and anterior horn cells of the spinal cord. Thus, patients with familial spastic paraparesis constitute an ideal population for studying the effects of L-threonine on spasticity for at least two reasons: first, the neuro-pathological substrate of spasticity in this condition is uncontaminated by other CNS lesions, and second, there is preservation of interneurons whose inhibitory actions are mediated by glycine.

b. Inclusion and exclusion criteria

The inclusion criteria are:

- i. Men and women between the ages of 21 and 65 years old.
- ii. Diagnosis of familial spastic paraparesis.
- iii. Good general health.
- iv. Signed consent form (see consent form Appendix A).

The exclusion criteria are:

- i. Cancer or serious underlying medical illnesses (e.g., serious cardiac disease, renal failure, poorly controlled diabetes mellitus).
- ii. Required use of psychoactive drugs, including hypnotics, sedatives, antidepressants, or neuroleptics.
- iii. Concomitant use of drugs prescribed for spasticity,

including diazepam and other benzodiazepines, dantrolene, and baclofen.

2. Drugs

L-threonine is a purified amino acid that is prepared by the pharmacy at the Massachusetts General Hospital in capsules containing 500 mg of L-threonine. The treatment schedule of 1.5 g tid was selected based upon the open label study. The total dose of 4.5 g/day is within the daily range of L-threonine that individuals might consume from dietary protein alone.

L-threonine is dispensed by Dr. John Growdon of the Movement Disorder Clinic at the Massachusetts General Hospital, according to the provisions of IND #24812 assigned to him by the FDA.

a. L-threonine capsule formulation

The L-threonine bulk powder is obtained from Ajinomoto U.S.A., Inc. with a certificate of guaranteed analysis as described below:

Specific Rotation (D-line): 20 C: -28.6

State of Solution (T%): 99.9%

Chloride (Cl): not more than 0.02%

Amonium (NH₄): not more than 0.02%

Sulfate (SO₄): not more than 0.02%

Iron (Fe): not more than 10 ppm

Heavy Metals (Pb): not more than 10 ppm

Arsenic: not more than 1.0 ppm

Loss on Drying: 0.02%

Residue on Ignition: 0.04%

Assay: 99.3 %

Other Amino Acids: (10mcg) not detectable

Upon receipt by the Pharmacy Research/Manufacturing Division of the MGH Pharmacy Department, and prior to encapsulation, the bulk L-threonine powder is analyzed for purity by the pharmacy.

Capsules containing 500 mg of L-threonine were prepared by the pharmacist by filling L-threonine powder into size 0 yellow capsules using the Chemical and Pharmaceutical Co., Inc. capsule machine (capacity 300). The weight of the capsules is checked by using the Sauter Balance and allowing a fill range of 475-525 mg. The completed capsules are polished with muslin using a light film of Light Mineral Oil. Analysis of the L-threonine contained within the capsules is determined using a Beckman Amino Acid Analyzer (see Results). The L-threonine capsules were packaged in polyethylene containers with a safety cap and labeled with an investigational drug label (sample 1).

The placebo capsules contain lactose NF and were prepared in the identical manner as the L-threonine

capsules and labeled with an investigational drug label. L-threonine and placebo capsules have an identical appearance, texture, and taste when intact.

The pharmaceutical manufacturers are as follows:

L-threonine powder from Ajinomoto U.S.A., Inc., Raleigh, N.C. Size 0 yellow gelatin capsules, Eli Lilly & Co., Indianapolis, IN Polyethylene containers and safety caps from Israel Amdler & Sons, Inc., Everett, MA. Lactose Nf (hydrous) from Ruger Chemical Co., Inc., Irvington, NJ

b. Drug dispensation

The Dispensing Pharmacy of the MGH dispensed the L-threonine and matching placebo capsule in a 160 cc polyethylene bottle with a 43 mm plastic cap. The appearance of the label on the container was the same for both products when dispensed to the patient. There was a detachable portion of the drug label (Sample 1) which contained the drug code. The code key for the study drug was as follows: Drug A (L-threonine); Drug B (placebo). The detachable portion of the drug label was removed by the pharmacist before dispensing, and affixed to the prescription. The detachable drug code key on the bottle label is for proper drug identification and selection by the dispensing pharmacist.

The subjects were assigned to treatment randomly, using the pharmacy computer system to generate the random list. The random list was generated in blocks of 10 patients (Table 8), and incorporated the crossover phase of the study as well. For example, drug A ----> B means drug A was dispensed for the first phase and drug B for the second treatment, and vice versa.

The capsules were stored in the dispensing pharmacy, which is located in the investigational drug section. The research orders for the L-threonine clinical trial were entered into the pharmacy computer system. Before dispensing medication, the containers were labeled with pharmacy standard computer generated labels for outpatient prescriptions (Sample 1). The first two labels on the sample (for drug container and box for the containers) are for patient use; the last label is a quality assurance sticker to be affixed to the prescription by the dispensing pharmacist. The L-threonine clinical trial material was inventoried monthly by the Research Pharmacist. The protocol, patient consent form, dispensation records, and other pertinent drug information regarding the L-threonine study were kept on file in the pharmacy.

TABLE 8

Regimen Assignment Lists for L-threonine Administration
 (This is the actual sequences generated when the study
 began and remained in the confidence of the dispensing
 pharmacist.)

Sequence 1: Sample Space = 2 No. of variables = 10

Pt. 1:	drug A--->B	Pt. 2:	drug B--->A
Pt. 3:	drug B--->A	Pt. 4:	drug B--->A
Pt. 5:	drug A--->B	Pt. 6:	drug A--->B
Pt. 7:	drug B--->A	Pt. 8:	drug A--->B
Pt. 9:	drug A--->B	Pt. 10:	drug B--->A

Sequence 2: Sample Space = 2 No. of Variables = 10

Pt. 11:	drug B--->A	Pt. 12:	drug A--->B
Pt. 13:	drug B--->A	Pt. 14:	drug B--->A
Pt. 15:	drug A--->B	Pt. 16:	drug B--->A
Pt. 17:	drug B--->A	Pt. 18:	drug A--->B
Pt. 19:	drug A--->B	Pt. 20:	drug A--->B

3. Laboratory Studies

a- Standard laboratory studies

Prior to beginning the research protocol, subjects were examined in order to obtain a complete medical history, undergo physical examination and neurological examinations, and complete the following laboratory examinations:

chest x-ray (if none in the past
year)

electrocardiogram (EKG)

complete blood count

platelet count

blood sugar

blood urea nitrogen

electrolytes

calcium

phosphorus

plasma protein

bilirubin

alkaline phosphatase

SGOT

amylase

plasma amino acids

CSF amino acids

All x-rays, EKGs, and blood tests were performed in the appropriate MGH laboratory; the amino acid analyses was performed at MIT (see Amino acid analyses in plasma and CSF, below). All laboratory blood tests were repeated at the end of each treatment (<a> in Fig. 10 and two weeks after completion of the drug study. Only one chest x-ray and one EKG were obtained.

b. Amino acid analyses in plasma and CSF

CSF was collected via lumbar puncture (LP) and blood via the antecubital vein toward the end of each drug treatment period (A and B; <a> in Fig. 10). Blood was centrifuged to separate plasma from cells, and both CSF and plasma frozen at -70°C until assayed for amino acid content. Preparation for analysis involved thawing at room temperature followed by the addition of equal volumes of 5% sulfosalicylic acid and centrifugation at 3,000 RPM (Beckman table top centrifuge) for 10 min. at 4°C. Aliquots of the protein-precipitated samples (100 uL) were then analyzed for amino acid content using a Beckman Automatic Amino Acid Analyzer. This method utilizes a 30 cm heated ion-exchange column, lithium citrate buffers, and postcolumn ninhydrin derivatization. Detection of these derivatized amino acids is accomplished by colorometric

analysis at 440 and 570 nm. Typical retention times with quantitatively acceptable peak resolution for threonine, serine, and glycine are 47, 49, and 88 minutes respectively. Peak areas are automatically integrated and compared with reference standards (Beckman), and the concentration of amino acids expressed as nmoles/ml of CSF or plasma.

4. Protocol

Patients with familial spastic paraparesis (see table 5) related a complete history and underwent physical examination and laboratory tests during the week before treatment ("intake" in Fig. 10). Subjects who met the inclusion and exclusion criteria were invited to participate in the research project. They indicated their understanding the project and desire to participate by signing the informed consent form. It took two years for the twenty patients to complete the study.

The study was conducted according to a double blind crossover design (Fig. 10). During the eight weeks of the study each patient received L-threonine 1.5 g tid by mouth for two weeks and placebo 1.5 g tid by mouth for two weeks (The last nine patients received 2.0 g tid); the treatment periods were separated by a two week washout period. A final assessment was conducted two weeks after completing the final treatment in order to detect any

delayed side effects of medication, and to document changes in spasticity. Patients were instructed in standardized daily diets that contain 75 g of protein, yielding an estimated 2-3 g of threonine per day (311). Half of the patients received L-threonine during the first two weeks and placebo during the fifth and sixth weeks of the study; the other half received medications in the reverse order.

Treatment group assignment was performed by the dispensing pharmacist according to a pre-scheduled randomized computer-generated order of administration (cf. table 8). Within each block of 10 patients, 5 received L-threonine and the other 5 placebo during the first treatment period; neither the clinical investigators nor the patients knew the order in which the treatments were being given.

Sixteen of the patients were examined every two weeks during the course of the study. Because of long distance travel, Four patients were only examined at the beginning of the study period and at the end of each treatment period thus omitting the exam after the wash out period. A detailed neurological examination was performed on each visit in order to assess signs and symptoms of spasticity, including muscular tone, strength, reflexes, and the ability to walk, run, and hop on one foot (see examination form, Appendix A). At each examination, a global

assessment of the patient's condition was obtained, and the patients were asked to report their own evaluation of treatment by answering the patient questionnaire (cf. neurologist evaluation form and patient questionnaire in Appendix A). Only 10 patients were admitted to the GCRC during the last two days of each treatment period (<a> on Fig. 10) for comprehensive examinations, including neurological examination and assessment forms; (the other 10 patients refused the Lumbar Puncture procedure, either because they had been subjected to it during their participation in the open label phase of the study, or because they were just not willing to have it done. They were therefore assessed as outpatients). In the GCRC, Blood pressure, pulse, temperature, and respiratory rate were recorded two times a day. Assessment included full laboratory tests as well as simultaneous collection of blood and CSF for amino acid analysis. Blood was collected by standard venipuncture; 5 cc of plasma will be frozen at -70oC and stored for amino acid analysis. CSF was obtained by a conventional LP (lateral decubitus position; L3-4 interspace; #22 gauge spinal needle) at 10 AM. Patients remained at bedrest for 8 hours prior to LP in order to avoid potential effects of mixing amino acid pools due to physical activity or time of day. The first 2 ml of CSF were always sent for routine studies (cell count; protein; glucose) and the next 2 ml aliquot were frozen and stored

at -70°C for amino acid analysis. Collection of blood and CSF under rigid standardized conditions (diet, level of activity, time of day, and aliquot of fluid) is necessary in order to minimize variations in the biochemical data that might be caused by factors other than the single variable of treatment.

Chronic L-threonine Treatment in Patients With Familial Spastic Paraparesis.

Patients who, at the completion of the double blind study, were judged to have derived substantial benefit during one treatment period compared to the other, may elect to receive L-threonine on for six months. This situation raised several scientific questions, including: Does spasticity suppression improve with L-threonine treatment longer than two weeks? Does the CNS adapt to threonine-induced elevation in glycine so that signs of spasticity recur despite continued treatment?

Five patients participated in this study. They were followed up on a monthly basis in a similar follow up protocol as described in the double blind phase except that they were all seen as outpatients and no lumbar punctures were performed.

5. Data Management and Statistical Considerations

The purpose of this study is to determine if treatment with L-threonine increases CNS glycine levels and produces symptomatic improvement in patients with spasticity. The study uses a double blind, two treatment period crossover design. Patients were randomized between two groups; those receiving placebo and those receiving L-threonine in the first treatment period. Each patient was crossed over to the other treatment in the second period. A total of 20 patients were enrolled in the study.

Clinical and biochemical data obtained during the course of the study were entered into the CLINFO system established on the GCRC VAX 11/750. Data forms for collecting this information were prepared and tested in the open label study (see Appendix A). The data was analyzed using methods similar to those described in Armitage (151). First the hypothesis that there is no period-treatment interaction (carry-over effect) is tested. Since this hypothesis is accepted, then both periods were used in the statistical analyses. The analysis of CSF glycine, which is a continuous and normally or lognormally distributed variable, was analyzed exactly as described in Armitage. One calculates the difference between a patient's response

in the first period and in the second period. These differences are compared between the randomized groups using a t-test. The other endpoints are measurements of motor function which are ordinal scales. For example, one of the measures is "Gait" which is measured as: 0=normal, 1=slightly stiff; 2=stiff with circumduction; 3=stiff with circumduction and effort; 4=needs cane or other support. Since these data are not normally distributed, they were analyzed using a permutation test rather than a t-test.

A crossover rather than parallel protocol design was selected because the number of patients available for the study is limited to 20 and cannot readily be increased due to rarity of the condition. The crossover design gives more information from a limited number of patients. Since all subjects received both treatments, the power for statistical analyses is greatly enhanced. The two major problems with crossover designs are carryover effects and patient dropouts. A long washout period (two weeks) has been incorporated in order to minimize carryover effects.

IV. RESULTS

1. Preclinical Studies.

Effects of Threonine and Threonine Plus Large or Small Neutral Amino Acids on Plasma Amino Acid Concentration.

The administration of the amino acids threonine, serine and valine intraperitoneally, led to a significant 250-400% increase in the amino acid being administered, whether the amino acid was given alone or in combination with the other amino acids. It had no effect on other amino acids, except that serine and serine plus threonine led to a significant 48 and 44% increase in glycine respectively. (figures 15-18).

Effects of Threonine and Threonine Plus Large or Small Amino Acids on Spinal Cord Amino Acid Concentrations.

Threonine administration (400 mg/kg) produced a 190% increase in threonine ($p < .01$) and a 47% increase in glycine ($p < .01$) concentrations in the spinal cord. There was no effect on serine or valine concentrations (figure 19).

Valine administration (400 mg/kg) produced a 390% increase in valine ($p < .01$). There was no significant effect on threonine, glycine or serine (Figure 20).

Threonine plus Valine administration (400 mg/kg each) produced an 85% increase in threonine ($p < .05$) with a 390% increase in valine ($p < .01$) but no significant effect on serine or glycine (Figure 21).

Serine administration (400 mg/kg) produced a 122% increase in serine ($p < .01$). Threonine, glycine and Valine showed no significant change (Figure 22).

Threonine and Serine administration (400 mg/kg each) produced a 92% increase in serine ($p < .01$) and a 38% increase in threonine ($p < .05$). Glycine and valine showed no significant changes (Figure 23).

2. Clinical Studies.

a. Documentation of Chemical Purity of Capsules Contents.

L-threonine is obtained from the Ajinomoto Company in bulk as purified amino acid. Methods to encapsulate threonine have been developed at the Massachusetts General Hospital. The purity and shelf stability of threonine capsules have been examined. Analysis of the threonine bulk powder as well as methods of encapsulation are described in the Methods section. Both bulk powder and stored capsules made in the MGH Pharmacy were analyzed using a Beckman Automated Amino Acid Analyzer 119C. This contained a 6 x 300 mm column at a temperature of 40°C with an AA-20 ion exchange resin. The flow rate was 35 ml/hour with detection of ninhydrin derivatives at 570 and 440 nm. The placebo capsule was free of amino acids. The stored threonine capsule contained only threonine. Threonine in the capsule may be identified by comparison with threonine standard and distinguished from the related amino acid serine (figures 1-3).

b. Results of the Single Drug Open Label Study of L-threonine in Treating Patients with Familial Spastic Paraparesis

Six patients with spastic paraparesis participated in this study to investigate whether L-threonine administration would increase CSF glycine levels and improve spasticity. Their characteristics are listed in table 9.

The patients' ages were between 52 and 68. Three were listed as having a Severe condition, and three Moderate. All but one were males. Their duration of illness was between 20 and 28 years except for one in whom it was 9 years.

Of the 6 patients, 5 received 500 mg tid, all 6 received 1000 mg tid, and 4 of them received 1500 mg tid of L-threonine. In each case, treatment with threonine lasted 1 month. All data were collected according to the methods described (cf. Methods, and Appendix A). L-threonine was well tolerated; there were no signs of clinical or laboratory toxicity, and all patients completed each trial they began. Spasticity scores before and after treatment were compared, and the results for each dose level are shown in Tables 2, 3, and 4. With doses of 1.5 g/day, only

1 patient (RoE) showed clinical improvement; the others were either marginally improved or showed no benefit. Clinical improvement was more substantial with higher doses of L-threonine. With 3.0 g/day, 6 of 6 patients derived benefit in one or more aspect of spasticity. In 1 of these 6 (WV), tone increased but the patient improved in three other aspects (strength, walking, and less brisk tendon reflexes). With a dose of 4.5 g/day, all patients improved: one patient had improvement in all aspects of the examination and all patients improved in at least four categories of spasticity. Because clinical improvement was more substantial with the 4.5 g/day than 3.0 g/day dose, and both doses were tolerated equally well, the higher dose of L-threonine was administered in the double blind p^o protocol. This is a higher dose of threonine than Barbeau et al. used in their studies with familial spinocerebellar degeneration (148), suggesting that the amount of threonine needed to suppress spasticity may vary according to different etiologies of spasticity. Barbeau et al. gave 500 mg daily of threonine; the patients in this study, did not improve until they received 3000 mg a day.

c. Biochemical Results in the Open Label Study.

Plasma and CSF amino acid analyses were performed on samples obtained from 3 patients who received both the 1.5 g/day and 3 g/day doses (Figs 4-7). These were patients number 1, 3, and 5 in table 9. They all were listed as moderate in severity and the duration of their illness was 20, 22 and 20 years, respectively.

Neither plasma nor CSF levels of threonine or glycine increased significantly with administration of 1.5 g/day. These data are consonant with the clinical results: most patients did not improve during 1.5 g/day and CSF glycine levels did not increase. In the only patient with mild improvement (RoE), there was a slight increase in CSF glycine (Fig. 7). In contrast to the effect of lower doses of threonine, a dose of 3 g/day caused a significant 2-4 fold increase of threonine and glycine levels in both plasma and CSF. These initial biochemical data supported the hypothesis that L-threonine administration increases glycine levels in humans as in rats and encouraged us to conduct the double blind study. Furthermore, there was a direct significant correlation between plasma threonine levels and CSF glycine and threonine levels (Figs. 8 and 9).

RESULTS OF THE DOUBLE BLIND CROSS OVER STUDY

1. Patients General characteristics.

20 Patients were enrolled in the double blind study. Their demographic characteristics are listed in table 5. They were between the ages of 23 and 68, 5 women and 15 men. One woman had to be dropped from the study because she became pregnant during the first phase of the protocol. One man was dropped because he hurt his back at the very onset of the protocol and had a steroid injection in the vertebral joints. The remaining 18 patients completed the protocol as described in the methods section except that 10 of them refused to undergo a lumbar puncture. Their duration of illness varied between 4 years and 35 years. The Global Severity of the disease based on the scale described in table 5, was as follows:

- 5 patients (28%) were described as severe; their ages were between 52 and 65 yrs with a mean age of 56.8; the duration of their illness was between 14 and 35 yrs with a mean duration of 21.8.

- 8 patients (44%) were described as moderate; their ages were between 32 and 68 yrs with a mean of 50.1; their duration of illness ranged from 6 to 25 yrs with a mean of 15.6.

- 5 patients (28%) were described as mild; their ages

were between 23 and 41 with a mean of 31; their duration of illness was between 4 and 10 years with a mean of 6.8.

The specific aspects of spasticity (hyperreflexia, rigidity, bladder incontinence...), varied from patient to patient with no significant correlation with either Age, Sex, Duration of illness or Severity. The patients listed as Severe, however, had a significantly ($p < .05$) larger number of symptoms (e.g. they all had difficulty in walking, bladder symptoms, and spontaneous spasms).

There was a highly significant correlation between the patients' Ages and their Duration of illness (figure 24), $p < .005$. The Duration of illness also correlated significantly with the Severity of the patients' condition at the onset of the study (figure 25).

2. Composite Scores of response.

Of the 26 variables studied, 17 changed at least once. The remaining 9 showed no change. Those 9 were either related to upper extremity signs and symptoms or were included as part of a general assessment of potential side effects of threonine administration. Only 17 variables were thus included in the Composite scores. (The patients with Familial Spastic Paraparesis have no abnormalities in the upper extremities. This presumably explains why the parameters related to the upper extremities were not affected by either placebo or L-threonine.)

Calculation of the Composite scores

The Composite score for an individual patient is the Sum of the individual Variable scores for that patient. A particular Variable Score equals the score obtained from the threonine response minus the score obtained from the placebo response for that Variable.

A Composite Score for a particular patient (CSp) is calculated with the following formula:

$$\text{CSp} = (\text{TvN} - \text{PvN})$$

Tvn = Score for Variable n following threonine administration to that patient.

Pvn = Score for Variable n following placebo administration to that patient.

A Composite Score for a particular Variable (CSV) equals the sum of all the patients Variable Scores for that one particular variable. It is calculated as follows:

$$CSv = (Tpn - Ppn)$$

Tpn = Score for that particular variable in patient n following threonine administration.

Ppn = Score for that particular variable in patient n following placebo administration.

A decrease in any one of the Spasticity variables (except for Strength), is an indication of improvement. In order to make all the variables move in the same direction, the change in Strength was multiplied by -1. The subjective reports and impressions were also rated as follows: -1 = improved, 0 = no change and, +1 = deteriorated.

Individual scores were thus obtained for each observation and computed to become individual Variable scores and Composite Scores as described above.

The range of possible scores during one observation are as described in the Neurological Exam Data form, (see Appendix A).

The individual scores were analyzed for significance with the sign ranked test.

3. Individual Variables Response to Treatment. (see table 7).

The Variables that showed significant response to L-threonine are listed below: (a negative score indicates improvement in response to L-threonine. i.e. decrease in spasticity. In order to maintain the same scoring system Strength has also been assigned a negative score as an indication of improvement).

1-Strength: 14 patients had a decrease in strength at the onset of the study. Ten improved during threonine with a change ranging from -1 to -6; two improved during placebo and they had scores of +1 and +2. ($p < .05$). The CSV = -17.

2-Deep Tendon Reflexes (DTR): Eighteen patients had increased DTR's in the legs. Eleven improved during threonine (-2 to -8) and two during placebo (+2 and +4). $p < .01$. CSV = -38.

3-Abnormal Reflexes: sixteen patients had abnormal reflexes. Four patients improved during threonine (-8, -8, -8, -16). None during placebo. $p < .05$. CSV = -40.

