BAYLOR
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31 March 1986

Philip J. Migliore, M.D.
Research Director
The Moran Foundation
Department of Pathology
Baylor College of Medicine
One Baylor Plaza
Houston, Texas 77030

Dear Dr. Migliore:

Enclosed please find the updated annual report and request for continued support for our project "Applications of HPLC Amino Acid Determinations". As you suggested, the proposal is submitted as a continuation of last year's work rather than a new project. I would like to thank you and the research committee members for your patience and constructive comments during the evolution of this proposal.

If I can be of any further assistance, please feel free to contact me.

Sincerely yours,

J. Clay Goodman, M.D.

TITLE: APPLICATIONS OF HPLC AMINO ACID DETERMINATIONS

Annual Report Update and Request for Continued Support

INVESTIGATORS: Principal Investigator: Clay Goodman

Co-investigators: Ching-nan Ou

Claudia S. Robertson

Over the past year, we have used Moran Foundation funds for the measurement of serum amino acids in head injuried patients using high pressure liquid chromatography with post-column ninhydrin derivatization. This technique permits accurate detection of some 40 serum constituents including essential and nonessential amino acids. Here we report the results of our studies of systemic and cerebral amino acid metabolism in 12 head injuried patients in the first 5 days following injury. A consistent pattern of metabolic derangements occurs raising the possibility of therapeutic benefit from early nutritional support in such patients. Additional studies of the amino acid alterations and the effects of intervention on metabolic patterns and clinical outcome form the basis of our request for continued support.

METHODS

Our preliminary study contained 12 patients in coma from head injury in the absence of other serious injury (Table 1). All patients were treated in the Neurosurgical Intensive Care Unit at Ben Taub General Hospital under the direction of an internist with training in critical care (C.S.R.) with neurosurgical interventions being under the direction of other Neurosurgery Department faculty and house officers. Management emphasized early evacuation of intracranial hematomas, monitoring of intracranial pressure with treatment of pressures exceeding 20 mmHg,

controlled ventilation, and avoidance of secondary injury of the brain. Routine medications included morphine, phenytoin, and antibiotics in penetrating injuries. Intracranial hypertension was treated with hyperventilation, mannitol, and barbiturates. Corticosteroids were not routinely administered. As dictated by standard neurosurgical care, all patients received 5% dextrose in 1/2 normal saline as their only caloric source for the first 3 days after admission. On day 4 alimentation commenced, enterally if bowel sounds were present or parenterally if ileus persisted. The feeding goal was 3500 kcal/day with 20 gm nitrogen/day.

Cerebral blood flow (CBF) using the Kety-Schmidt technique with nitrous oxide as the diffusable indicator, and cerebral amino acid, glucose, oxygen, and lactate AV differences were measured at least daily for the first 5 days following injury. The cerebral metabolic rate (CMR) of each substrate of interest was computed by multiplying the arterial-jugular venous difference of the substrate by the CBF. Systemic metaboic rates were computed using cardiac output measured by thermodilution multiplied by arterial and mixed venous differences of metabolites. Daily urinary nitrogen loss was measured using the chemiluminescence technique (Antek Instruments, Houston, Tx). Additionally, serum catecholamines were measured by HPLC with electrochemical detection.

Pickering Laboratories of Mountain View, California, was the source of columns and reagents for a post-column ninhydrin derivatization method of measuring amino acids in physiological fluids. Chromatographic conditions included a 3mm X 250mm 10µM lithium cation exchange column at 42°C with a guard column and a three component lithium salt mobile phase (flow rate 0.3 ml/min) gradient with an elution and regeneration cycle of 210 minutes (figure 1). Following separation, the amino acids

were mixed with ninhydrin (flow rate 0.3 ml/min) at a mixing tee and subsequently reacted for 100 seconds at 120°C in a Pickering CRX390 post column reactor resulting in chromophores monitored at 546 nm in a 12µl cell (figure 2). An injection of standard and a serum sample on the system in its current configuration are shown (figures 3 & 4). Sample preparation involved simple deproteinization followed by autoinjection of a 20 µl sample. We injected 20 standards and 112 serum samples with good preservation of constituent resolution and peak areas.

RESULTS

The systemic hypermetabolic response of these 12 patients was typical of the head-injury pattern and is shown in detail in Tables 2 and 3. Systemic oxygen consumption was elevated, as was cardiac output, and urinary nitrogen losses averaged 14.8 gm/day. Systemic oxygen consumption and cardiac output were maximal on day 1; the oxygen consumption declined toward normal over the 5 day study interval whereas the cardiac output remained high. The patients were moderately hyperglycemic and arterial lactates were elevated, with the highest levels being on the first day and declining thereafter. The arterial branched chain amino acids (leucine, isoleucine, valine) were decreased with lowest levels occurring on day 2. Arginine, threonine, and serine were also decreased, whereas methionine, tyrosine, glutamic acid, taurine, and phenylalanine were elevated. Catecholamines were elevated throughout.

Studies of the cerebral metabolic response disclosed that cerebral blood flow was elevated relative to the patients' pCO_2, but that cerebral utilization of oxygen and glucose were consistently reduced. Cerebral lactate production was increased, being maximal on day 1 and decreasing toward normal by day 5.

In uninjured persons, the brain is normally in net positive amino acid

balance, but in our study we found that the uptake of amino acids by the injured brain varied with time following injury. On day 1 after injury, there was significant cerebral uptake of all amino acids except threonine which reached its arterial nadir on day 1 in contrast to most of the other amino acids which reached their lowest arterial concentrations on day 2. On day 2 when most amino acid concentrations reached their lowest levels, none showed significant net uptake by the brain; and there was net loss of aspartic acid, glycine, and tyrosine. Following day 2, there was a gradual restoration of normal positive balance. Each patient followed the same evolution of brain exchange of amino acids; that is, of uptake on day 1, no uptake or even loss on day 2, followed by return of amino acid uptake. The time course of the resumption of uptake was variable, and was independent of cerebral blood flow, and cerebral metabolism of oxygen, glucose, and lactate. Although the groups are small, there were no siginificant differences in the amino acid patterns of the patients who had good neurological recovery from those who had poor outcome. In fact the only parameter which influenced by uptake of amino acids by the brain was the arterial concentrations of the amino acids.

DISCUSSION & PROPOSAL

Following isolated severe head injury, the systemic metabolic response is similar to that seen in severe multi-organ injury, sepsis, and burns. Early comprehensive nutritional support of such critically ill patients has a demonstrable beneficial impact on mortality, wound healing, and immunocompetence. Standard neurosurgical practice, however, has dictated minimal nutritional support during the first days following injury due to fears of fluid loading from nutritional support adversely impacting intracranial pressure. We have shown, however, that immediately following injury, the brain is in net negative amino acid

balance, and that there is a shift toward anaerobic glucose utilization with increased lactate production. It is possible that measures taken to provide the brain with amino acids during this period or to blunt the systemic reduction in amino acids might have favorable effects on outcome on neurological injury. Of course, the leakage of amino acids and shift to anaeobic glucose use might reflect the dying metabolism of irreversibly damaged brain tissue for which our therapeutic measures would be of no value. We prefer the more optimistic hypothesis that manipulation of the systemic metabolic environment can affect cerebral metabolism, and that provision of substrates required for the repair of injury may favorably influence outcome.

To that end, we propose continuing our studies as outlined above to gather more data about the systemic and cerebral metabolic response in head injury in the context of earlier enteral and parenteral nutritional support in the environment of the intensive care unit where any adverse effects of such interventions on intracranial pressure can be monitored and corrected. Continued Moran Foundation support for the amino acid analysis component of the project would be most helpful.

We have demonstrated that this method of amino acid analysis can be implemented in a clinical laboratory with gradient high pressure liquid chromatographic capability. The pre-mixed reagents are reasonably inexpensive further facilitating service laboratory implementation. A drawback of the method is the analysis time of 210 minutes which limits the total number of samples which can be processed to about 7/day. We propose reducing this analysis time to 120 minutes by using a shorter cation exchange column with smaller particle size packing material (Pickering Laboratories High Speed Amino Acid Analysis Lithium Column, 3mm X 150mm, 7µM). Some resolution of closely eluting amino acids may

be lost employing the faster analysis time, but we hope to manipulate chromatographic conditions to offset this loss.

Samples will consist of plasma spiked with amino acid standards, as well as plasma from head injuried hypercatabolic comatose patients with and without early hyperalimentation. The samples from head injuried patients will be obtained in the course of an ongoing project investigating systemic metabolic responses to injury (no additional blood drawing will be required and this ongoing project is sanctioned by institutional human investigation committees).