4-Hopping right and left legs: Sixteen patients had difficulty hopping. Five patients improved during threonine (-1, -1, -1, -1, -2). None during placebo. $p < .01$. CSV = -6.

5-Clonus : Fifteen patients had clonus. Ten patients improved during threonine (-1 to -4). One during placebo (-1). $p < .01$. CSV = - 20.

6-Bladder control: Ten patients had difficulty with bladder control. Four improved during threonine (-1, -1, -1, -2). None during placebo. $p < .05$. CSV = -5.

None of the patients admitted to this study had detectable signs or symptoms of spasticity in the upper extremities and there was no change noted in muscle strength or tone or DTR's following placebo or threonine administration.

There were no patients with bowel symptoms and no patients reported change in their bowel habits following either treatment.

Two patients reported that their sleeping had improved following threonine intake and two felt it improved following placebo. One said his sleeping deteriorated with intake of threonine and one during placebo.

There was no significant effect on sleeping habits, mood

and behavior, energy or activity levels as evaluated by the patients.

Seventeen of the eighteen patients had increase in their muscle tone in the legs at admission. Four improved during threonine (-2, -2, -2, -3) and two during placebo (+1, +4). This was not statistically significant.

Sixteen patients had difficulty running. One improved during threonine (-1) and one during placebo (+1).

Thirteen patients had difficulty walking. Four improved during threonine (-1, -1, -1, -2), and one with placebo (+1). Not statistically significant. Eight patients improved their walking time during threonine and three during placebo. Not significant.

Sixteen patients reported having experienced spontaneous leg spasms. Five improved during threonine (-1, -1, -1, -2 and -2). One improved during placebo (+1).

The Composite Scores for the other variables ranged from 0 to -6. All the Composite scores for the 17 variables went in the predicted direction except for one (Running) which showed a total score of 0.

Patient Composite Scores; CSp (see table 7).

The composite Scores for the individual patients ranged from +18 to -28. There were fourteen patients (78%) who responded in the predicted direction. One patient (5.5%)

had no change, and three patients (16.5%) changed in the opposite direction. The total composite score for all variables and all patients is -181.

The overall response to threonine was statistically significant $p < .01$.

The CSp was found to be lowest (most improvement) in patients listed as having a Severe condition. It showed no correlation with Sex. There was a significant ($p < .05$) difference in CSp in patients listed as Mild versus Severe (Figure 26).

4. Response to threonine as assessed by Overall Physician Evaluation and Overall Patient Evaluation.

At the end of each treatment period (placebo or threonine), the physician and the patient described their assessment of the response as: Improved; No change; or Deteriorated.

+1 = Placebo better than threonine

0 = No difference between placebo and threonine

-1 = threonine better than placebo

Based on the Overall Physician Evaluation, there was a significant response to threonine ($p < .01$) but not to placebo. Of the 18 patients, 11 (61%) did better on threonine. In 4 (22%), there was no difference between placebo and threonine. 3 (17%) did better on placebo.

Based on the Overall patient evaluation there was a higher response to threonine when compared with placebo. Eight (44%) did better on threonine. five (28%) reported no difference. five (28%) did better on placebo.

Table 6, summarizes these findings and shows the combinations of physician and patient evaluation in respect to the response to treatment.

5. Biochemical Results.

Plasma and CSF threonine and glycine levels are shown in table 9. Threonine administration significantly increased plasma threonine levels by 225% and CSF threonine levels by 200% ($p < .05$). It led to a 12% overall increase in glycine levels in the CSF which was not statistically significant (figures 27 and 28). There was a lot of variability in plasma glycine in response to threonine; it doubled in three patients; it decreased almost by half in three others; and it changed only very little in the remaining. Changes in plasma glycine had no relationship with CSF glycine or threonine. Mean plasma glycine was unchanged.

When the patients who did not respond clinically were excluded from the analysis, the increase in CSF glycine (from 8.54 to 10.28 nmoles/ml or 20% increase), in response to threonine intake, became statistically significant ($p < .05$), and the correlation between the increases in CSF threonine and glycine became significant ($p < .05$). (figures 29-31). The responders had significantly higher CSF glycine response when compared to non-responders ($p = .03$; figure 31).

The correlation coefficient of the changes in blood levels of threonine and changes in CSF threonine or glycine was not statistically significant in either responders or non-responders.

6. Biochemical results and patients characteristics.

There was no significant relationship between blood threonine or glycine, or changes in blood threonine or glycine with either Age, Sex, or Severity either in responders or non-responders.

Baseline levels of threonine or glycine in the CSF had no significant correlation with either Age, Sex, Severity or Duration of illness.

7. Biochemical Results and the Patients Composite Scores CSp.

There was a significant correlation ($p < .05$) between changes in CSF glycine and CSp and a very strong correlation ($p < .005$) between CSF glycine levels following L-threonine administration and CSp (figure 32).

Other biochemical data obtained such as baseline blood or CSF levels of threonine showed no significant correlation with CSp.

There was no relationship between the individual variable scores such as Strength, Tone, DTR etc. with any of the biochemical parameters studied.

Chronic Study.

Only four patients were enrolled in a 6 month chronic L-threonine study, to examine the effects of long term threonine administration on the signs and symptoms of spasticity. The design was the same as for the double blind study except that it was performed on an outpatient basis. No admissions and no Lumbar punctures. Patients were followed up on a monthly basis in contrast to the bi-weekly basis of the double blind protocol. There were no additional benefits experienced by the patients or seen by the physician from long term L-threonine intake. The benefits experienced during the double blind phase continued with the chronic treatment. There was no suggestion of habituation to threonine.

One patient experienced improvement during the first part (drug A) of the double study (pt# 2; E. P.) and experienced deterioration during the second part (drug B). He had started symptoms of depression during the wash out period (between drug A and drug B) which became severe during drug A. When he joined the chronic study, he had completely recovered from his depression and was put on drug A as the drug he responded to initially. On the one month follow up, he reported no benefits at all and requested a change of medication. He was then put on drug B on which he and the physicians noticed substantial improvement. The labels later showed that drug A was the placebo.

Side Effects.

None of the physiological or biochemical variables studied for harmful side effect showed any change with threonine intake. This included ECG, Chest X-ray, Blood Chemistry profiles and Urinalysis (as described in the methods section. One patient mentioned a mild upset stomach during the first 3 days of threonine intake which subsided on its own and he persisted with the medication without any complaints. The same patient mentioned that he was feeling "jittery" during the intake of the second drug which turned out to be placebo. One patient described floaters in one of her eyes for about 3 weeks, after chronic l-threonine administration for 6 months. She had never experienced them before, and had no ophthalmologic signs or symptoms otherwise. Examination by an ophthalmologist was non revealing. The floaters subsided spontaneously and the patient was not experiencing them at the one month follow up examination.

5 patients experienced minor headaches following the lumbar punctures. Headaches subsided with bed rest, minor analgesics and fluid intake. 2 patients experienced severe headaches upon assuming the erect position for 3 days following the lumbar puncture. They had to be bed ridden. One of them required a 25 mgs Demerol injection. No other side effects were reported or detected and no long term complaints were expressed.

V. DISCUSSION

Preclinical Study

The animal studies clearly confirm that threonine administration can increase glycine levels in the spinal cord of rats.

Maher and Wurtman (144), had shown this to be a dose dependent response which could be inhibited by the simultaneous administration of the large neutral amino acids valine and tyrosine.

Threonine administered intraperitoneally leads to an increase in blood concentration of threonine. Once in the circulation, threonine crosses the blood-brain barrier and enters the central nervous system (79). Wurtman and coworkers (159) had shown that amino acids, like threonine, transported by the Large Neutral Amino Acid (LNAA) carrier at the blood-brain barrier, are subject to competition with other LNAA that are circulating in the plasma. The amount of competition a LNAA will experience is determined by the sum of the LNAA (160). This carrier is also referred to as the L-system in contrast with the alanine-serine-cysteine (ASC)-preferring system, which is sodium dependent. The ASC system is also referred to as the small neutral amino acid carrier. Recently Tews (158) suggested that threonine could also be transported through the ASC system.

When animals were given threonine 400 mg/kg, alone or in

combination with equimolar amounts of either valine or serine, blood levels of threonine increased by about 400%. Threonine alone significantly increased spinal cord threonine and glycine. Spinal cord threonine levels increased significantly less, when threonine was administered with either serine or valine. Both serine and valine inhibited the rise in glycine produced by threonine. This shows that threonine can be transported through both the L- and the ASC-systems and is subject to competition for transport by both the large as well as the small neutral amino acids.

Although serine is a precursor of glycine, and its administration increased spinal cord serine and plasma glycine, it failed to increase spinal cord glycine. This result is in agreement with the findings of other investigators (300, 301). It is possible that serine and threonine could serve different metabolic/neurotransmitter pools of glycine, thus accounting for why serine increases plasma glycine and threonine increases spinal cord glycine but not vis versa. Although the formation of glycine from serine has been studied in the rat CNS as well as the distribution of STHM, the enzyme catalyzing this reaction (92-94, 98), no data is available regarding the K_m and V_{max} of either serine or threonine in the rat or human CNS. The K_m for serine in the various tissues studied, varies between .5 and 1.25 mM (see table 10) and serine

concentration in the spinal cord is .4 to .9 mM (.76 in the present study). On this basis alone, it is not possible to determine exactly why serine does not lead to an increase in glycine. The possibilities are either that STHM has different kinetics in the CNS, serine is not readily available to CNS STHM under the experimental conditions used, or it is subject to inhibition by other factors such as product inhibition (glycine in this case).

Clinical Studies

In the preliminary human study, there were two major findings: For the first time, it was demonstrated that L-threonine can increase CSF threonine and glycine levels in humans and that threonine suppressed the manifestations of spasticity in patients with Familial Spastic Paraparesis. Both the increase in the CSF glycine and the improvement in spasticity were dose dependent.

In a regular 75 gm protein diet, a person consumes on the average about 2-3 gms of threonine (311). Since the patients were all being seen as inpatients for a period of one week, it was possible to control their diet and keep their daily protein intake constant. This avoided any significant fluctuations in threonine CSF levels secondary to changes in blood levels of competing amino acids as a result of changes in diet. An additional 3 gms of purified L-threonine had to be given before a significant increase in CSF glycine or a significant response in spasticity

occurred. When 4.5 gms were given, the response was further improved. It is based on this that the dose for the double blind study was selected.

Drugs used in the treatment of spasticity (132-133) have aimed at either enhancing presynaptic inhibition (benzodiazepines, such as diazepam; baclofen) or at diminishing muscular contraction to excitatory stimulation (dantrolene). Benzodiazepines increase presynaptic inhibition by increasing the affinity of GABA receptor sites for the endogenous ligand; at therapeutic doses, they do not normally affect synaptic events mediated by other transmitters such as glycine (134,135). By increasing presynaptic inhibition, benzodiazepines decrease release of excitatory transmitters from afferent fibers with a resultant decrease in the gain of the stretch reflex. Baclofen was originally thought to function as a GABA agonist because it is a lipophilic derivative of GABA that crossed the blood-brain barrier and entered the CNS (133). Although baclofen does slow synaptic transmission in the cord, its electrophysiological and pharmacological profile is quite different from GABA (136-140). Baclofen reduces excitatory transmitter release, apparently by activating GABA b receptors and restricting calcium influx into presynaptic terminals (141,142). As with benzodiazepines, the net effect of reducing excitatory transmitter release

by afferent fibers (and presumably by interneurons) is to suppress reflex activity. Dantrolene's therapeutic effect occurs directly on contractile mechanisms within muscle, without any specific action on reflex pathways (132,133). It reduces the depolarization induced calcium efflux into the sarcoplasm caused by conducted muscle action potentials. Because sarcoplasmic calcium is necessary in order to activate myosin-ATPase and cause actin and myosin filaments to slide past one another, the force produced by muscle in response to its electrical activation is diminished by dantrolene, although the electromyogram is unchanged. These current modalities for treating spasticity are only partially effective in altering muscle tone and reflexes, and have significant side effects. Diazepam and baclofen do suppress flexor spasms in many patients, but neither drug improves walking (132,133,136). Side effects of these drugs include drowsiness, light-headedness, and confusion; furthermore, additive CNS depression may occur when other agents, such as alcohol, are taken concomitantly (132). Dantrolene administration lessens muscle tone and hyperreflexia to the extent that it produces muscular paralysis. Thus, its use is counterproductive in those patients whose spasticity masks limb weakness; these individuals benefit from increased extensor tone that enables them to stand and walk on otherwise weak legs. Hepatic toxicity is a serious

potential side effect that tempers physicians' inclination to prescribe dantrolene (143).

An alternate approach to the treatment of spasticity, as shown by the present studies, is to increase inhibitory (glycinergic) tone in spinal cord interneurons. This is a rational neuropharmacological strategy, but was impossible prior to the discovery that threonine increased glycine levels in the cord (144). The present studies offer a novel approach to treating spasticity based upon enhancing glycinergic neurotransmission; because the drug is an amino acid, few if any side effects have been observed.

Although there is a strong anatomical and biochemical basis supporting the view that glycine or its relative lack could be involved in the pathophysiology of spasticity (34, 93, 122, 126, 127), there was prior to these studies no pharmacological tool to test this belief. The present investigations not only provided a possible treatment; they also strongly suggest that glycine is indeed a neurotransmitter in the spinal cord of humans, playing a role in the reflex arc and muscle tone and amenable to pharmacological modification. The specific manifestations of spasticity affected by glycinergic neurotransmission could not be assessed from the preliminary study because of the small number of patients and the subjective component

due to the one drug open label aspect of the protocol. Nevertheless, the most noted changes were seen in Strength, Deep Tendon Reflexes and the ability to stand unsupported or hop on one foot.

Double Blind Study

The availability of patients with Familial spastic Paraparesis (FSP) provided a neuropathological condition with pure involvement of the corticospinal (pyramidal) tracts (117), uncontaminated by other CNS lesions and with preservation of interneurons whose inhibitory actions are mediated by glycine (see section II; M and 178, 190-192).

Patient Characteristics.

As could be expected from the history of the disease, the older the patients, the longer the duration of illness and the more severe the condition. It is not understood why the disease manifests mostly in the third and fourth decades of life. Aging itself, even normally, carries aspects of degeneration of function and tissue due to both environmental influences as well as genetic predispositions.

The exact nature of the disease, as with other primary neuronal degenerations, is obscure. At first sight, it appears to show, in common with other degenerations, the phenomenon of 'dying-back'--that is, a progressive

withering of axons beginning at their terminations and proceeding towards the cell body, which eventually disappears (192). This 'dying-back' is a recognized nature of the corticospinal tracts in motor neurone disease, of the pontocerebellar fibers in olivopontocerebellar atrophy, and of the posterior spinocerebellar tracts in Friedreich's ataxia (192). On the other hand, there is no evidence, in FSP, of the phenomenon of trans-synaptic, or 'chain' degeneration, seen in olivopontocerebellar degeneration and Friedreich's ataxia. It is worth noting, too, that the two tracts which are constantly affected in FSP contain the longest fibers in the central nervous system (190-192); and that, in spite of the long duration of the disease, the cells of origin of these tracts--in the cerebral cortex and posterior root ganglia respectively--show little, if any, depletion at the time of death. It may well be that the mechanism of distal fibre degeneration in FSP is different from that in the so-called 'system' degenerations, in which collections of neurones are affected regardless of the length of their axons. The findings, in fact, could be explained as the result of a generalized failure of nerve cells to maintain the vitality of axons of more than a certain length, rather than as a specific disease of particular types, or systems, of neurons. This conjectured difference may or may not be real; in any case, it cannot be assumed that the basic cause is the same in all kinds of

primary neuronal degeneration.

Greenfield (193) commented the pathogenesis of the spinocerebellar degenerations and suggested that the lesion would ultimately be found to be an enzyme deficiency. It has been shown that accidental organic phosphate poisoning in man has resulted in degeneration of long tract fibers--corticospinal and gracile--especially at the distal ends (194); and experimentally Cavanagh (195), using tri-ortho-cresyl phosphate, and Fenton (196) with di-iso-propyl-fluorophosphonate, were able to reproduce similar but more acute changes in chickens. The organic phosphates destroy pseudochoolinesterase, an enzyme which has no known function in the central nervous system; but affection of other enzymes as well is a possibility. A premature aging process has been suggested as the pathogenesis of various spinocerebellar disorders, a concept proposed by Raymond (197) and then expanded to include a wide variety of familial or hereditary disorders which show distal axonal atrophy. In FSP, no excess of the signs commonly associated with aging in the central nervous system has been reported in the literature.

One of the features of these disorders is the age of onset. It is interesting to speculate on how a system which has functioned well from birth to 30 years should then fail. In the case of metachromatic leukodystrophy one

can understand why the disorder is not seen in infants before 2 1/2 or 3 years of age, because it is known that aryl sulphatase-A deficiency will not produce an effect until myelin formation has occurred (198). There is no explanation, however, for similar cases of this disorder starting in adulthood. Whether or not what is inherited in FSP is a tendency to 'switch-over' enzyme production from normal to abnormal at a certain age, cannot be determined. Since the flow of nutrients is from perikaryon to axonal termination, it is understandable that the distal parts of the long axons would be affected first if there were any derangement in cell nourishment.

The inheritance of FSP points to heterogeneous autosomal dominant characteristics (278-280). A suspected viral disease, Tropical Spastic Paraparesis (TSP), causing similar onset of symptoms with no suggestion of genetic predisposition has been observed in the tropical islands. Neuropathologic examination of TSP (223-224) show that the basic lesion is a bilateral degeneration of the corticospinal tracts and dorsal funiculi, with preservation of Betz cells and dorsal root ganglia neurons and, in addition, an inflammatory process characterized by proliferative vasculopathy, perivascular cuffing, and chronic leptomeningitis. The peripheral nerves also show evidence of axonal and myelin involvement, which could be due in part to an ischemic or compressive component. The

neuropathologic changes in the spinal cord are similar to those observed in Jamaican tropical myeloneuropathy (218, 225) and the vacuolar myelin changes resemble those of subacute combined degeneration due to vitamin B12 deficiency, and those of the myelopathy recently described in AIDS (226-228). TSP is obviously a different pathologic and epidemiologic condition from FSP.

In conclusion, uncomplicated FSP is a well-defined clinical and pathological entity.

Overall physician and patient evaluation.

Based on the overall physician evaluation, there was a significant response to threonine but not to placebo; 61% versus 17%. 44% of the patients estimated their condition as having improved during threonine versus 28% during placebo with no difference in the others.

The patients evaluation was based on a questionnaire they filled at the end of each treatment period. The only subjective parameter (based on patient evaluation) that showed statistical significance was the improvement in bladder function. Other symptoms that improved were stiffness in the legs, spontaneous spasms, strength and walking ability. Although not statistically significant, some of these changes were very helpful to the patients. One patient, for example, used to need 3-4 pads per day

because of bladder incontinence. When she started treatment, her symptoms subsided to the point of needing only one pad per day. Another patient, walked 18 holes of golf for the first time in two years, his wife noticed that she could not hear his feet dragging on the floor anymore while he walked at home.

The patients assessment of what it means to have improved was entirely subjective. Two patients for example, said they experienced no overall improvement. When asked, among other things, about their spontaneous spasms at night, they reported having had either none or almost none, whereas they used to have spasms 2-4 times per week.

The physician's estimate based on the clinical exam (which was attempted to be made as objective as possible by grading the different variables into objective scores), shows significance. i.e. the physician's estimate is that threonine helps in spasticity. This evaluation agrees with the composite scores. It was expected that the physicians overall impression, agreed with the Composite Scores because the Composite score weighs heavily on the physician's evaluation of each variable score.

In a condition like spasticity, it is possible for the physician to detect changes that the patients might not experience (e.g. decrease in Deep Tendon Reflexes). This, together with the objective reference scale available to the physician makes the physician evaluation more

significant in detection of mild changes and evaluation of overall response.

Composite Scores

The Composite Scores provided a tool to estimate the power of change for individual variables and in individual patients. The range of variation for each variable was 0 to 4 except for Strength. Strength could vary from 0 to 80 but never went under 70 except in one patient; in any case it never changed by more than six points under any circumstances. This gave all the variables an equal contribution to the Composite Scores and allowed comparison (from a descriptive level) between variable changes.

The Composite Scores for every variable (summing the scores for that variable across patients) went in the predicted direction. The variables showing significant improvement with threonine were Strength, DTR, Abnormal Reflexes, Hopping, clonus, and Bladder control.

DTR, Abnormal Reflexes and Clonus responses could be explained by a possible increase in glycine availability to the inhibitory interneurons in the spinal cord grey matter with more inhibitory input on the motor neuron and lessening of its hyperexcitability. Although the exact mechanism of the different manifestations of spasticity have not been elucidated, there is evidence that the Ia

inhibitory interneurons are under a diminished input from the pyramidal tract leading to a decrease in inhibition possibly contributing to spasticity (see section II; J and 272). There is also strong evidence that these interneurons are glycinergic (34, 93, 122, 126, 127). This could explain the finding that an increase in glycine through threonine administration improves some of the manifestations of spasticity.

Hopping on one foot is a complex motor function which involves the reflex arc as well as position sense, balance and voluntary motor coordination. Glycine could influence hopping either solely as it is described above or in combination with other unknown mechanisms.

The improvement in Strength could be related to the ability to inhibit the antagonistic muscle groups that otherwise create a weakening effect on muscle movement in a particular direction. This effect could also be related to decrease in general muscle tension, providing rest for the muscle tissue and subsequent increase in the ability for voluntary contraction.

Patient Composite Scores

The Composite Scores for the individual patients showed a significant improvement during threonine intake versus placebo demonstrating the effectiveness of L-threonine in treating Spasticity (table 7).

The largest improvement was seen in patients with illness described as Moderate or Severe (fig 26). There was no correlation with Sex.

As the disease advances the deficit becomes more pronounced and the signs and symptoms more noticeable (179, 180, 278-280). Since the disease does not affect the alpha motor neuron itself or the muscle tissue (178-192), progression in the disease does not lead to a decrease in the potential for response. In fact, this gives more room to possible improvement. Assuming that the spinal interneurons involved in spasticity respond to "denervation" as predicted by Wurtman (159), and increased their firing rate, they will thus, presumably increase their response to precursor availability (159, 170). This could explain why patients with more severe manifestations responded better. The autopsy studies on Familial Spastic Paraparesis do not suggest a loss in the density of the spinal cord interneurons (178-180, 189-192). Therefore, the local apparatus required for improvement is available, although the severity of the condition is increasing and

there is an increase in the loss of pyramidal control over spinal segmental levels (178, 189, 192)

Biochemical Results

The administration of threonine led to two fold increases in blood threonine in all patients. This was reflected by an overall doubling of CSF threonine (table 9).

There was a lot of variability in plasma glycine in response to threonine: it doubled in three patients, decreased almost by half in three others and changed little in the remaining. Changes in plasma glycine had no relationship with CSF glycine or threonine. Mean plasma glycine was unchanged. The variability in plasma glycine could be related to diet.

One of the draw backs of the protocol is that the diet of the patients could not be controlled. Because of the length of the study (8 weeks), it was not possible to put the patients on a strict 75 gm protein diet as was done in the open label protocol. As demonstrated in the animal studies, the ability of threonine to enter the blood-brain barrier depends on its plasma concentration and on the level of competition with other large or small neutral amino acids. It is possible therefore that diet might explain why changes in blood threonine were not mirrored case per case with changes in CSF threonine or glycine.

CSF glycine showed an overall 12% increase and a 20% (significant) increase in responders with no change in non-responders (fig. 31). There was a strong correlation between CSF glycine levels and the composite scores (fig

32). This suggests that the clinical response could be related to CSF glycine levels or to changes in CSF glycine concentration. It gives support to the hypothesis that increase in glycinergic neurotransmission is related to improvement in spasticity.

If this hypothesis is true, as these studies seem to indicate, it is not surprising that glycine levels did not increase in non-responders. Furthermore, CSF threonine levels in the two non-responders, on whom CSF data was obtained, showed only a modest 1.3 x increase in CSF threonine as compared to an overall more than 2-3 x increase. Also, their CSF glycine levels showed a slight decrease rather than the 10-25 % increase observed in responders. Although no statistical conclusion could be made because of the small number of subjects in the non-responder subgroup (n=3), it is possible that there were other factors like diet that could have played a role in biochemical and thus clinical response to L-threonine.

There were no significant correlations between the biochemical results and the patients general characteristics except that CSF glycine levels post threonine administration were higher in the older and the more severe cases. This correlates well with the finding that Age, Severity and the biochemical changes were related in the same direction to clinical response.

The best biochemical marker of response, based on the correlation coefficient with CSp, was the CSF levels of glycine post threonine administration. This lends support to the hypothesis (fig. 32).

Unfortunately, no blood marker that could be used as a predictor of response was identified. However, threonine did not show any noticeable side effects in the population examined in the present study; its use in patients who, potentially, might not respond to it should not present a major drawback. The need for a predictor of response is more urgent in treatments where side effects might outweigh the benefits.

VI. SUMMARY AND CONCLUSIONS

1- Threonine increases blood and CSF threonine levels and CSF threonine and glycine levels in laboratory animals and in humans. It is subject to competition for entry into the central nervous system by the large as well as the small neutral amino acids.

2- Increases in CSF glycine produced by threonine lead to an improvement in spasticity.

3- Not all patients and not all manifestations of spasticity respond equally to threonine. The best overall response is in the group of patients with a moderate to severe condition. Strength, Deep Tendon Reflexes, Hopping, Clonus and Bladder control were among the variable that responded best.

4- The studies suggest that Threonine has the potential to be used as a safe long term medication with little or no side effects and habituation.

TABLE A

*SHOWS THE EFFECT OF L-THREONINE ADMINISTRATION
ON THREONINE AND GLYCINE CONCENTRATIONS
IN RAT SPINAL CORD
AND BRAIN.*

*MAHER AND WURTMAN
(144).*

TABLE A

Effect of L-threonine Administration on Threonine and
Glycine Concentrations in Rat Spinal Cord and Brain
Maher and Wurtman (144)

Dose of Threonine (mg/kg)	Spinal Cord		Brain	
	Threonine (umoles/g)	Glycine (umoles/g)	Threonine (umoles/g)	Glycine (umoles/g)
0	0.49 + 0.03	2.62 + 0.04	0.28 + 0.03	0.50 + 0.06
50	0.56 + 0.04	2.60 + 0.11	0.41 + 0.09	0.50 + 0.02
100	0.67 + 0.04**	3.13 + 0.16**	0.47 + 0.02**	0.64 + 0.06
200	0.80 + 0.04*	3.08 + 0.07**	0.53 + 0.05*	0.65 + 0.09
400	1.09 + 0.04*	3.29 + 0.10*	0.69 + 0.03*	0.62 + 0.04

L-threonine was administered intraperitoneally at different doses and animals were killed one hour later. Tissues were analyzed for threonine and glycine contents. Data are expressed as means + standard error of 5 determinations.

* p < 0.01, differs from control.

** p < 0.05, differs from control.

TABLE 1
SHOWS THE PATIENTS CHARACTERISTICS
IN THE OPEN LABEL STUDY.

Table 1

PATIENT CHARACTERISTICS.

INITIALS	PT#	AGE	SEX	DURATION	GLOBAL	<u>CHIEF SYMPTOMS*</u>		
				OF ILLNESS(y)	SEVERITY*	GAIT	BLADDER	SPASMS
R.F	1	68	M	20	MODERATE	3	0	2
E.P.	2	49	M	9	MODERATE	2	0	0
R.E.	3	52	M	22	SEVERE	4	4	4
L.K.	4	58	F	23	SEVERE	4	3	3
WV	5	64	M	20	MODERATE	3	2	0
Ra.E	6	67	M	28	SEVERE	4	3	3

*GAIT: 0=Normal 1=Stiff 2=Stiff+Circumduction

3=Stiff+Circumduction+Effortful 4=Needs cane or other support

*BLADDER: 0=Normal 1=Frequency 2=Urgency 3=Incontinence 4=Severe

Incontinence

*SPASMS: 0=Normal 1=Rare 2=1-2x/Wk 3=2-4x/Wk 4=Every Night

*SEVERITY: MILD= (GAIT<2) MODERATE= (GAIT=2-3) SEVERE= (GAIT=4)

*TABLES 2, 3, AND 4,
SHOW THE CLINICAL RESPONSE TO 1.5, 3.0 AND 4.5 GM OF L-THREONINE
IN PATIENTS WITH FAMILIAL SPASTIC PARAPARESIS
IN THE OPEN LABEL SINGLE DRUG STUDY.
THESE RESULTS ARE BASED ON THE PHYSICIAN EVALUATION
AS DESCRIBED IN THE METHODS SECTION.*

*THE SCORES SHOWN ARE THE DIFFERENCE IN SCORES BETWEEN THE BASELINE
EXAMINATION AND THE EXAMINATION FOLLOWING L-THREONINE
ADMINISTRATION.*

- A NEGATIVE SCORE INDICATES A DECREASE IN SPASTICITY AND THUS
REFLECTS IMPROVEMENT.*
- 0 (ZERO) MEANS NO CHANGE.*
- A POSITIVE SCORE MEANS DETERIORATION.*

*THERE WAS A SIGNIFICANT DOSE RESPONSE ($P < .05$). THE HIGHER THE DOSE,
THE BETTER THE CLINICAL RESPONSE.*

Table 2. Clinical Response to L-threonine in Patients with Spastic Paraparesis: 1.5 g/day

NAME	STRENGTH	TONE	DEEP TENDON REFLEXES	RUNNING	WALKING	HOPPING R. LEG	HOPPING L. LEG
EP	0	0	0	0	0	0	0
RF	0	-2	-2	0	0	0	0
RoE	-4	0	0	0	0	-1	-1
LK							
WV	0	0	0	0	0	0	0
RaE	-2	0	0	0	0	0	0

Table 3. Clinical Response to L-threonine in Patients with Spastic Paraparesis: 3.0 g/day

NAME	STRENGTH	TONE	DEEP TENDON REFLEXES	RUNNING	WALKING	HOPPING R. LEG	HOPPING L. LEG
EP	0	0	-1	0	-1	-1	-1
RF	0	-4	-6	-1	-1	-1	-1
RoE	-8	-2	-4	0	-1	-1	-1
LK	0	0	-2	0	0	-1	-1
WV	-2	+4	-2	0	-1	0	0
RaE	-2	0	0	0	0	0	0

Table 4. Clinical Response to L-threonine in
Patients with Spastic Paraparesis: 4.5 g/day

NAME	STRENGTH	TOPE	DEEP TENDON REFLEXES	RUNNING	WALKING	HOPPING R. LEG	HOPPING L. LEG
EP	-1	-2	-2	-1	-1	-1	-1
RF							
RoE	-2	0	-4	0	-1	-1	-1
LK	-6	-2	-2	0	0	-1	-1
WV							
RaE	-2	0	-2	0	-1	-1	-1

TABLE 5
SHOWS A SUMMARY OF THE PATIENTS' CHARACTERISTICS
IN THE DOUBLE BLIND CROSS-OVER STUDY.

- 5 patients (28%) were described as severe; their ages were between 52 and 65 yrs with a mean age of 56.8; the duration of their illness was between 14 and 35 yrs with a mean duration of 21.8.

- 8 patients (44%) were described as moderate; their ages were between 32 and 68 yrs with a mean of 50.1; their duration of illness ranged from 6 to 25 yrs with a mean of 15.6.

- 5 patients (28%) were described as mild; their ages were between 23 and 41 with a mean of 31; their duration of illness was between 4 and 10 years with a mean of 6.8.

Table 5
PATIENT CHARACTERISTICS.

INITIALS	PT#	AGE	SEX	DURATION OF ILLNESS (y)	GLOBAL SEVERITY*	CHIEF SYMPTOMS*		
						GAIT	BLADDER	SPASMS
R.F.	1	68	M	20	MODERATE	3	0	2
E.P.	2	49	M	9	MODERATE	2	0	0
E.S.	3	49	M	20	MODERATE	3	0	3
F.S.	4	54	M	14	SEVERE	4	1	3
J.E.	5	63	M	6	MODERATE	3	1	3
R.E.	6	52	M	22	SEVERE	4	4	4
L.K.	7	58	F	23	SEVERE	4	3	3
N.T.S.	8	65	M	35	SEVERE	4	3	3
N.T.J.	9	32	M	8	MODERATE	3	0	1
M.T.	11	28	F	10	MILD	0	2	1
P.M.	12	35	F	7	MILD	1	0	1
L.D.	13	44	M	25	MODERATE	3	0	1
E.S.	15	50	M	21	MODERATE	3	1	4
G.P.	16	23	M	6	MILD	1	0	0
M.P.	17	28	M	4	MILD	0	1	1
J.M.	18	46	F	16	MODERATE	3	2	4
J.F.	19	41	M	7	MILD	1	0	1
F.S.	20	55	M	15	SEVERE	4	1	3

*GAIT: 0=Normal 1=Stiff 2=Stiff+Circumduction
3=Stiff+Circumduction+Effortful 4=Needs cane or other support

*BLADDER: 0=Normal 1=Frequency 2=Urgency 3=Incontinence 4=Severe
Incontinence

*SPASMS: 0=Normal 1=Rare 2=1-2x/Wk 3=2-4x/Wk 4=Every Night

*SEVERITY: MILD= (GAIT<2) MODERATE= (GAIT=2-3) SEVERE= (GAIT=4)

TABLE 6

SHOWS THE OVERALL PHYSICIAN AND PATIENT AVALUATION OF RESPONSE.

At the end of each treatment period (placebo or threonine), the physician and the patient described their assessment of the response as: Improved; No change; or Deteriorated. For the purpose of this analysis only, these assessments were given scores based on the following table:

- +1 = Placebo better than threonine*
- 0 = No difference between placebo and threonine*
- 1 = threonine better than placebo*

Based on the Overall Physician Evaluation, there was a significant response to threonine ($p < .01$) but not to placebo. Of the 18 patients, 11 (61%) did better on threonine. In 4 (22%), there was no difference between placebo and threonine. 3 (17%) did better on placebo.

Based on the Overall patient evaluation there was a non-significant response to threonine when compared with placebo. Eight (44%) did better on threonine. five (28%) reported no difference. five (28%) did better on placebo.

TABLE 6

OVERALL PHYSICIAN AND PATIENT EVALUATION OF RESPONSE

P A T I E N T E V A L U A T I O N	PHYSICIAN EVALUATION			TOTAL
	+1	0	-1	
+1	17%	5.5%	5.5%	28%
	(3)	(1)	(1)	(5)
0	0%	5.5%	22.5%	28%
		(1)	(4)	(5)
-1	0%	11%	33%	44%
		(2)	(6)	(8)
TOTAL	17%	22%	61%	100%
	(3)	(4)	(11)	(18)

+ 1 = PLACEBO BETTER THAN THREONINE

0 = PLACEBO SAME AS THREONINE

- 1 = THREONINE BETTER THAN PLACEBO

TABLE 7
SUMMARIZES THE FINAL SCORES FOR EACH VARIABLE
AND SHOWS THE SIGNIFICANCE IN IMPROVEMENT
FOR EACH VARIABLE:

Of the 26 variables studied, 17 changed at least once. The remaining 9 showed no change. Only 17 variables were thus included in the Composite scores.

A Composite Score for a particular patient (CS_p) is calculated with the following formula:

$$CS_p = (T_{vn} - P_{vn})$$

T_{vn} = Score for Variable n following threonine administration to that patient.

P_{vn} = Score for Variable n following placebo administration to that patient.

A Composite Score for a particular Variable (CS_v) equals the sum of all the patients Variable Scores for that one particular variable. It is calculated as follows:

$$CS_v = (T_{pn} - P_{pn})$$

T_{pn} = Score for that particular variable in patient following threonine administration.

P_{pn} = Score for that particular variable in patient n following placebo administration.

A decrease in any one of the Spasticity variables is an indication of improvement. The subjective reports and impressions were also rated as follows: -1 = improved, 0 = no change and, +1 = deteriorated. Individual scores were thus obtained for each observation and computed to become individual Variable scores and Composite Scores as described above. The range of possible scores during one observation are as described in the Neurological Exam Data form, (see Appendix A).

11 VARIABLES WERE BASED ON PHYSICIAN EVALUATION: STRENGTH, TONE, DEEP TENDON REFLEXES (DTR), ABNORMAL REFLEXES (BABINSKI AND CROSSED ADDUCTORS), RUNNING, WALKING, WALKING TIME (15 YARDS), HOPPING ON THE RIGHT AND LEFT LEGS (HOP R, HOP L), CLONUS, AND PHYSICIAN IMPRESSION (PHY. IMP.).

6 VARIABLES WERE BASED ON THE PATIENTS' EVALUATION (SU=SUBJECTIVE): STRENGTH (STR SU), WALKING (WALK SU), SPASM (SPASM SU), BLADDER CONTROL (BLADDER SU), STIFFNESS OR TIGHTNESS IN THE LEGS (STIFF SU) AND PATIENT IMPRESSION (PT. IMP.).

DTH = DIFFERENCE IN CSF THREONINE BETWEEN PLACEBO AND TREATMENT.

DGL = DIFFERENCE IN CSF GLYCINE BETWEEN PLACEBO AND TREATMENT.

The Variables that showed significant response to L-threonine were the following: 1-Strength: $p < .05$. Twelve patients had a change in strength ranging from +2 to -6. The $CS_v = -17$. 2-Deep Tendon Reflexes (DTR): $p < .01$. Thirteen patients had a change ranging from +4 to -8. $CS_v = -38$. 3-Abnormal Reflexes: $p < .05$. Four patients had a change ranging from -8 to -16. $CS_v = -40$. 4-Hopping right and left legs: $p < .01$. Five patients had a change ranging from -1 to -2. $CS_v = -6$ and -5 respectively. 5-Clonus : $p < .01$. Eleven patients had a change ranging from -1 to -4. $CS_v = -22$. 6-Physician Impression : $p < .02$. Fourteen patients had a change ranging from +1 to -2. $CS_v = -14$. 7-Bladder control: $p < .02$. Four patients had a change ranging from -1 to -2. $CS_v = -5$.

TABLE 7

Summary of scores and significance for Spasticity Variables

Pt. #	1	2	3	4	5	6	7	8	9	11	12	13	15	16	17	18	19	20	CSV	P value	
<u>STRENGTH</u>																					
<u> </u> TONE	-2	+1	-2	-2	-2	-6	-1		-1		-1	-1	-2		-2	-2		+2	-17	<.05	
<u> </u> DTR		+1	-4	-4	-3	-6	-6	-8		+4		-3	-2		-2	-3		-2	-4	NS	
<u> </u> ABN.REFL.		+4	-16	-8		-8	-8	-8	-2		-2	-3	-2		-2	-3		+2	-38	<.01	
<u> </u> RUN																			-40	<.02	
<u> </u> WALK		+1			-1														0	NS	
<u> </u> WAL.TIME	-1	+4	-1	-1	-2	-1	-2		-1		-1	-2	-1		-1	-1		+1	-4	NS	
<u> </u> HOP.R.		-1		-1	-2	-1	-1			+1								+1	-4	NS	
<u> </u> HOP.L.																			-6	<.01	
<u> </u> CLONUS	-2	-1	-3	-2	-2	-2	-1			-2		-1			-1	-1			-5	<.01	
<u> </u> PHY.IMP.	-1	+1	-2	-2	-2	-2	-4		+1		-1	-2			-2	-2		+1	-22	<.01	
<u> </u> STR. SU.		+2																	-14	<.02	
<u> </u> WALK SU.	+1	+2		-1	-2		-1		-1		-1	-1			-1	-1		+1	-2	NS	
<u> </u> SPASM SU.				-1	-2			-1	-1						-2	-2			-4	NS	
<u> </u> BLADDER SU.				-1	-2			-1	-1	+1					-2	-2			-6	NS	
<u> </u> STIFF SU.		+2		-1	-2				1		1	1	1	1	-1	-1			-5	<.02	
<u> </u> PT.IMP.	+1	+2		-1	-2														-5	NS	
<u> </u> C.S.P.	-4	+18		-28	-25	-21	-2	-18	-1	+1	+1	-1	-1	-1	-1	-2		+1	-4	NS	
<u> </u> DTH	26		73	116		43	65		30	30	35	119				15	28	6		NS	
<u> </u> DGL	15		28	5.4		2.4	1.4		3.3	3.3	3.3	4				1.4	1.9				

TABLE 8

***SHOWS THE REGIMEN ASSIGNMENT LISTS
FOR L-THREONINE ADMINISTRATION
IN THE DOUBLE BLIND STUDY.***

TABLE 8

Regimen Assignment Lists for L-threonine Administration
(This is the actual sequences generated when the study began and remained in the confidence of the dispensing pharmacist.)

Sequence 1: Sample Space = 2 No. of variables = 10

Pt. 1:	drug A--->B	Pt. 2:	drug B--->A
Pt. 3:	drug B--->A	Pt. 4:	drug B--->A
Pt. 5:	drug A--->B	Pt. 6:	drug A--->B
Pt. 7:	drug B--->A	Pt. 8:	drug A--->B
Pt. 9:	drug A--->B	Pt. 10:	drug B--->A

Sequence 2: Sample Space = 2 No. of Variables = 10

Pt. 11:	drug B--->A	Pt. 12:	drug A--->B
Pt. 13:	drug B--->A	Pt. 14:	drug B--->A
Pt. 15:	drug A--->B	Pt. 16:	drug B--->A
Pt. 17:	drug B--->A	Pt. 18:	drug A--->B
Pt. 19:	drug A--->B	Pt. 20:	drug A--->B

TABLE 9

**SHOWS THE BIOCHEMICAL RESULTS
OF THE DOUBLE BLIND STUDY.
BLOOD THREONINE AND GLYCINE WERE CALCULATED
FOLLOWING PLACEBO AND THREONINE ADMINISTRATION ON 16 PATIENTS.
CSF DATA WERE OBTAINED ON 10 PATIENTS.
THE MEAN + STANDARD ERROR (SEM)
ARE SHOWN FOR EACH TREATMENT.
THE CLINICAL RESPONSE BASED ON
THE OVERALL PHYSICIAN (PHY. EVAL.)
AND PATIENT (PT. EVAL.) EVALUATION
ARE SHOWN FOR THE PURPOSE OF COMPARISON.
I=IMPROVED
S=SAME
D=DETERIORATED.
P=PLACEBO
T=THREONINE.**

Table 9

PLASMA AND CSF [THR] AND [GLY] nMoles/ml.

PT#	PLASMA				CSF				CLINICAL RESPONSE			
	PLACEBO		THREONINE		PLACEBO		THREONINE		PHY. EVAL.		PT. EVAL.	
	THRE	GLY	THRE	GLY	THRE	GLY	THRE	GLY	P.	T.	P.	T.
1.	171	226	416	256	25.4	4.55	51.8	6.06	D	I	S	S
2.	150	224	245	133					I	S	I	D
3.	216	266	555	232	28.5	7.46	101	10.26	S	I	S	S
4.	124	203	302	196	54.6	9.51	171	14.86	S	I	S	I
5.	109	202	403	211					D	I	D	I
6.	123	194	284	148	37.9	6.3	80.9	8.7	D	I	I	S
7.	406	540	673	503	40.4	12.6	105	14.0	D	I	D	I
8.									S	I	S	S
9.	147	197	360	274					S	S	S	I
11.	314	238	439	238	90.1	10.9	120	7.6	S	D	I	S
12.	280	173	338	372	43.8	7.5	78.6	7.2	I	I	I	S
13.	138	202	373	204					S	I	S	I
15.	178	229	654	241	55.6	9.2	175	9.6	S	S	S	S
16.	111	123	325	249					S	S	D	S
17.	151	223	301	189					S	I	S	I
18.	125	439	551	306	104	10.4	119	11.8	D	I	D	I
19.					63.8	4.4	91.6	6.3	S	I	I	I
20.	119	271	305	196					I	S	I	S
Mean	178	247	407	247	54.4	8.3	109	9.6				
*SEM	21	26	33	22	8.1	.86	12.4	.98				

P=PLACEBO
T=THREONINE

TABLE 10
IS A SUMMARY
OF THE ENZYMATIC STUDIES ON
SERINE TRANSHYDROXYMETHYLTRANSFERASE
(STHM).

TWO STUDIES ON RABBIT LIVER (104, 308)
SHOW THAT STHM CAN CLEAVE THREONINE
INTO GLYCINE AND ACETALDEHYDE.
RECENT STUDIES (166, 167) SHOW THAT
IT CANNOT IN RAT AND CAT LIVER.

TABLE 10

ENZYMATIC STUDIES ON SERINE TRANSHYDROXYMETHYLTRANSFERASE (STHM).