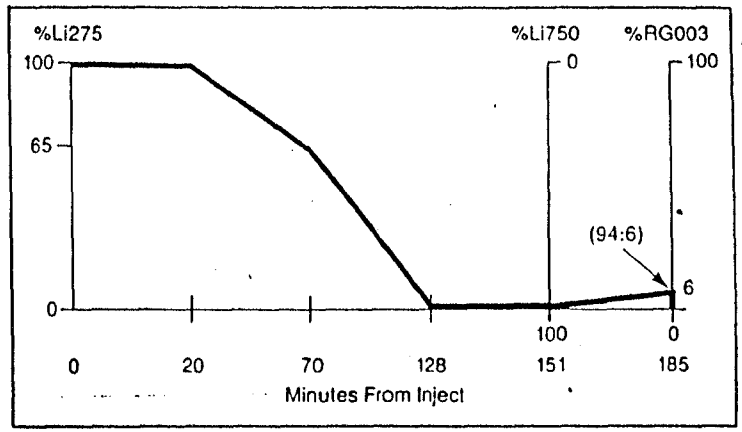
Budget Requirements: The following reagents, supplies, and equipment will be needed for this project:

Lithium Column, 3mm X 250mm, 10µM	$1100.00
Lithium Column, 3mm X 150mm, 7µM (x2)	$2600.00
Lithium Guard Columns (2)	$360.00
Lithium Eluent, 2.75 pH (2 cases)	$176.00
Lithium Eluent, 7.50 pH (2 cases)	$176.00
Lithium Column Regenerant (x2)	$30.00
Ninhydrin reagent (2 cases)	$400.00
Sample preparation reagents	$50.00
Calibration standard reagents	$37.00
Miscellaneous HPLC components	$400.00
ISCO UA-5 Absorbance Detector & Cell	$2800.00
TOTAL	$8129.00

The long and short columns, guard columns, eluent solutions, ninhydrin, sample preparation reagents, and standards are disposables essential for the execution of this project and cost a total of $4929.00. The mobile

phase is quite acidic through much of the analysis necessitating frequent seal replacement and plumbing repairs; hence, $400.00 is budgeted for miscellaneous HPLC components so that these repairs can be performed. The UA-5 detector is equipped with a 0.12 µl flow cell in contrast to the detector currently in use which has a 12 µl flow cell. The reduced cell volume may reduce band broadening in the detector permitting good resolution despite rapid column transit with the high speed column.

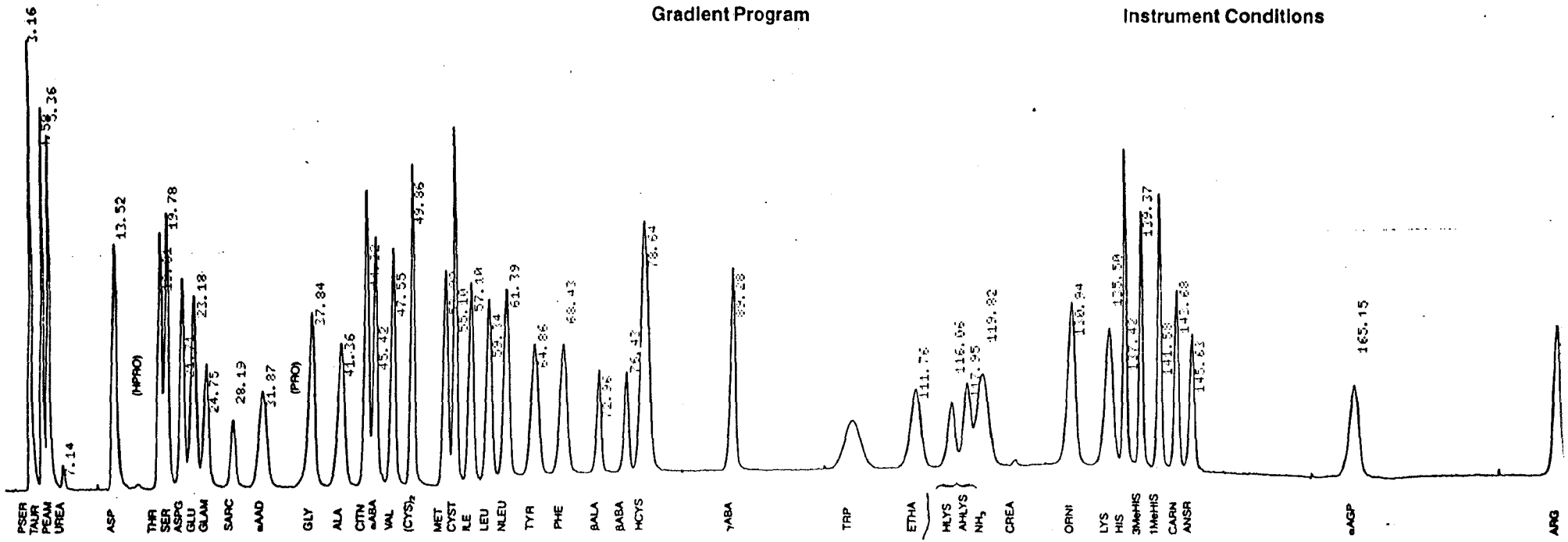
GRADIENT LC, AMINO ACIDS: Physiologic Fluid