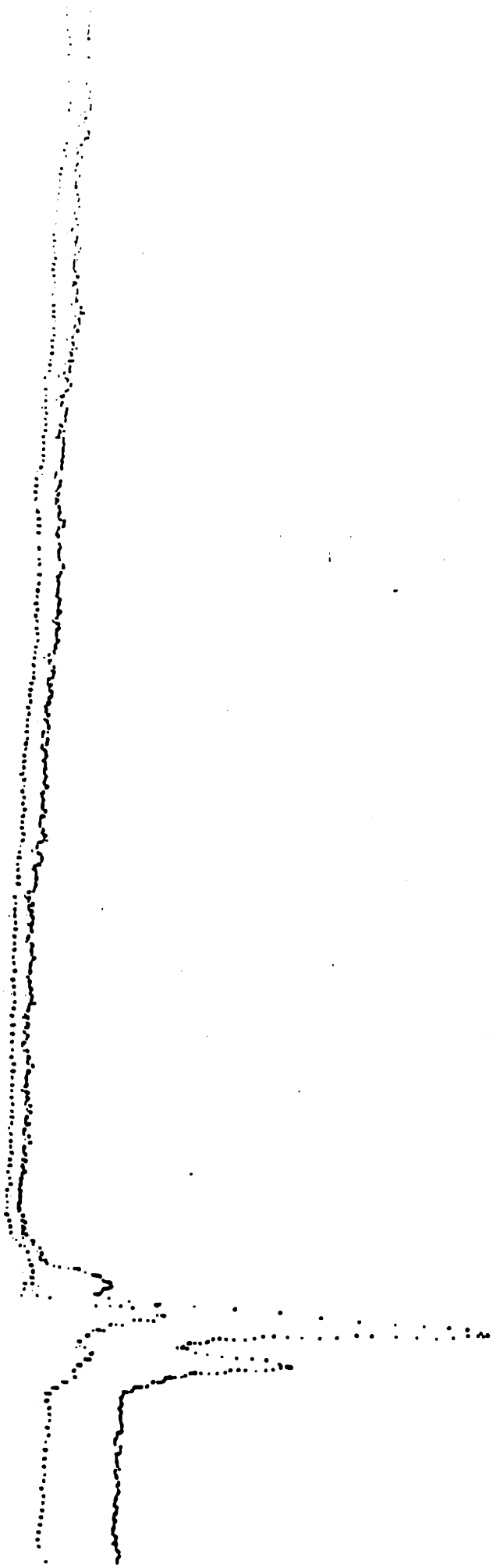
SOURCE	ENZYME	SUBSTRATE	Km (mM)	Vmax uMOL/MG/MN	REF.
S. cerevisiae	STHM	SERINE	.7		302
G- ROD	STHM	SERINE	1.25	.01	303
FISH LIVER	STHM	SERINE GLYCINE	5.6 3.7		304
RAT LIVER	STHM	L-SERINE L-ALLOTHR.	.45 .25		305
BOVINE BRAIN	STHM	L-SERINE	.67		306
E.coli	STHM	SERINE	.67		307
RABBIT LIVER	STHM	L-SERINE DL-ALLOTHR. L-THR.	.8 1.5 40.	9.6 2.4 .8	308
RABBIT LIVER	STHM	L-SER GLY DL-ALLOTHR. L-THREO.	.7 5.9 3.0 40.		104
RAT LIVER	STHM	L-SER GLY	.54 1.8		309
RAT LIVER	STHM	L-SERINE D-SERINE L-ALLOTHR D-ALLOTHR L-THREONINE D-THREONINE	.51 -- 2.0 -- -- --	48.9 0.0 12.2 0.0 0.0 0.0	166

FIGURES 1, 2 AND 3

*SHOW THE AMINO ACID ANALYSIS
OF A SAMPLE OF THE CAPSULES GIVEN TO PATIENTS
AND DEMONSTRATE THE PURITY
WITH RESPECT TO AMINO ACID CONTENT.*

Figure 1. Antino acid analysis: placebo capsule

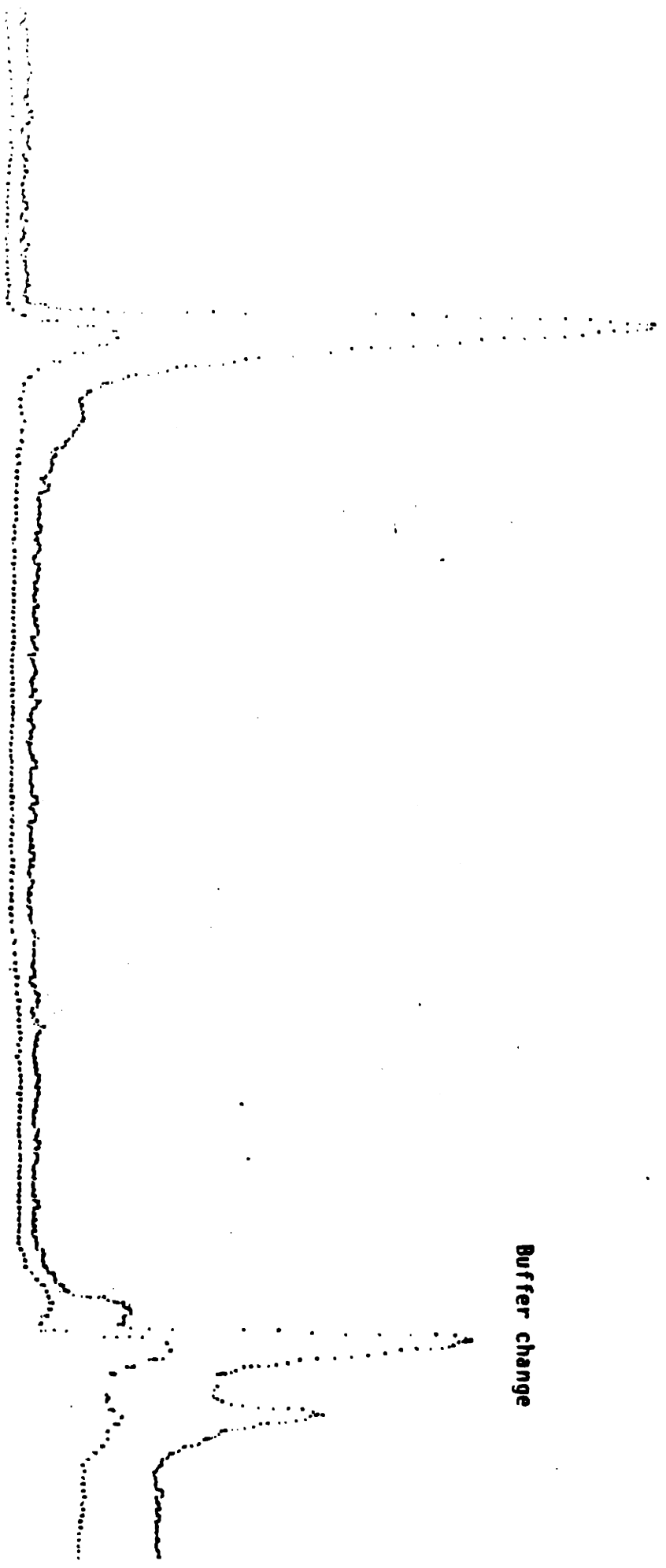
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Buffer change

Figure 2. Amino acid analysis: L-threonine capsule

Threonine Capsule (44.5 min)

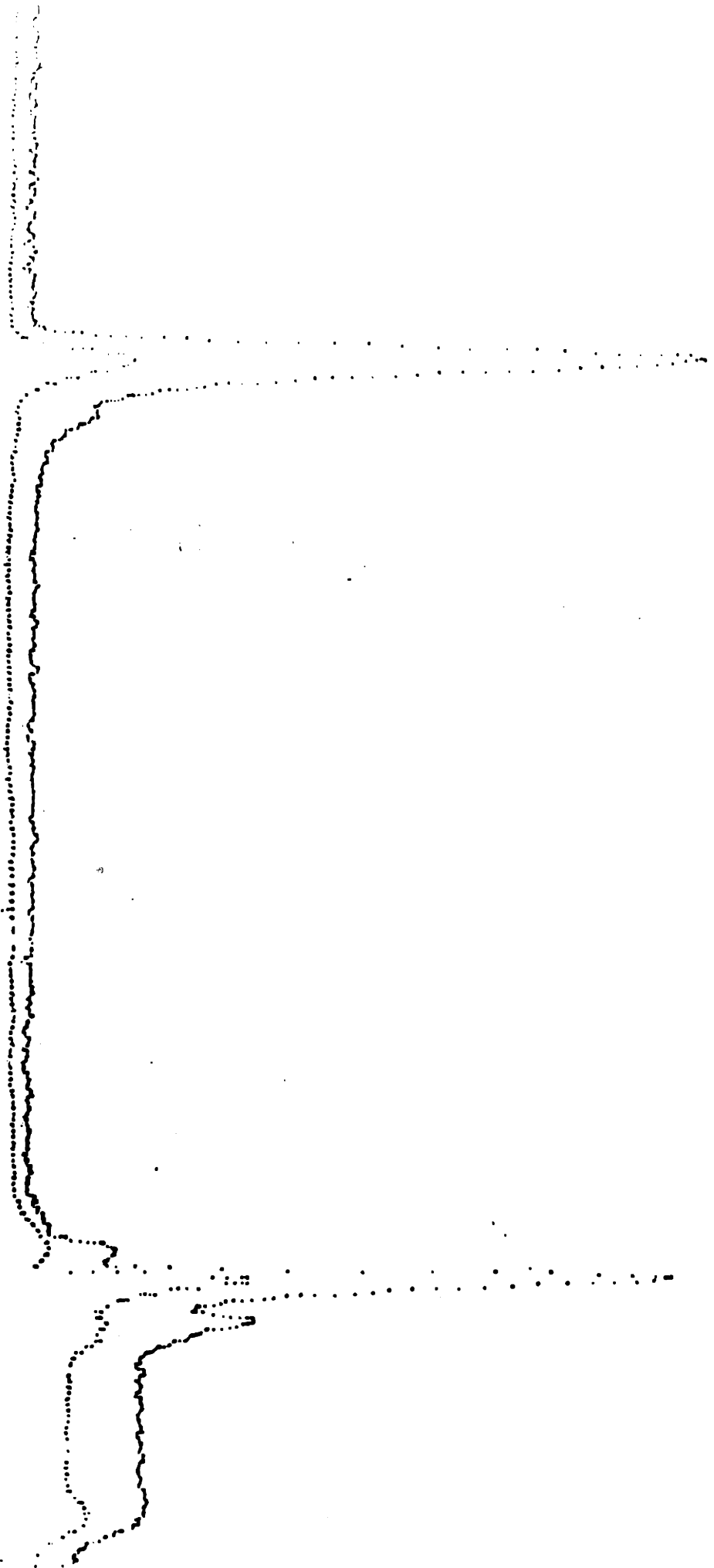


Buffer change

Figure 3. Amino acid analysis: threonine standard

Threonine Standard (44.6 min)

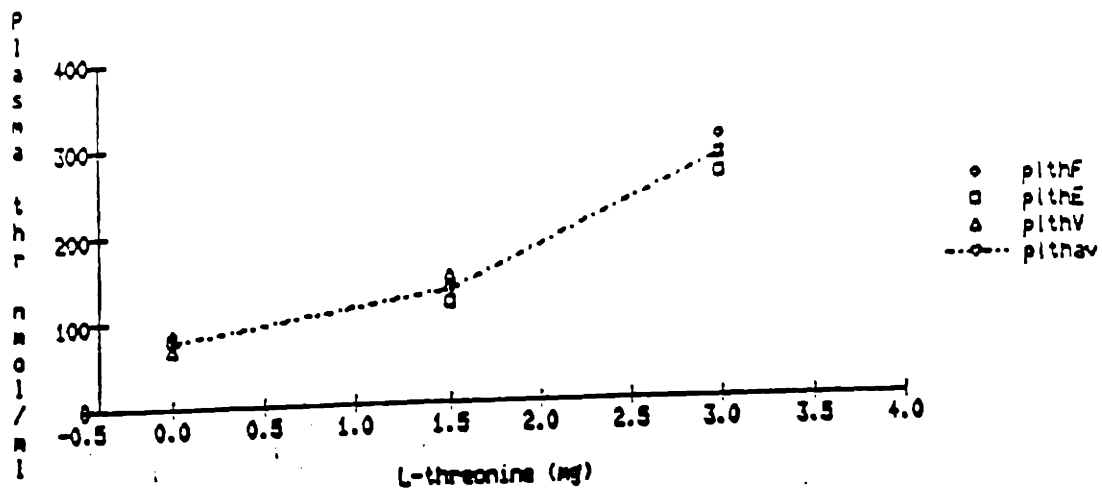
Buffer change



FIGURES 4 AND 5

*SHOW THE EFFECT OF L-THREONINE
ON PLASMA
THREONINE
AND
GLYCINE LEVELS
IN THE OPEN LABEL
STUDY.*

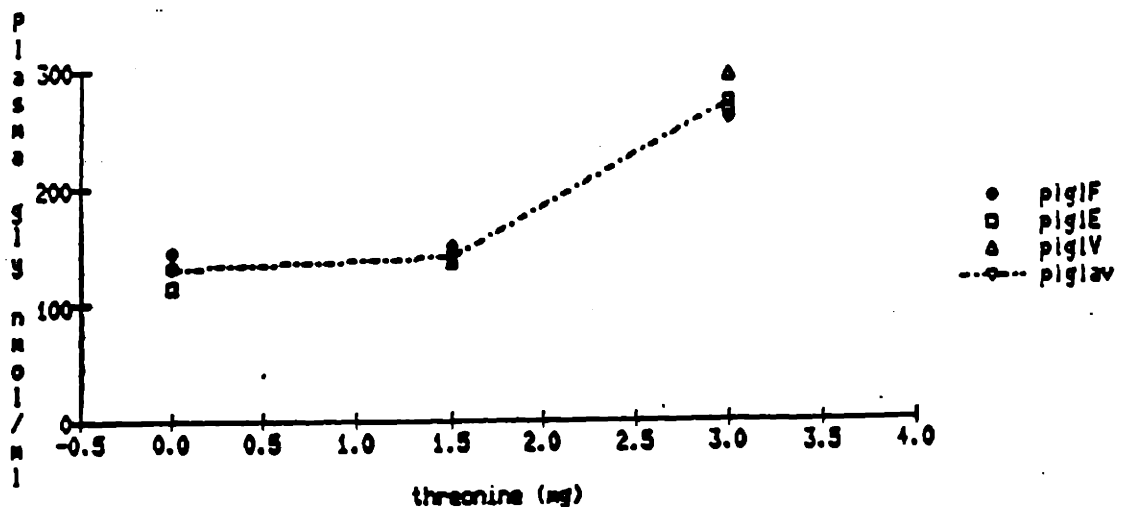
Figure 4. Plasma Threonine Levels after Oral Doses of L-threonine



There was a significant increase in plasma threonine levels (from 78.20 ± 5.75 to 280.50 ± 13.05 nmol/ml; $p < 0.05$) during oral ingestion of 3 g/day of L-threonine. The slight increase during 1.5 g/day of L-threonine was not statistically significant.

Symbols represent data from individual patients; the dotted line connects the mean values.

Figure 5. Plasma Glycine Levels after Oral Doses of L-threonine

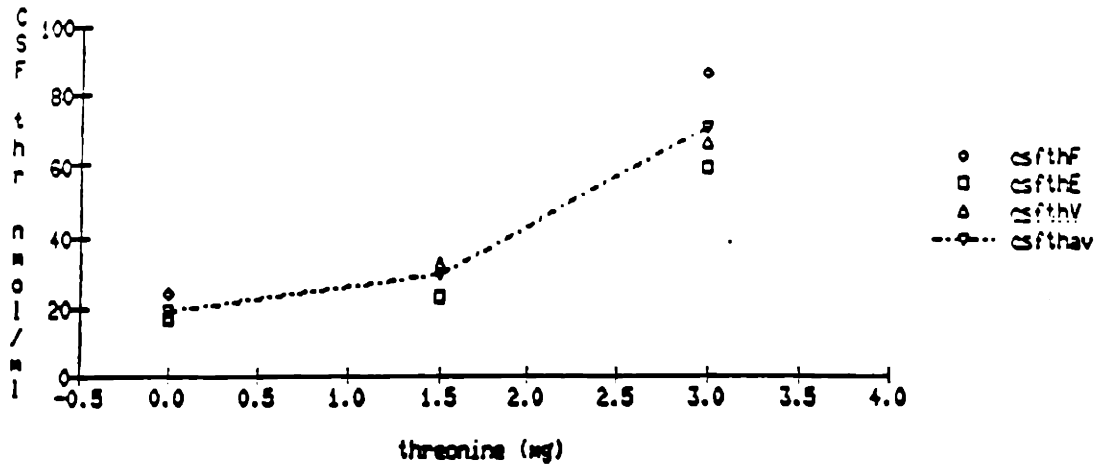


There was a significant increase in plasma glycine levels from 129.80 ± 8.47 to 270.80 ± 10.70 nmol/ml; $p < 0.05$) during oral ingestion of 3 g/day of L-threonine. The slight increase during 1.5 g/day of L-threonine was not statistically significant.

Symbols represent data from individual patients; the dotted line connects the mean values.

FIGURES 6 AND 7
SHOW THE EFFECT OF L-THREONINE
ON CSF
THREONINE
AND
GLYCINE LEVELS
IN THE OPEN LABEL
STUDY.

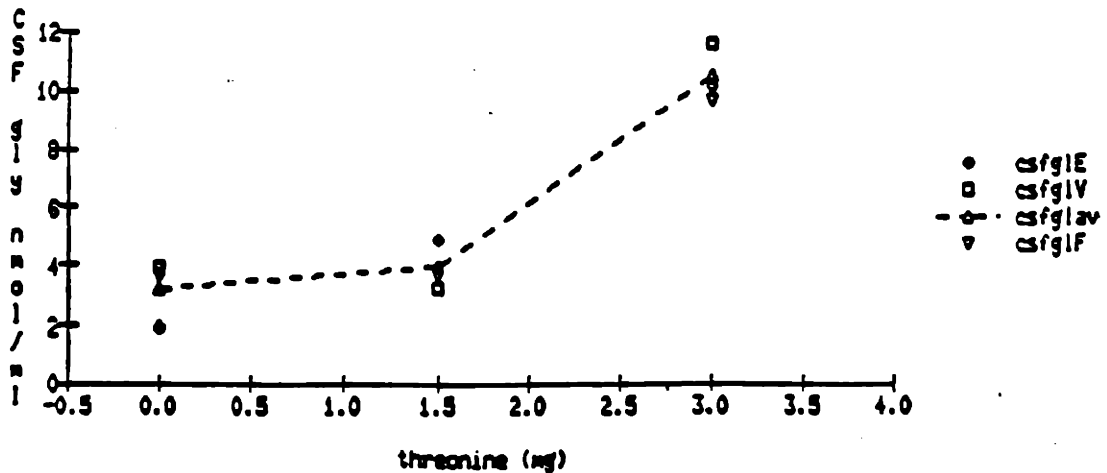
Figure 6. CSF Threonine after Oral Doses of L-threonine



There was a significant increase in CSF threonine levels (from 19.27 ± 2.30 to 70.22 ± 8.15 nmol/ml; $p < 0.05$) during oral ingestion of 3 g/day of L-threonine. The slight increase during 1.5 g/day of L-threonine was not statistically significant.

Symbols represent data from individual patients; the dotted line connects the mean values.

Figure 7. CSF Glycine Levels after Oral Doses of L-threonine



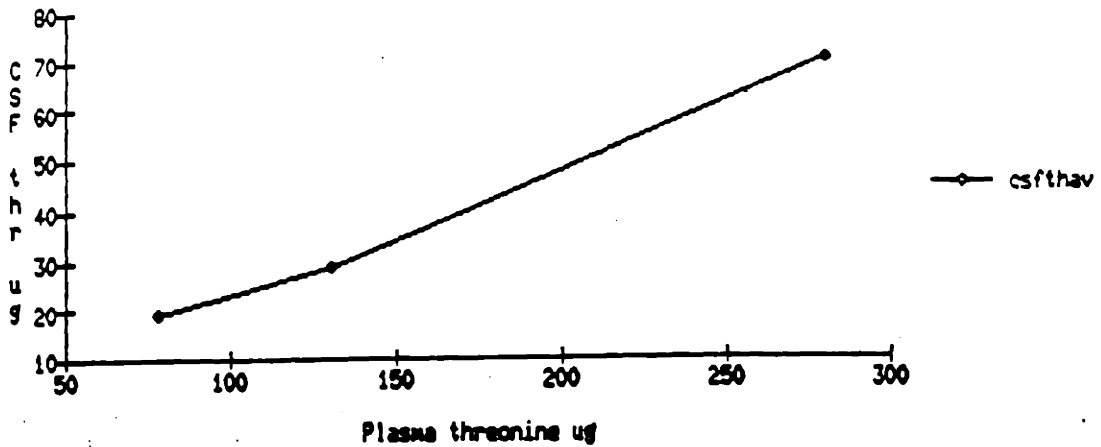
There was a significant increase in CSF glycine levels (from 3.17 ± 0.61 to 10.41 ± 0.58 nmol/ml; $p < 0.05$) during oral ingestion of 3 g/day of L-threonine. The slight increase during 1.5 g/day of L-threonine was not statistically significant.

Symbols represent data from individual patients; the dotted line connects the mean values.

FIGURES 8 AND 9

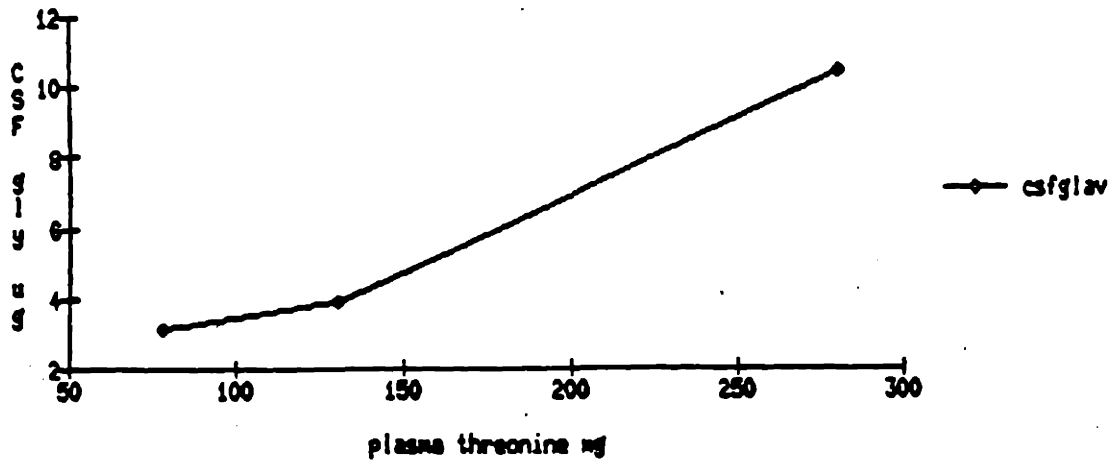
SHOW THE RELATONSHIP BETWEEN
PLASMA THREONINE
AND
CSF THREONINE
AND
CSF GLYCINE
LEVELS.