Gradient Program

Sample:	2.5 nM/Constituent
Flow Rate:	0.3cc/min, Eluent 0.3cc/min, Trione
Temperature:	42°C Isothermal
Development:	50 sec at 130°C
Detector:	0.5 AUFS 25µL × 12mm

Instrument Conditions

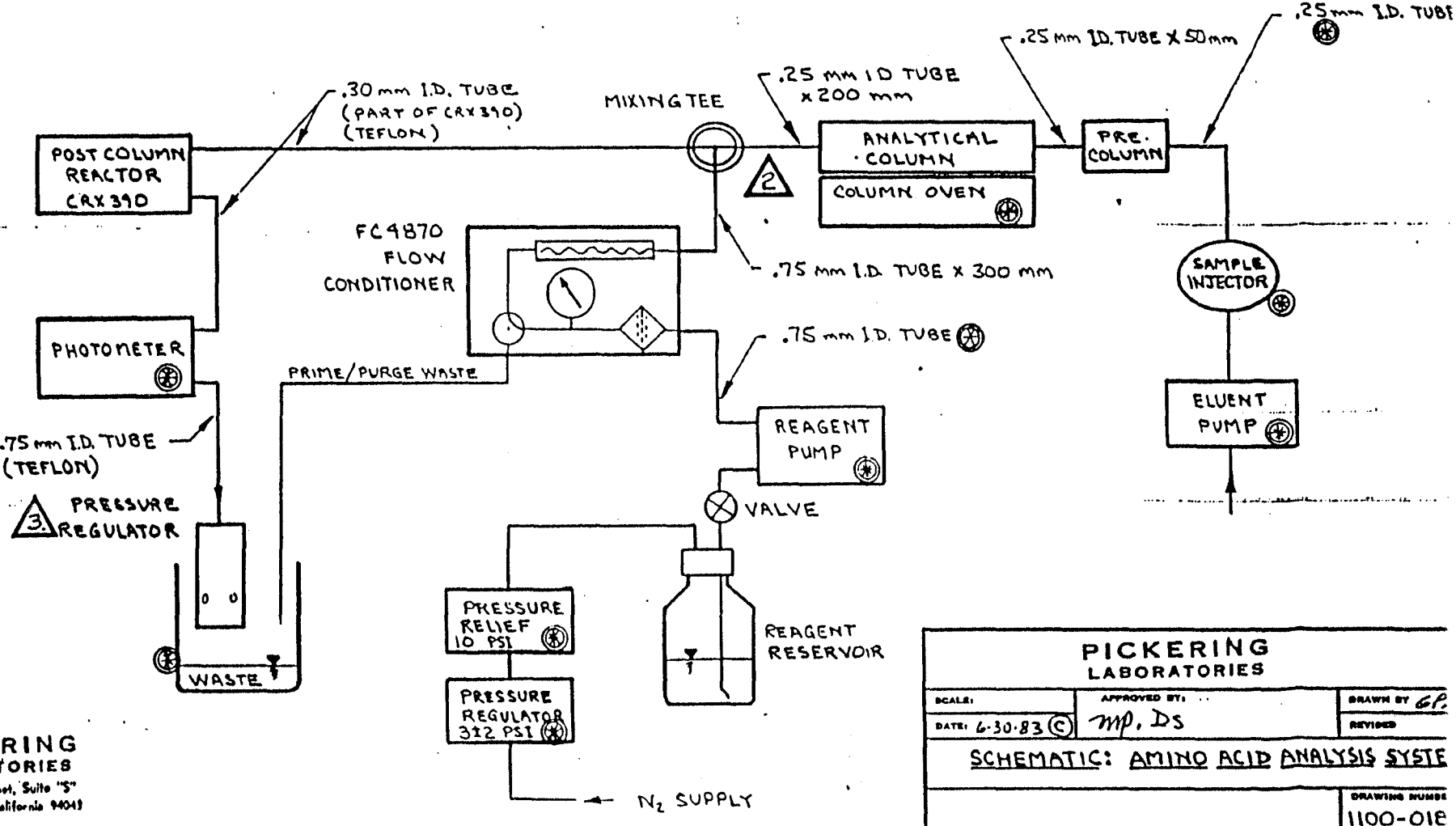


PICKERING
LABORATORIES

NOTES: 1. ⊛ ITEMS NOT SUPPLIED BY PICKERING LABORATORIES.

2. WARNING: DO NOT PERMIT REAGENT TO BACKFLOW INTO ANALYTICAL COLUMN. REAGENT WILL DAMAGE COLUMN.

3. WARNING: REAGENT AND ELUENT – AT 100 °C REACTOR TUBE WILL PLUG AND BURST IF OPERATED ABOVE 100 °C WITHOUT SPECIFIED REGULATED BACK-PRESSURE.



PICKERING LABORATORIES
1951 Colony Street, Suite "S"
Mountain View, California 94043

PICKERING LABORATORIES

SCALE:	APPROVED BY: mp, DS	DRAWN BY GP
DATE: 6-30-83 ©		REVISED

SCHEMATIC: AMINO ACID ANALYSIS SYSTE

DRAWING NUMBER 1100-01E

A-B-S-O-R-B-A-N-C-E a-t 5-4-6 n-m
STANDARD MIXTURE