Figure 8. The Relationship between Threonine Levels in Plasma and CSF



Increases in plasma threonine levels cause significant increases in CSF threonine levels. Each data point represents the mean value of three subjects.

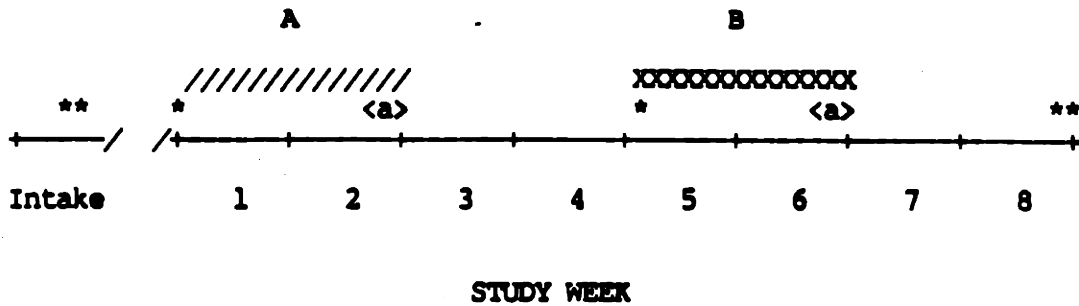
Figure 9. The Relationship between Plasma Threonine Levels and CSF Glycine Levels



Increases in plasma threonine levels greater than 150 nmol/ml produce significant increases in glycine levels in the CSF. Each data point represents the mean value for three subjects.

FIGURE 10
IS A
DIAGRAM
OF THE
DOUBLE BLIND PROTOCOL.

Figure 10. Diagram of Double-Blind Protocol for Testing Safety and Efficacy of L-threonine in Patients with Familial Spastic Paraparesis

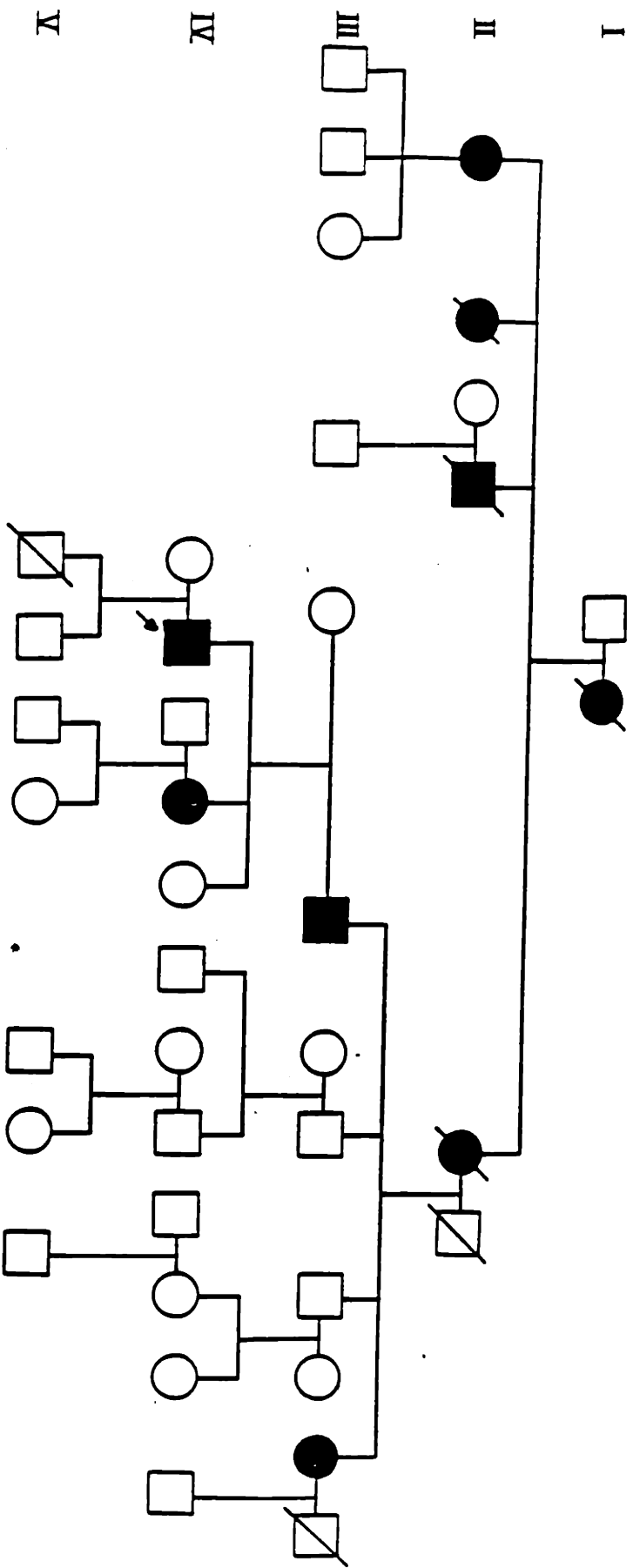


- A - L-threonine 1.5 g tid
- B - Placebo 1.5 g tid
- ** - Complete history, physical examination and laboratory tests
- * - Outpatient neurological examination
- <a> - 2 day inpatient admission for neurological assessment, global impressions, and laboratory tests, including plasma and CSF collection for amino acids

The order of drug administration (A and B periods) counterbalanced in blocks of 10 across subjects.

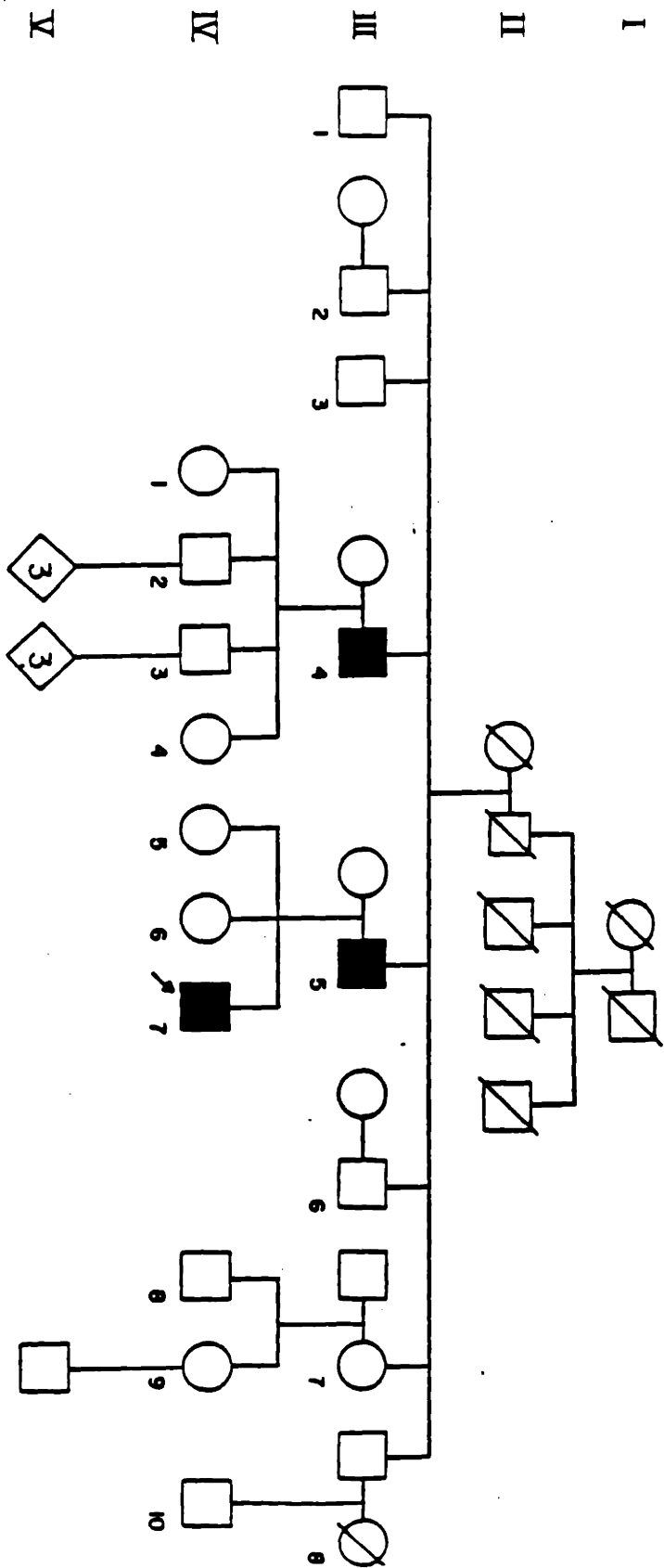
*FIGURES 11, 12, 13, AND 14,
SHOW
PEDIGREES OF DIFFERENT FAMILIES
WITH
FAMILIAL SPASTIC PARAPARESIS.*

Figure 12. Pedigree of a Family with Familial Spastic Paraparesis



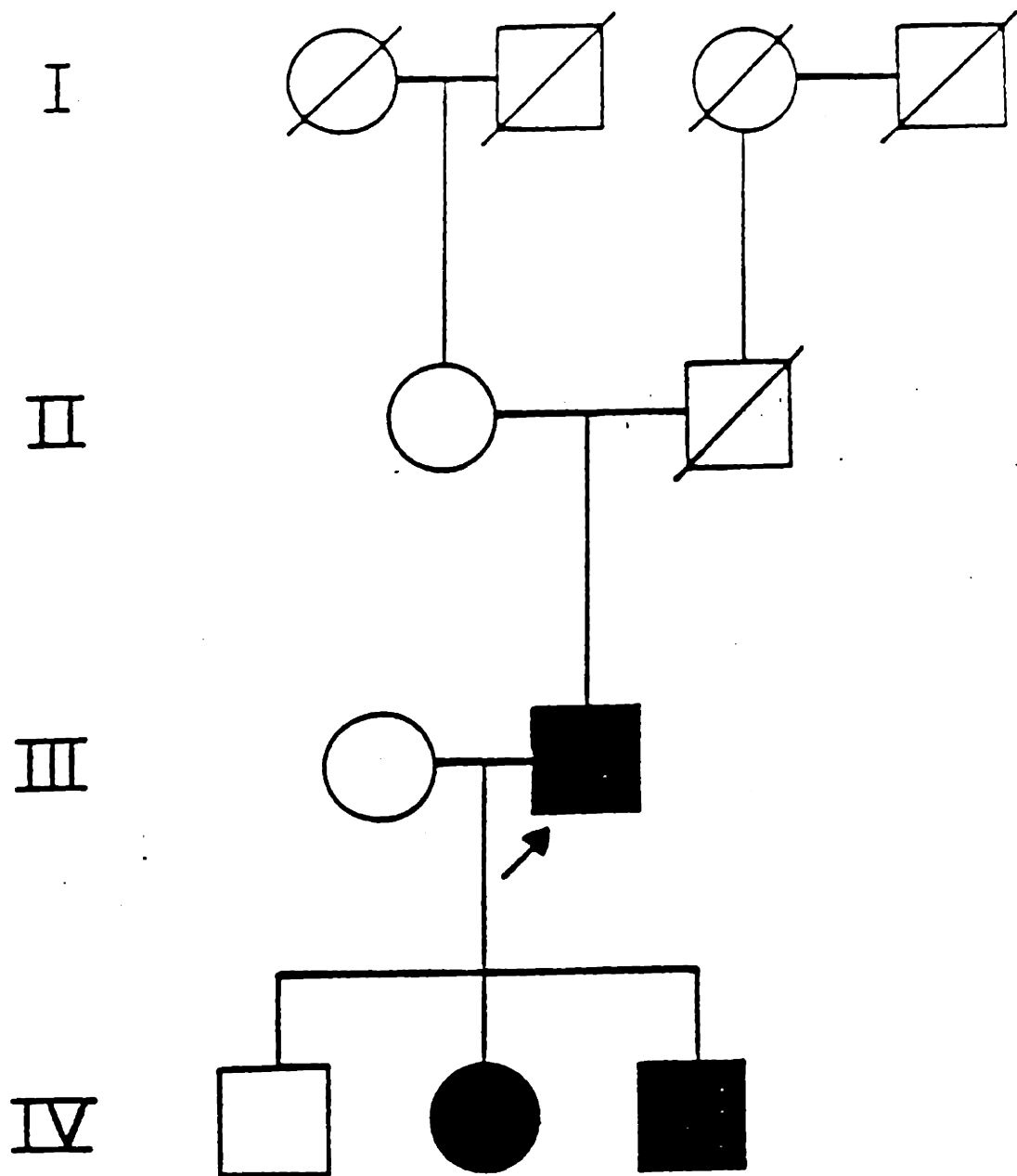
FAMILY 2

Figure 3. Pedigree of a Family with Familial Spastic Paraparesis



FAMILY 3

Figure 14. Pedigree of a Family with Familial Spastic Paraparesis



FAMILY 4

FIGURE 15

*SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
THREONINE (400 MG/KG),
VALINE (400 MG/KG),
THREONINE PLUS VALINE (400 MG/KG EACH),
SERINE (400 MG/KG) AND
THREONINE PLUS SERINE (400 MG/KG EACH)
ON PLASMA CONCENTRATIONS OF THREONINE.*

Figure 15

Effect of amino acids on plasma [THR]

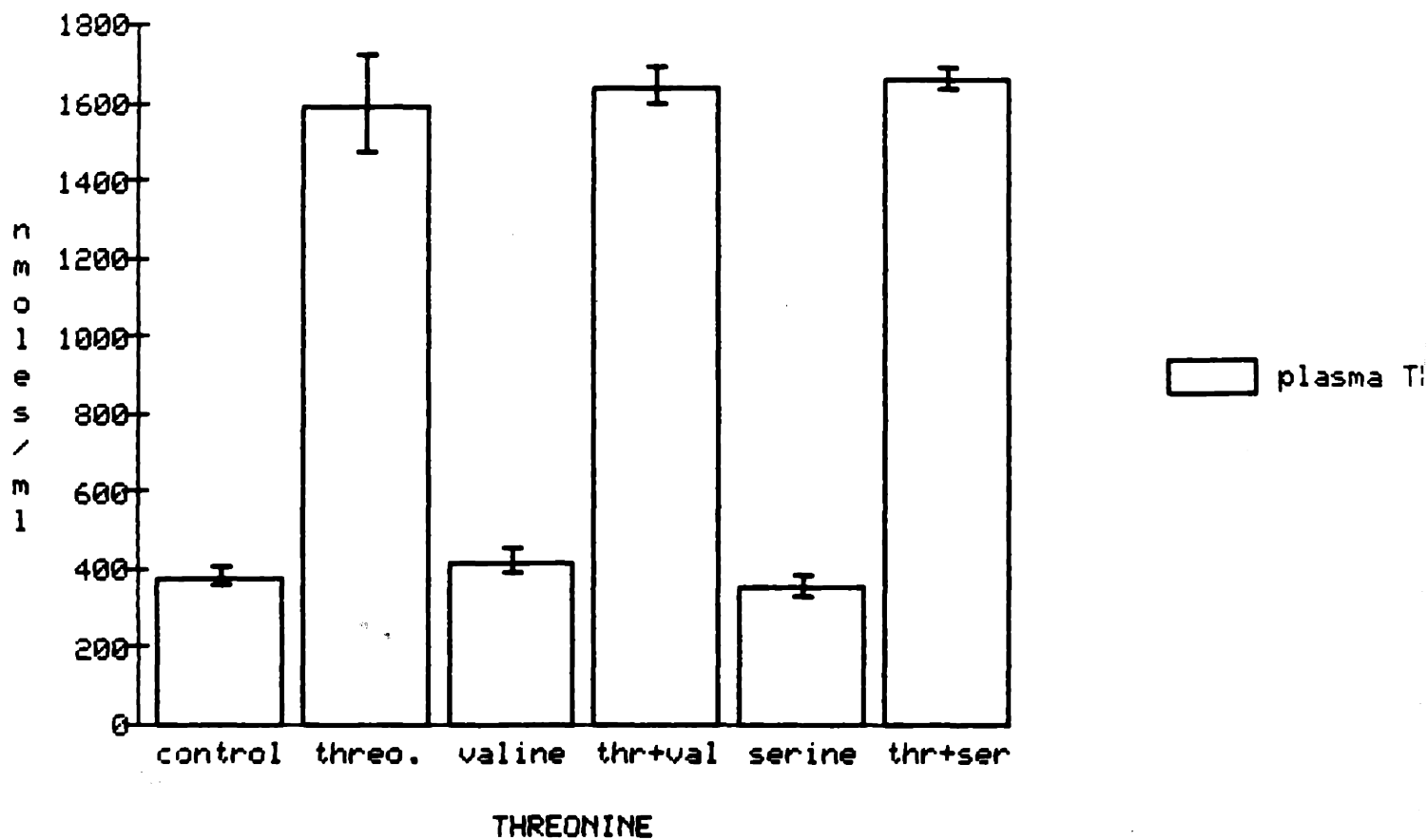


FIGURE 16

*SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
THREONINE (400 MG/KG),
VALINE (400 MG/KG),
THREONINE PLUS VALINE (400 MG/KG EACH),
SERINE (400 MG/KG) AND
THREONINE PLUS SERINE (400 MG/KG EACH)
ON PLASMA CONCENTRATIONS OF VALINE.*

Figure 16

Effect of Amino Acids on plasma [UAL]

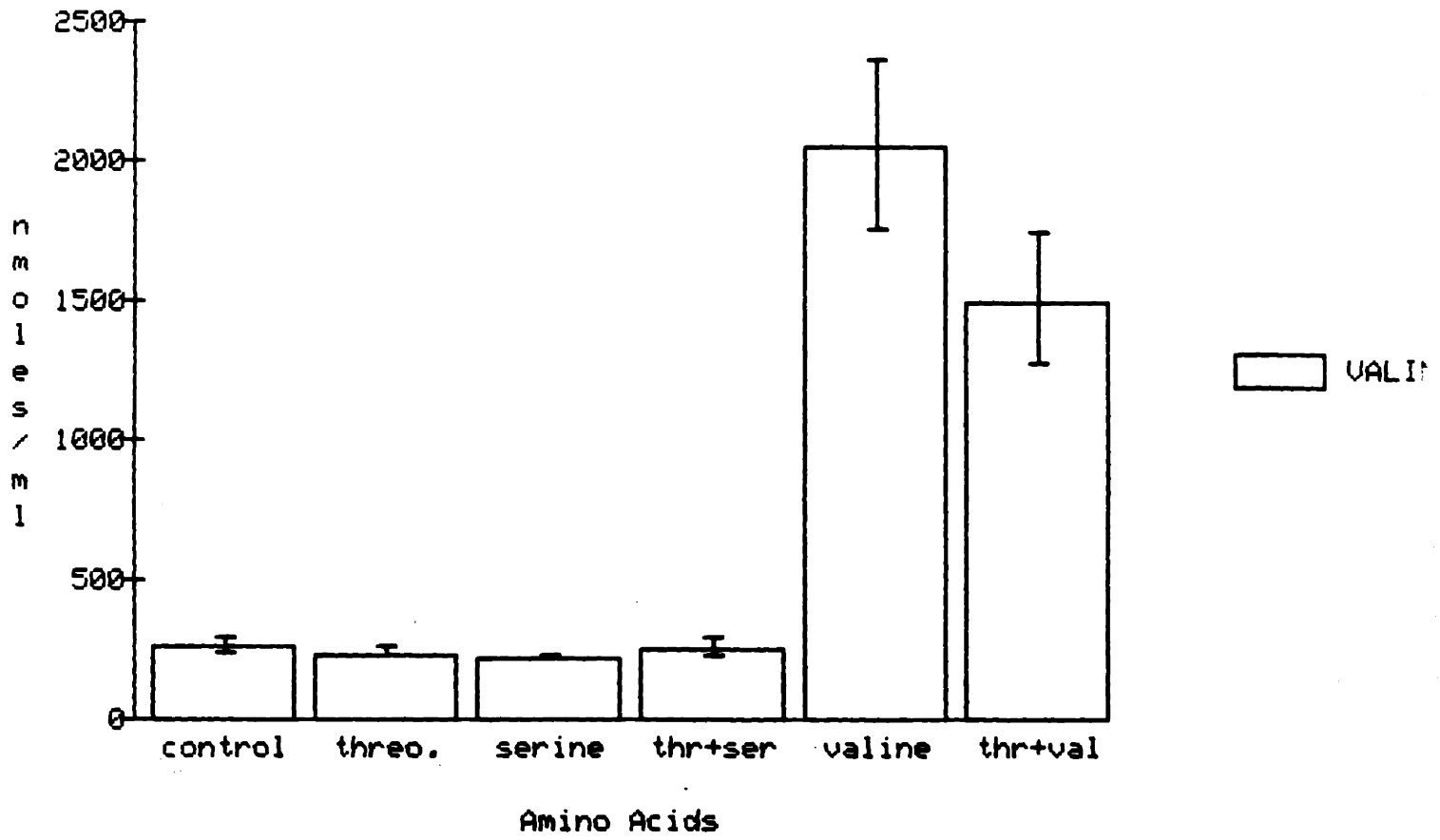


FIGURE 17

*SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
THREONINE (400 MG/KG),
VALINE (400 MG/KG),
THREONINE PLUS VALINE (400 MG/KG EACH),
SERINE (400 MG/KG) AND
THREONINE PLUS SERINE (400 MG/KG EACH)
ON PLASMA CONCENTRATIONS OF SERINE.*

Figure 17
Effect of amino acids on plasma [ser]

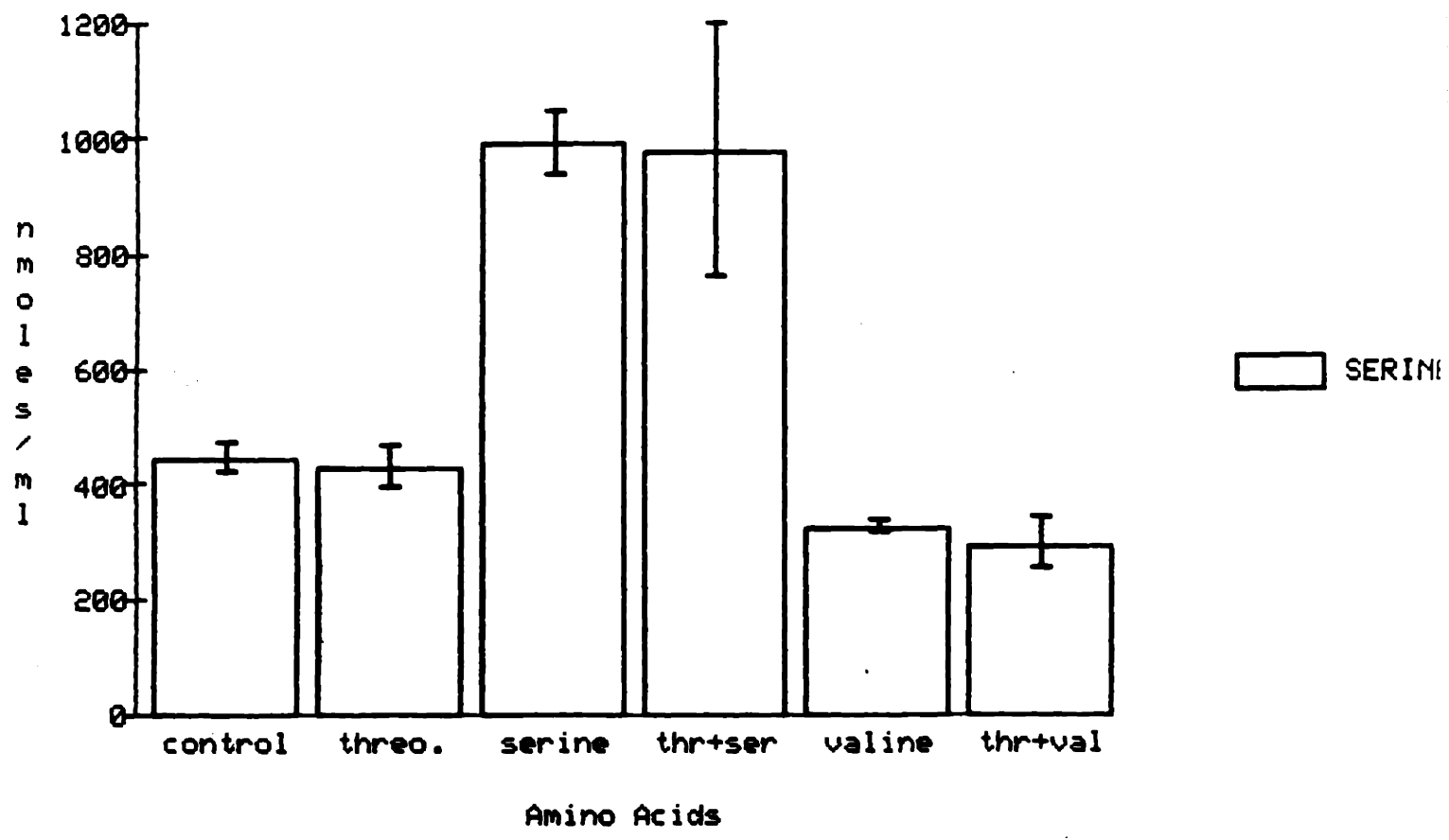


FIGURE 18

*SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
THREONINE (400 MG/KG),
VALINE (400 MG/KG),
THREONINE PLUS VALINE (400 MG/KG EACH),
SERINE (400 MG/KG) AND
THREONINE PLUS SERINE (400 MG/KG EACH)
ON PLASMA CONCENTRATIONS OF GLYCINE.*

Figure 18

Effect of amino acids on plasma [GLY]

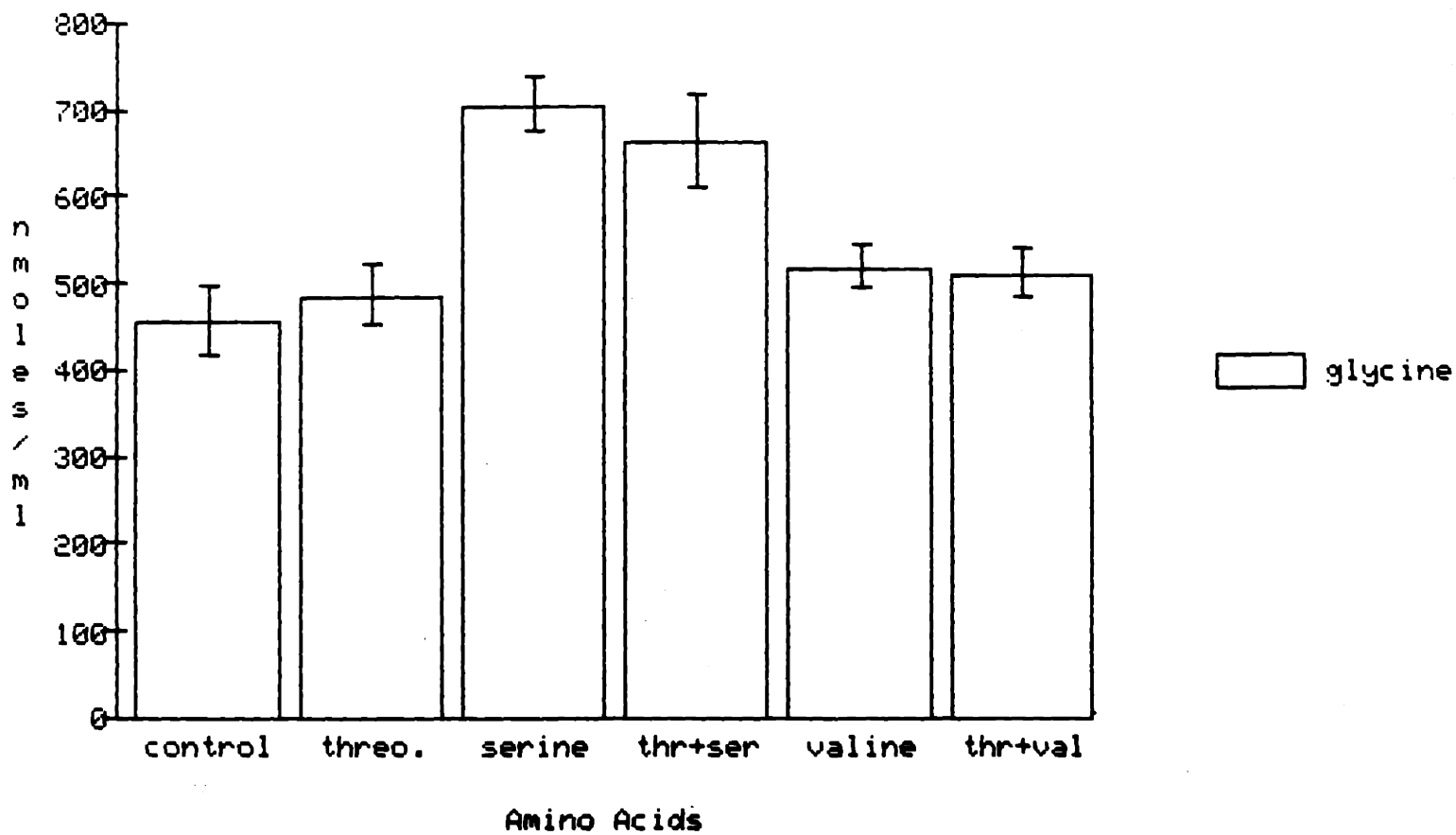
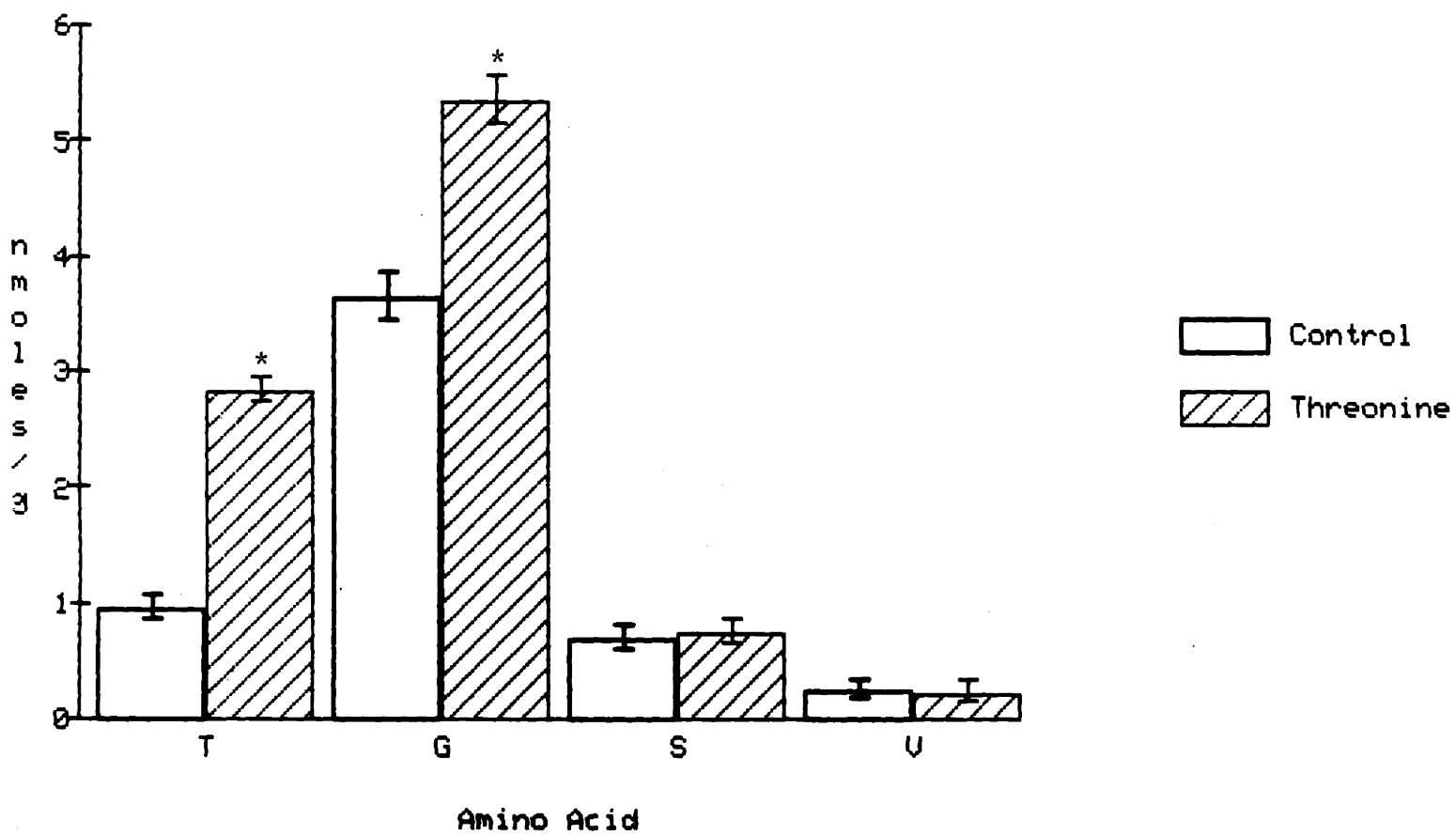


FIGURE 19
SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
THREONINE (400 MG/KG)
ON SPINAL CORD CONCENTRATION OF
THREONINE (T),
GLYCINE (G),
SERINE (S), AND
VALINE (V).

Figure 19
Threonine (400 mg/kg)

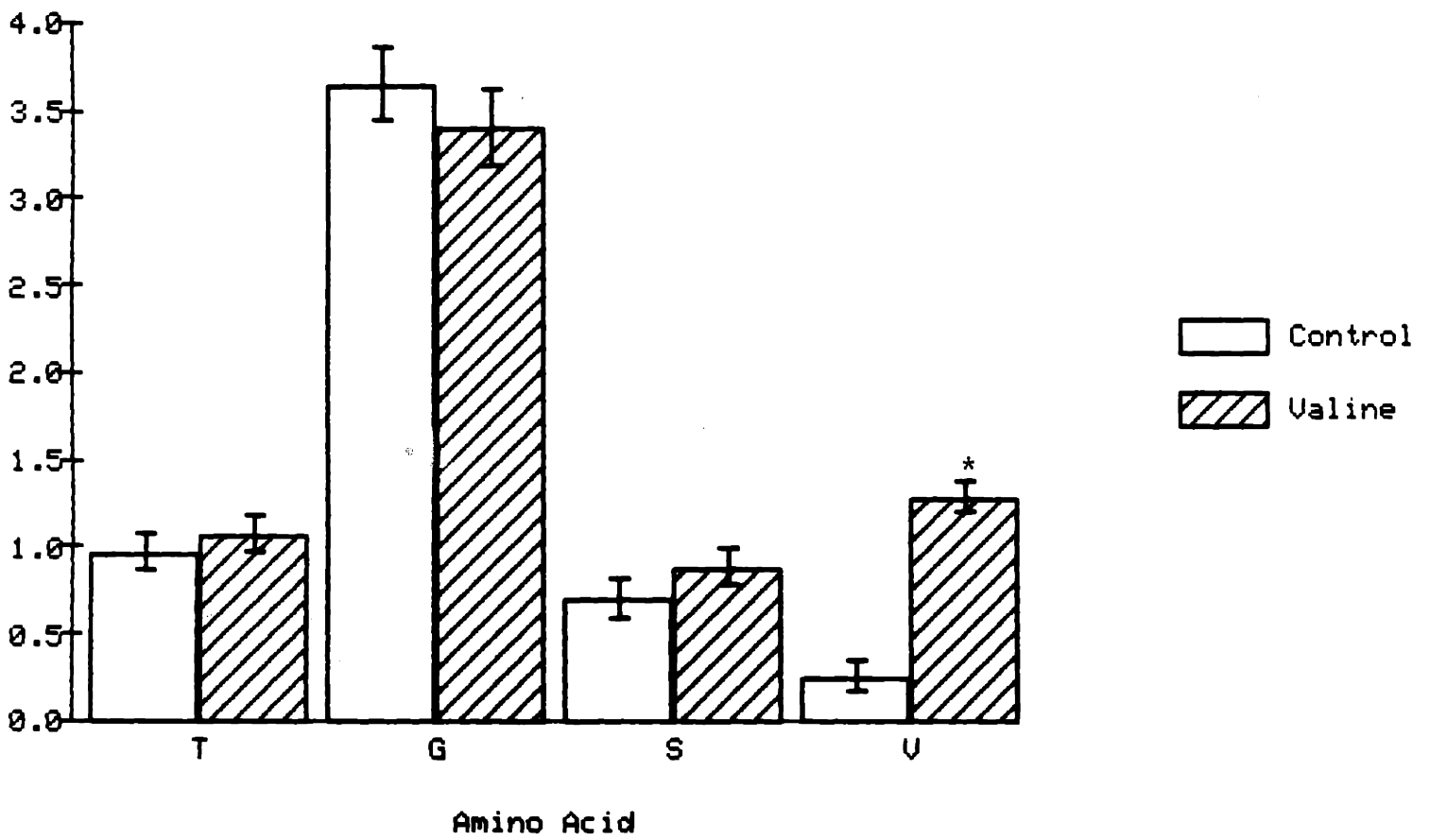


* $p < .05$

FIGURE 20
SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
VALINE (400 MG/KG)
ON SPINAL CORD CONCENTRATION OF
THREONINE (T),
GLYCINE (G),
SERINE (S), AND
VALINE (V).

Figure 20

Valine (400 mg/kg)



* p < .05

FIGURE 21

*SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
THREONINE PLUS VALINE (400 MG/KG EACH)
ON SPINAL CORD CONCENTRATION OF
THREONINE (T),
GLYCINE (G),
SERINE (S), AND
VALINE (V).*

Figure 21

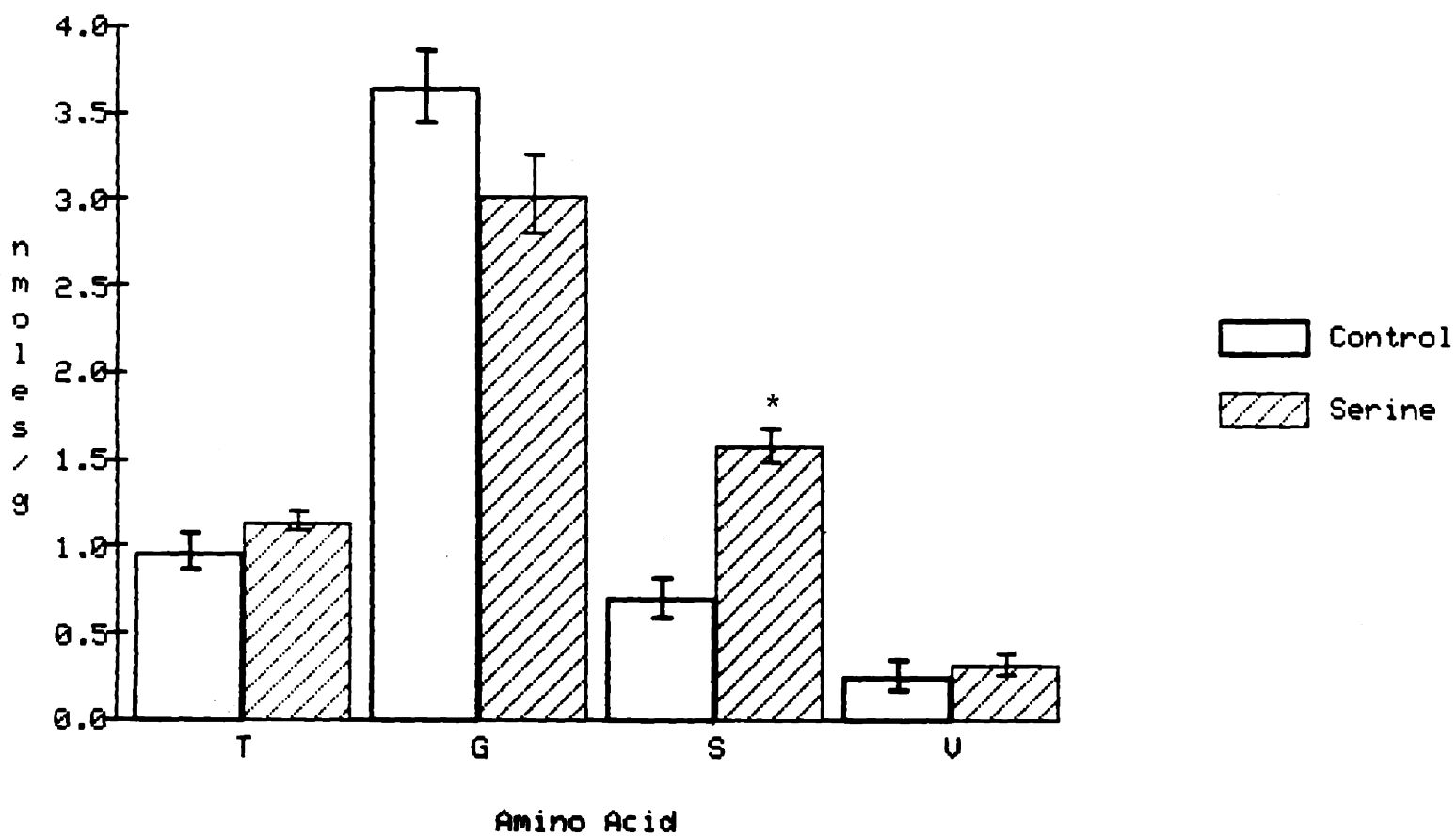
Threonine (400 mg/kg) + Valine (400 mg/kg)



*p < .05

FIGURE 22
SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
SERINE (400 MG/KG)
ON SPINAL CORD CONCENTRATION OF
THREONINE (T),
GLYCINE (G),
SERINE (S), AND
VALINE (V).

Figure 22
Serine (400 mg/kg)



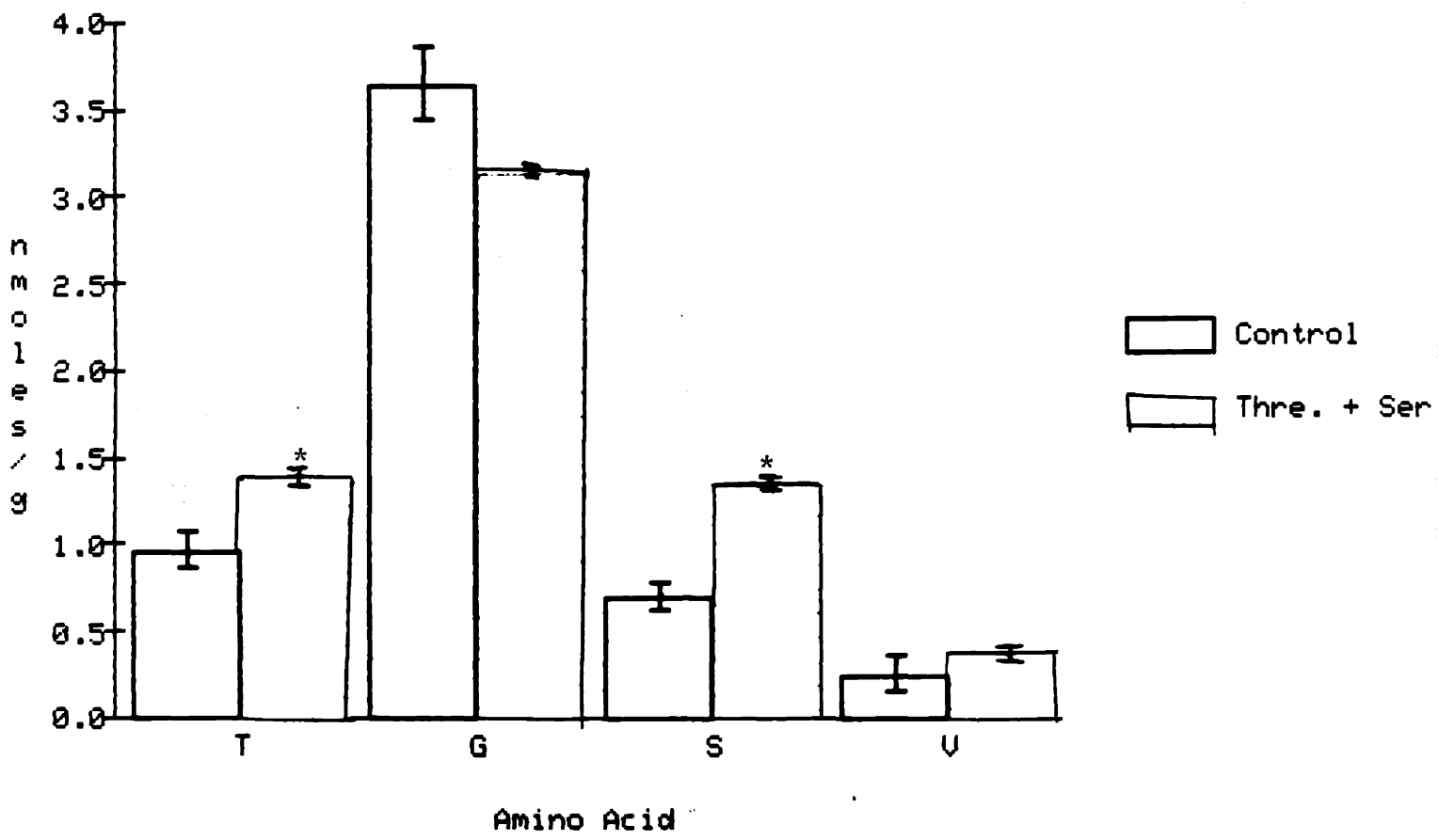
* $P < .05$

FIGURE 23

*SHOWS THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF
THREONINE PLUS SERINE (400 MG/KG EACH)
ON SPINAL CORD CONCENTRATION OF
THREONINE (T),
GLYCINE (G),
SERINE (S), AND
VALINE (V).*