PSER
TAUR
PEAM
UREA
ASP
HPRO
THR
SER
ASPG
GLU
SARC
AAAD
PRO
GLY
ALA
CITN
AABA
VAL
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MET
ILEU
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NLEU
TYR
PHE
BALA
BAIBA
HCYS
GABA
TRP
EDTA
HLYS
AHLYS
NH3
CRLA
ORN1
LYS
HIS
3MEHIS
1MEHIS
CARN
ANSR
AAGP
ARG
RETENTION TIME (min)

Table 1. Demographic characteristics of patients studied.

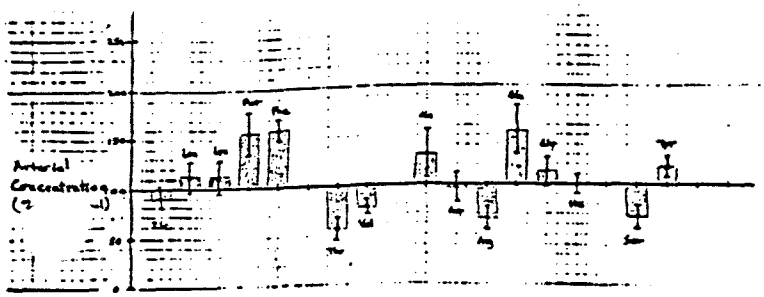
Age.	15-25:	7
	25-35:	3
	35-45:	1
	>45:	1
Sex.	male:	9
	female:	3
Injury.	Diffuse brain injury:	3
	Epidural hematoma:	1
	Subdural hematoma:	4
	Intracerebral hematoma:	3
	Gunshot wound:	1
Glasgow Coma Score.	3:	1
	4:	2
	5:	0
	6:	7
	7:	2
Glasgow Outcome Score.	Good recovery	3
	Moderate disability	3
	Severe disability	1
	Vegetative state	3
	Dead	2

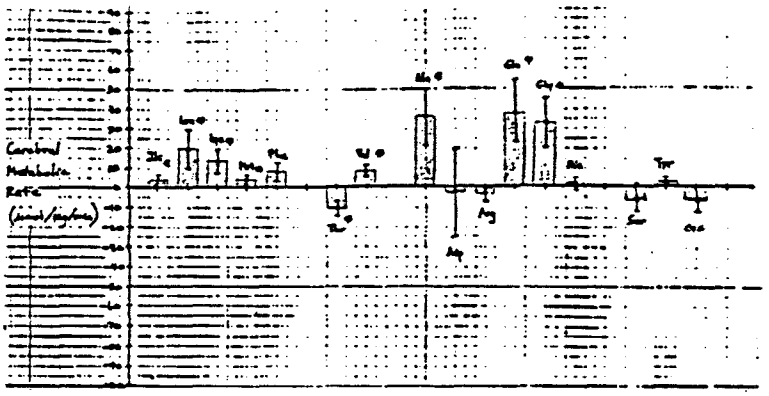
Table 2. Cerebral and Systemic Metabolism.