Figure 23

Threonine (400 mg/kg) + Serine (400 mg/kg)



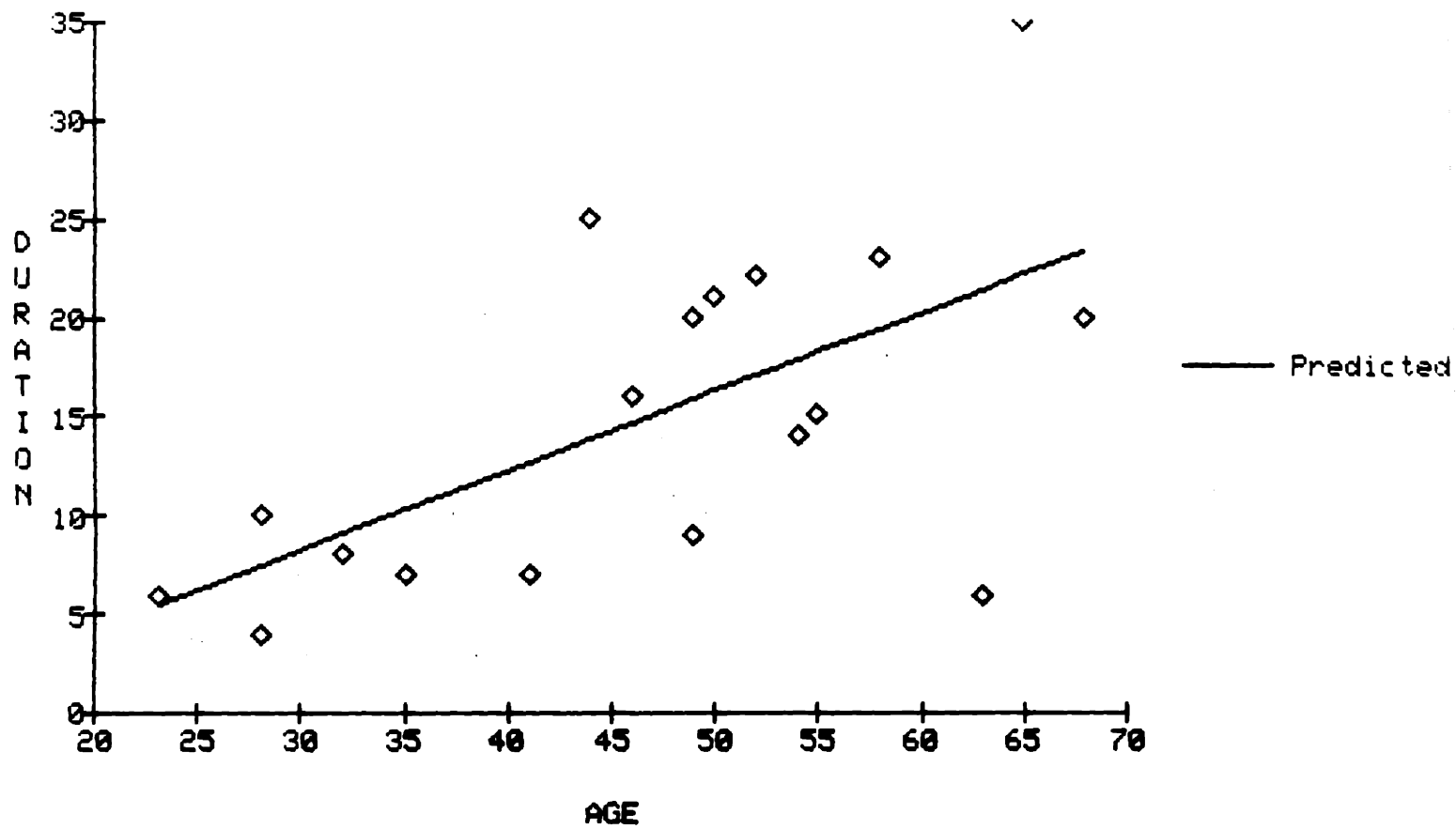
* P < .05

FIGURE 24

SHOWS
THE RELATION BETWEEN
AGE AND
DURATION OF ILLNESS
IN THE
DOUBLE BLIND
STUDY
POPULATION.

Figure 24

Relation between Age and Duration of illness



$p < .005$

FIGURE 25

SHOWS
THE RELATION BETWEEN
SEVERITY AND
DURATION OF ILLNESS
IN THE
DOUBLE BLIND
STUDY
POPULATION.

Figure 25

SEVERITY VS DURATION OF ILLNESS

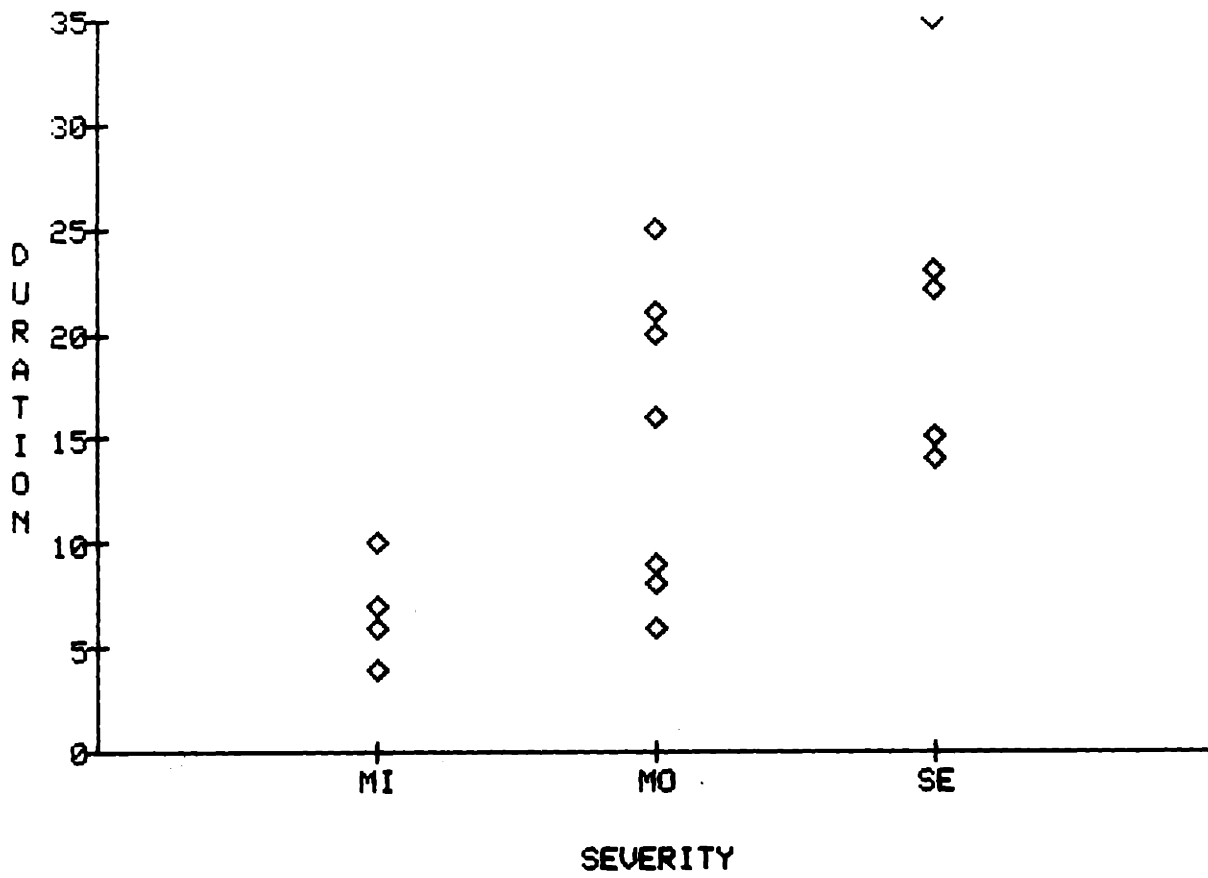


FIGURE 26
SHOWS THE COMPOSITE SCORES (CSP) SORTED BY SEVERITY.
MI=MILD,
MO=MODERATE,
SE=SEVERE.

Figure 26

Composite Scores sorted by Severity

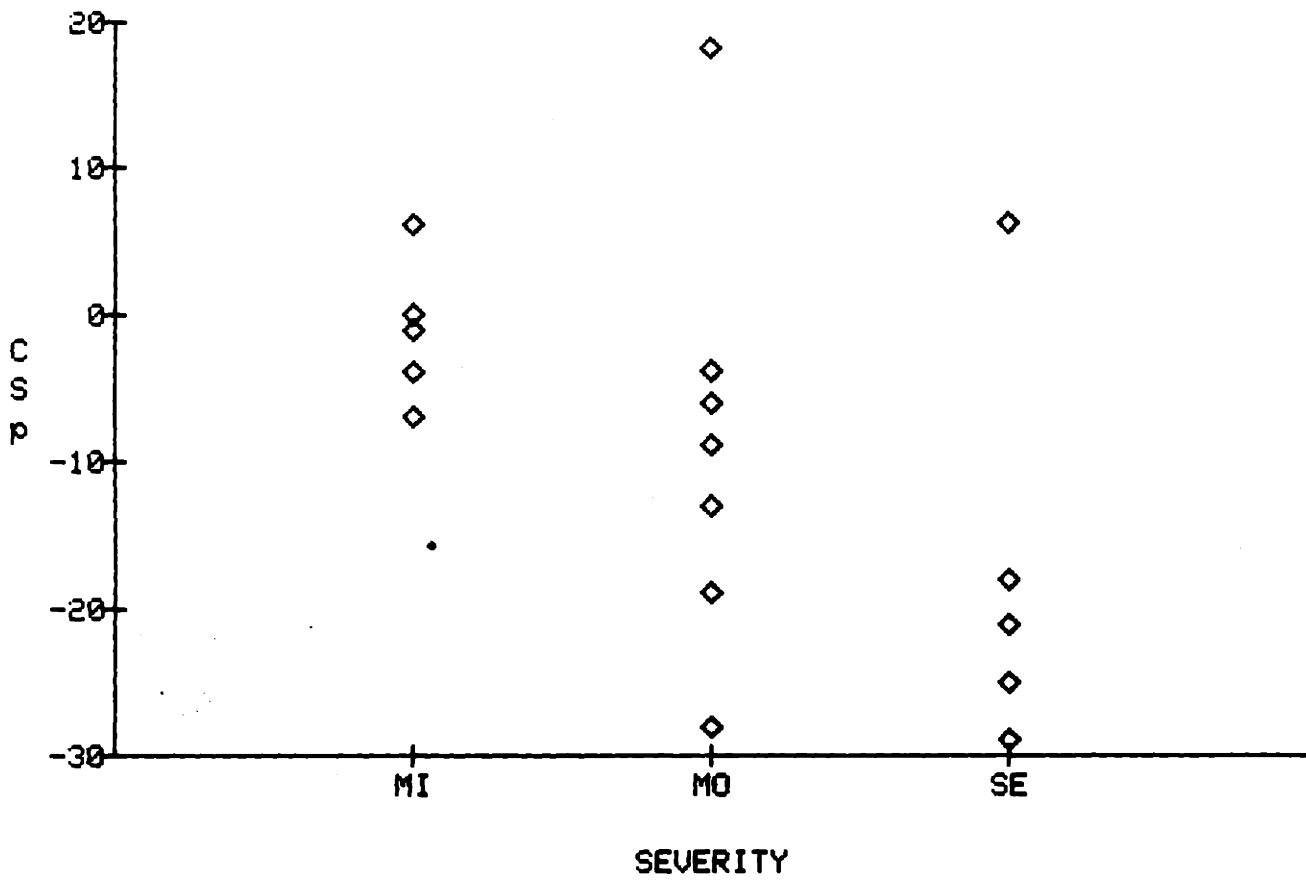
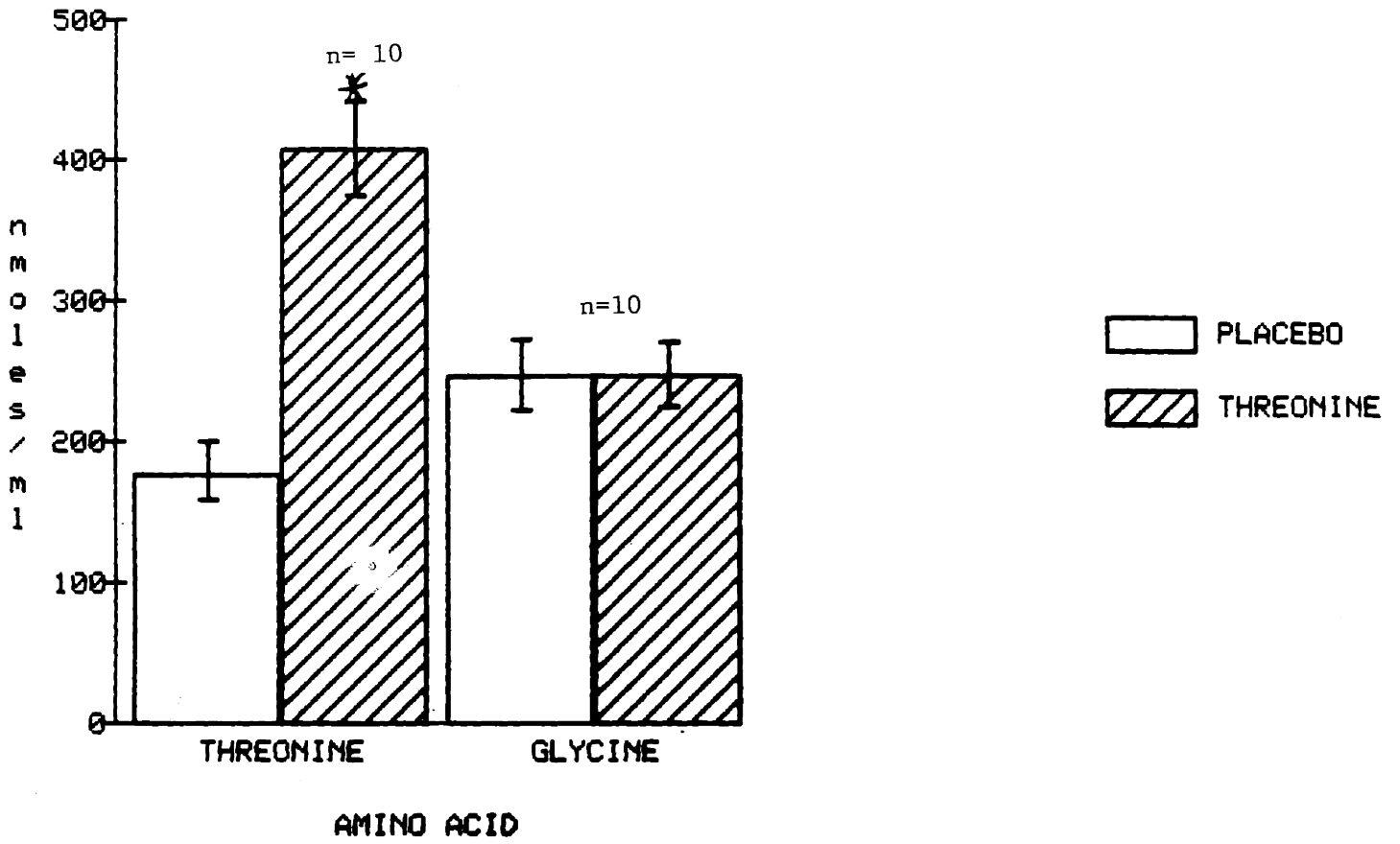


FIGURE 27

*SHOWS THE EFFECT OF
ORAL L-THREONINE ADMINISTRATION ON
PLASMA THREONINE (THR) AND
GLYCINE (GLY) CONCENTRATION.*

Figure 27

EFFECT OF THREONINE ON PLASMA [THR] AND [GLY]



$p < .05$

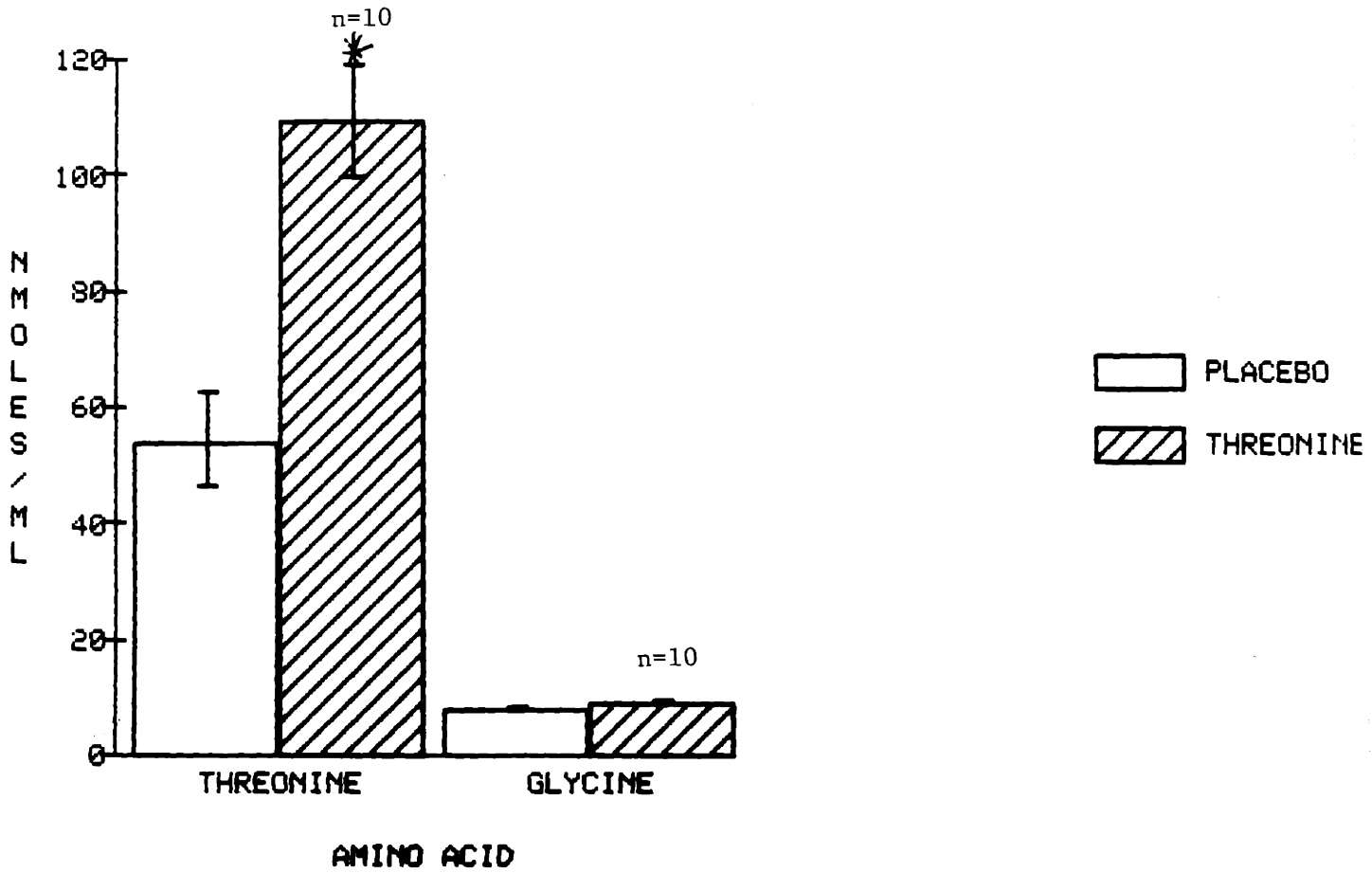
N = 10

FIGURE 28

*SHOWS THE EFFECT OF
ORAL L-THREONINE ADMINISTRATION ON
CSF THREONINE (THR) AND
GLYCINE (GLY) CONCENTRATION.*

Figure 28

THREONINE EFFECT ON CSF [THR] AND [GLY]



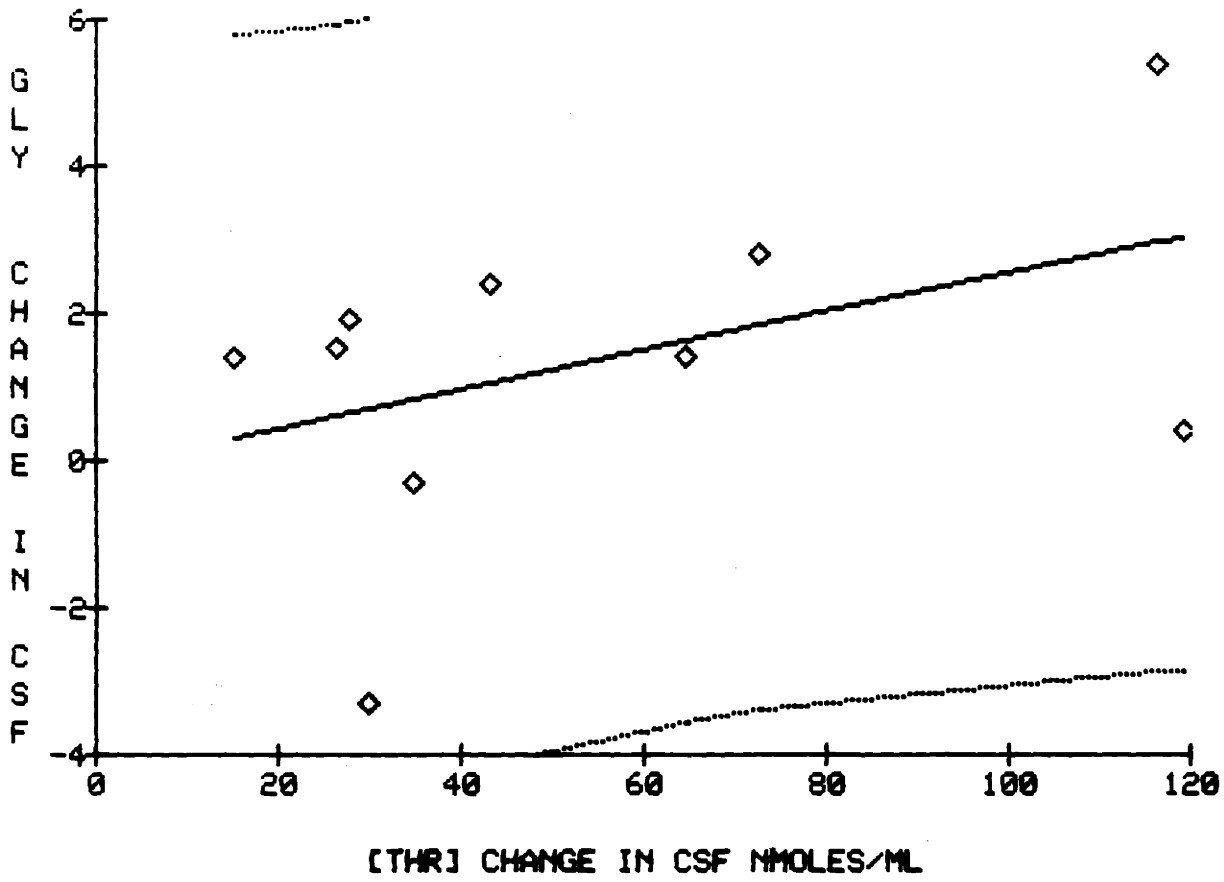
* p < .05

FIGURE 29

*SHOWS THAT THERE IS
NO SIGNIFICANT OVERALL CORRELATION
BETWEEN CSF GLYCINE AND CSF THREONINE LEVELS.*

Figure 29

CHANGE IN [GLY] VS [THR] IN CSF



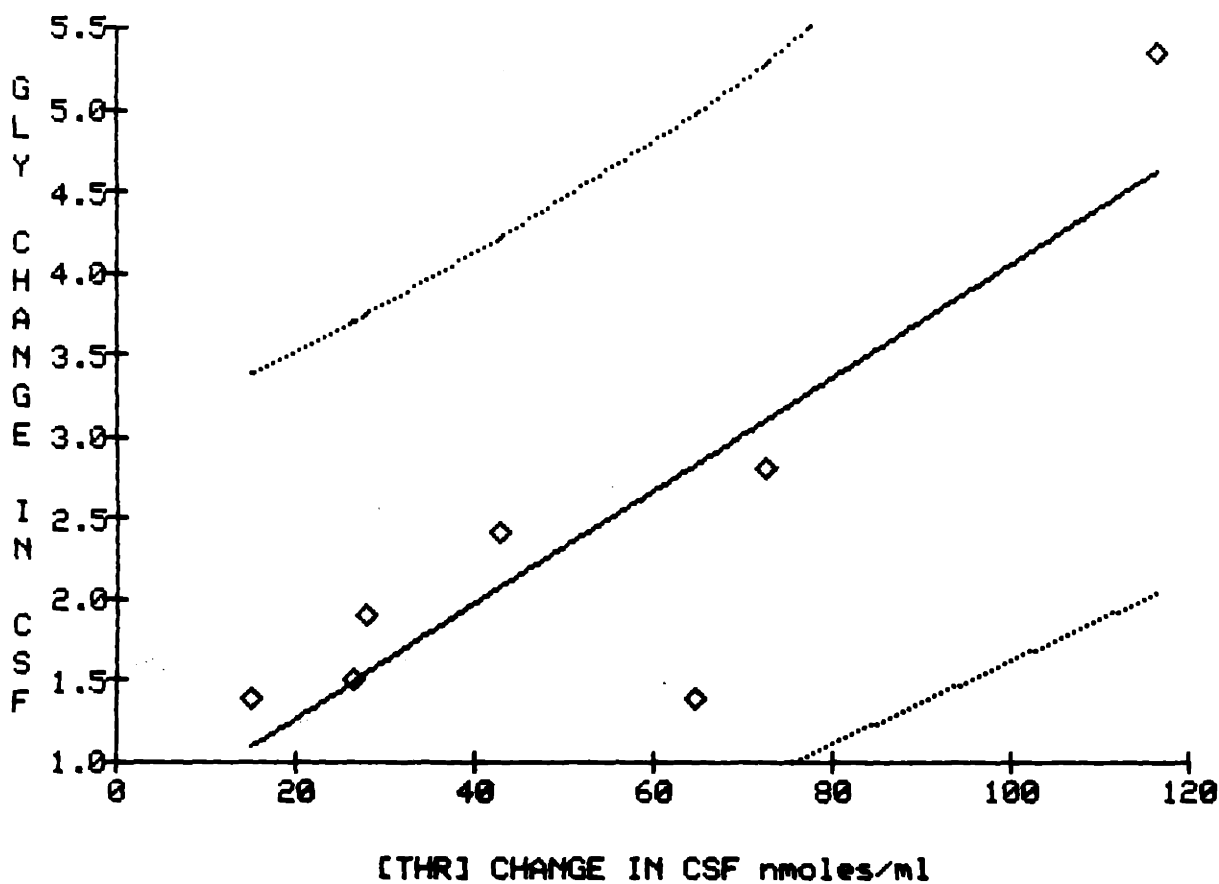
NS n=10

FIGURE 30

*SHOWS THAT THERE IS
A SIGNIFICANT CORRELATION ($P < .05$) BETWEEN
CSF GLYCINE AND CSF THREONINE LEVELS IN RESPONDERS.*

Figure 30

CHANGE IN [GLY] VS [THR] IN CSF IN RESPONDERS ONLY



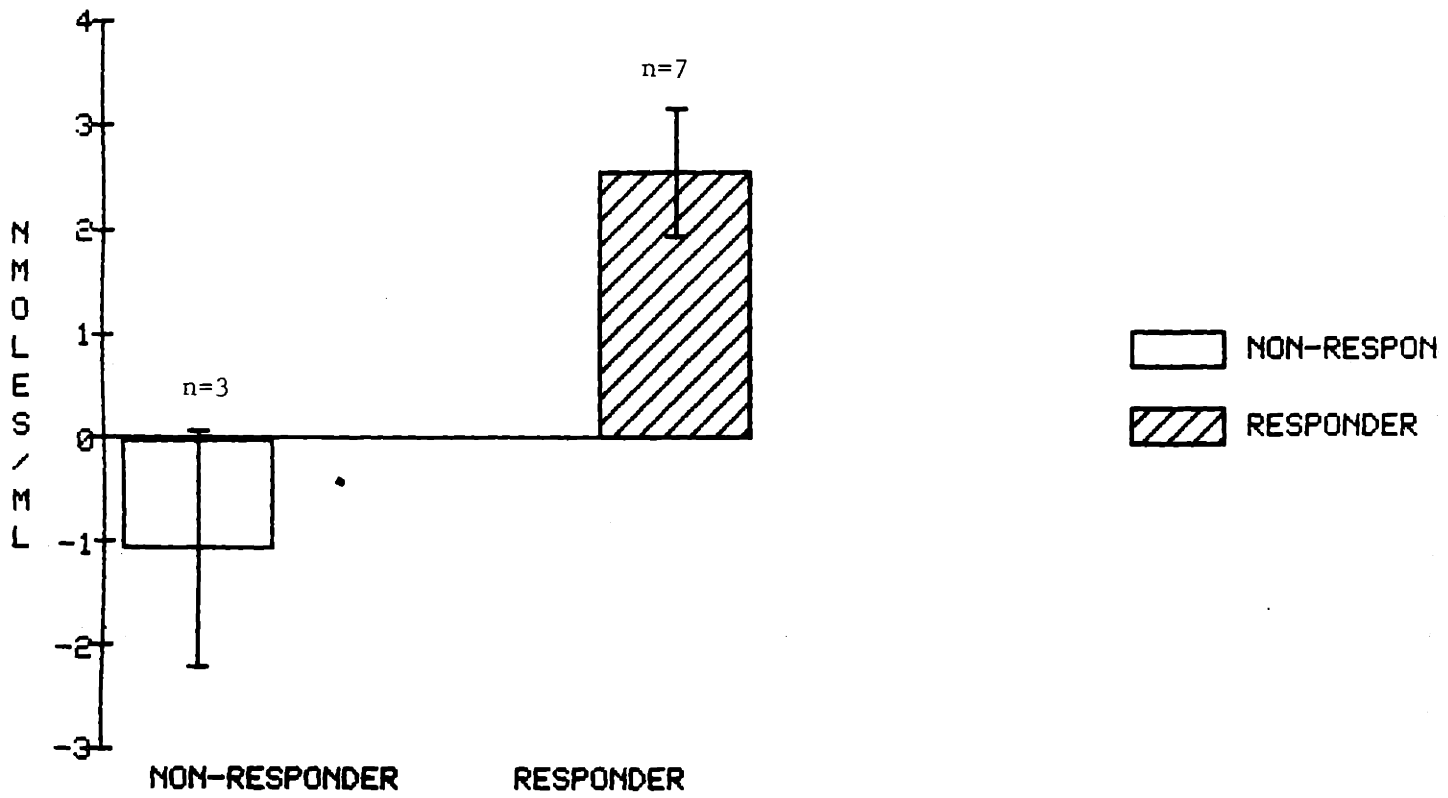
$p < .05$ $n=7$

FIGURE 31

*SHOWS A SIGNIFICANT DIFFERENCE IN
THE INCREASE IN CSF GLYCINE
FOLLOWING L-THREONINE ADMINISTRATION,
BETWEEN RESPONDERS AND NON-RESPONDERS.*

Figure 31

D [GLY] IN CSF; RESPONDERS VS NON RESPONDERS

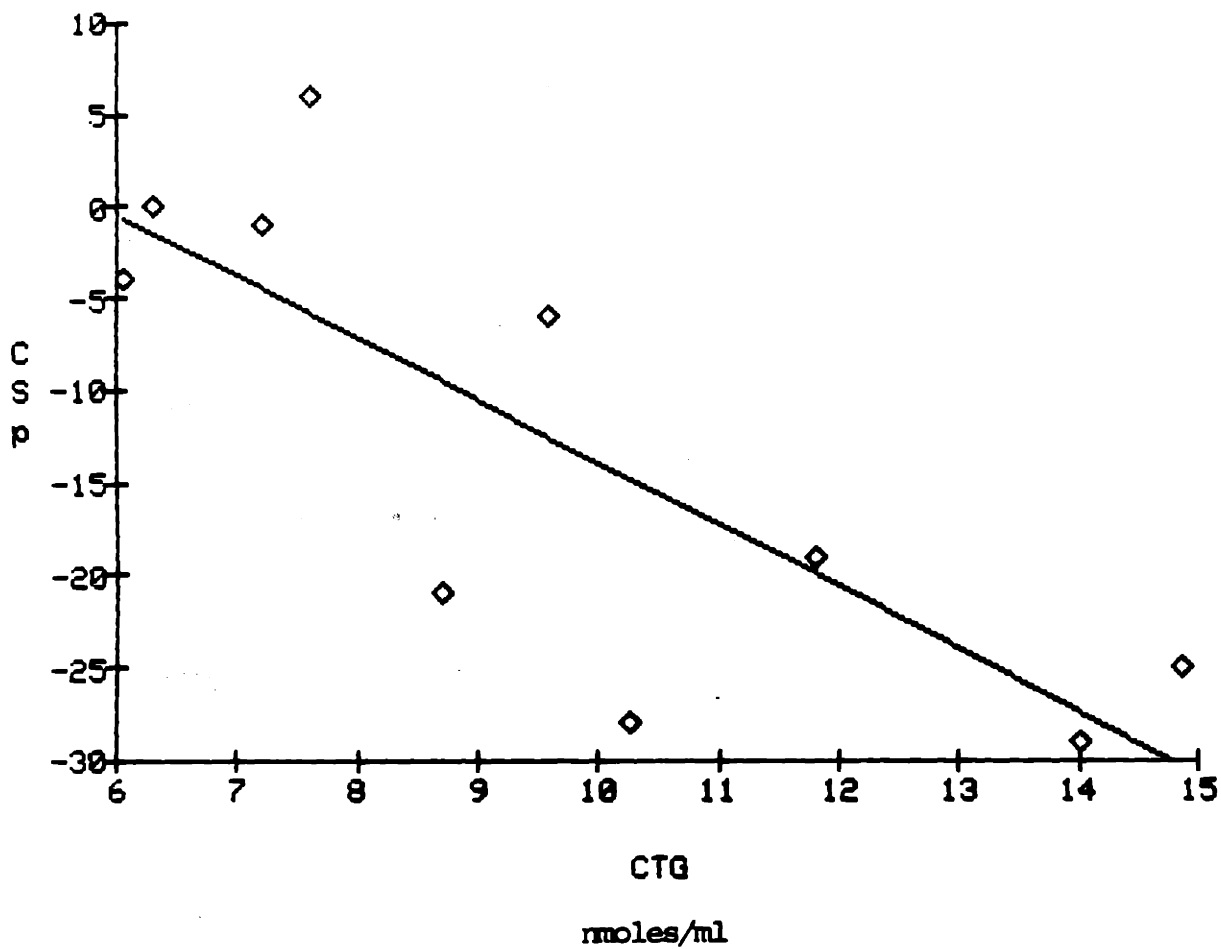


$p < .03$

FIGURE 32

*SHOWS A SIGNIFICANT CORRELATION ($P < .005$)
BETWEEN THE COMPOSITE SCORES AND THE CSF LEVELS OF GLYCINE
INCLUDING BOTH
RESPONDERS AND NON-RESPONDERS.*

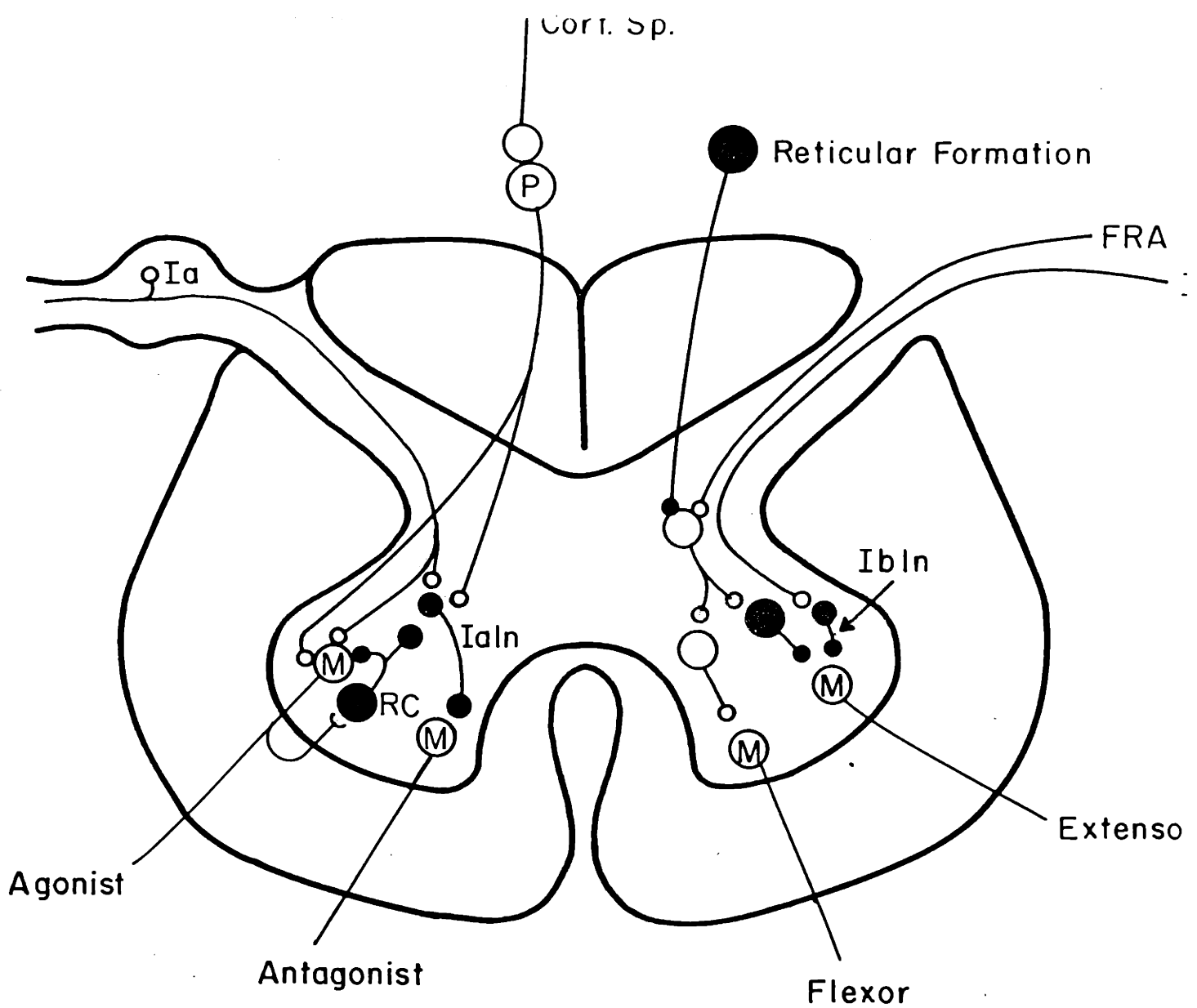
Figure 32
Composite Scores and CSF [GLY]



$p < .005$

$n=10$

FIGURE 33
SHOWS A DIAGRAM
OF THE
REFLEX ARCS
WITH EXAMPLES OF
CORTICOSPINAL AND
RETICULOSPINAL
INPUTS.



FRA = "FLEXOR REFLEX AFFERENTS" (groups II-IV muscle afferents, cutaneous afferents, joint afferents)

Rc = RENSHAW CELL

Ia = MUSCLE SPINDLE AFFERENT

IaIn = Ia INHIBITORY INTERNEURON

Ib = GOLGI TENDON AFFERENTS

M = MOTOR NEURON

P = PROPRIOSPINAL NEURON

ADAPTED FROM - Illert and Tanaka Exp. Brain Res. 31; 131-141, 1978

- Lundberg "Integration in the Nervous System"
Symposium Asanuma Ed., N.Y., 1979

- Burke Adv. Neuro. 47; 401-423 1988

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APPENDIX A

1. Neurological Examination Data Form
2. Laboratory Results Data Forms
3. Physician's Global Evaluation Data Form
4. Patient's Global Assessment Data Form

L-THREONINE - SPASTICITY RESEARCH

Last Name _____ First Name _____
 MGH Unit # _____ Date of Birth _____

STRENGTH (1 = flicker, 2, 3 = hold against gravity, 4, 5 = normal)

	Right Arm	Left Arm
Deltoid		
Bicep		
Tricep		
Extensors		
Flexors		

	Right Leg	Left Leg
Iliopsoas		
Quadriceps		
Hamstrings		
Anterior Tibial		
Gastrocnemius		
Extensor Hall.		

TONE: (0 = normal; 1 = only with remote activation; 2 = definite increase; 3 = moderate increase; 4 = stiff as a board)

Right Arm	Left Arm	Right Leg	Left Leg
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REFLEXES: (0 = absent; 1; 2; 3 = slightly hyperactive; 4 = abnormally hyperactive)

Right bicep	Left bicep
Right tricep	Left tricep
Right radialis	Left radialis
Right Hoffman	Left Hoffman
Right adductor	Left adductor
Right Crossed adductor	Left crossed adductor

Right knee
Right ankle
Right plantar
Right cremasteric
Right abdominal

Left knee
Left ankle
Left plantar
Left cremasteric
Left abdominal

GAIT: _____

RUN: _____

(0 = normal; 1 = slightly stiff; 2 = stiff with circumduction;
3 = stiff, circumduction, & effortful; 4 = needs cane or other
support)

HOP ON RIGHT LEG; _____

HOP ON LEFT LEG: _____

(0 = normal; 1 = slightly stiff; 2 = stiff with no spring;
3 = can't hop; 4 = can't stand unsupported on foot)

EXAMINER _____

DATE & TIME OF EXAMINATION _____

mo/ day/ yr

L-THREONINE - SPASTICITY RESEARCH

Last Name _____ First Name _____

MGH Unit Number _____ Date of Birth _____

LABORATORY STUDIES:

_____ EKG (0 = normal; 1 = non-specific abnormal;
(mo/date/year) 2 = abnormal)

_____ Urinalysis

_____ Chest X-Ray

CEREBROSPINAL FLUID STUDIES:

DATE _____
mo/date/year

cells
protein
sugar

alanine
arginine
asparagine
aspartic acid
cystine
glutamine
glycine
histidine
isoleucine
leucine
lysine
methionine
ornithine
phenylalanine
proline
serine
taurine
threonine
tryptophan
tyrosine
valine

PATIENT'S LAST NAME _____

FIRST NAME _____

MGH UNIT NUMBER _____

DATE OF BIRTH _____

DATE OF EVALUATION _____

Please describe how you have been feeling over the past two weeks by answering the following questions. Circle "improved" if you believe there was improvement, "s" if you believe there has been no change, and "deteriorated" if you believe your condition has worsened.

1. Do you think the muscle strength in your legs has:

Improved Same Deteriorated

2. Do you think your walking has:

Improved Same Deteriorated

3. Do you think the stiffness in your legs has:

Improved Same Deteriorated

4. Do you think the jumping in your legs at night or when you are lying down (flexor spasms) has:

Improved Same Deteriorated

5. Do you think that your bladder function (ease with which you empty your bladder, frequency of urination, bladder incontinence including dribbling and wetting underwear) has:

Improved Same Deteriorated

Describe: _____

6. Do you believe your level of energy and activity (e.g. distances you can walk, how soon you get tired, etc.) has:

Improved Same Deteriorated

7. Do you think your sleeping habits have:

Improved Same Deteriorated

8. Have you noticed any changes in your mood, emotional state, behavior or body function during the past two weeks (e.g. depressed, headache, dizzy, nausea, poor appetite, etc.)? If so, describe _____

9. On the whole, do you believe your condition has:

Improved Same Deteriorated

10. Please comment on any additional changes you have noticed or the people living (or working) with you have noticed. _____

PHYSICIAN'S GLOBAL EVALUATION

PATIENT'S LAST NAME _____ FIRST NAME _____

MGH UNIT NUMBER _____ DATE OF BIRTH _____

THIS FORM WAS COMPLETED BY _____ M.D.

DATE OF EVALUATION _____

Rate the following signs and symptoms according to this scale:

- I = improved
- S = stable
- D = deteriorated

1. STRENGTH			
Upper extremities:	I	S	D
Lower extremities:	I	S	D
2. WALKING:	I	S	D
3. RUNNING:	I	S	D
4. TONE			
Upper extremities:	I	S	D
Lower extremities:	I	S	D
5. DEEP TENDON REFLEXES:	I	S	D
6. FLEXOR SPASMS:	I	S	D
7. BLADDER FUNCTION:	I	S	D
8. OVERALL GLOBAL ASSESSMENT:	I	S	D

SPECIAL COMMENTS, INCLUDING SIDE EFFECTS OF MEDICATION: _____