		Day After Injury				
		1	2	3	4	5
Cerebral Metabolism:						
CBF	ml/gm/min	.485 ±.076	.520 ±.088	.523 ±.077	.720 ±.163	.580 ±.065
CMRO2	umol/gm/min	.83 ±.12	.83 ±.08	.83 ±.06	.86 ±.17	.95 ±.40
CMRG	umol/gm/min	.208 ±.065	.201 ±.041	.242 ±.053	.162 ±.059	.157 ±.022
CMRL	umol/gm/min	-.039 ±.022	-.097 ±.048	-.030 ±.017	-.055 ±.036	-.029 ±.023
Systemic Metabolism:						
CI	L/min-M2	4.7 ±.5	4.1 ±.4	3.8 ±.2	4.1 ±.7	4.6 ±.2
VO2	ml/min-M2	146 ±23	131 ±8	130 ±4	129 ±12	125 ±15
Nitrogen Loss	gm/d	13.8 ±2.5	14.9 ±2.8	14.0 ±1.9	13.0 ±2.3	17.1 ±3.0

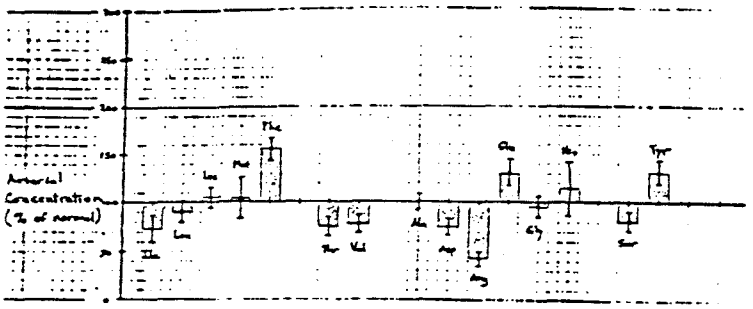
Table 3. Arterial Levels of Metabolic Substrates and Catecholamines.

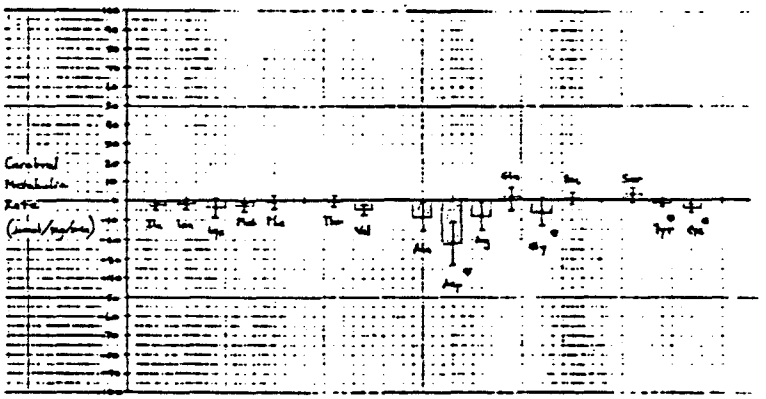
	Day After Injury				
	1	2	3	4	5
Lactate	2.3±.6	1.5±.2	1.2±.1	1.2±.2	1.2±.2
Glucose	9.6±.6	7.9±.4	8.1±.6	8.9±1.1	8.7±2.0
Essential Amino Acids (umol/L):					
Isoleucine	51±7	41±3	59±7	65±17	66±6
Leucine	121±14	99±11	141±14	129±29	129±26
Lysine	169±20	160±16	246±27	193±42	207±35
Methionine	36±5	24±5	37±6	51±23	36±7
Phenylalanine	73±6	73±4	88±6	115±31	86±12
Threonine	70±13	92±12	123±24	86±6	107±42
Valine	145±15	148±16	184±21	208±28	193±16
Nonessential Amino Acids (umol/L):					
Alanine	381±70	293±25	390±51	349±81	291±6
Aspartic acid	154±28	118±14	128±25	178±38	171±90
Arginine	40±8	26±5	31±4	38±5	24±1
Glutamic acid	297±45	253±34	262±100	243±50	344±82
Glycine	313±41	270±38	340±33	309±63	300±30
Histidine	75±7	87±21	87±6	79±16	79±10
Serine	90±18	104±13	128±11	91±9	116±40
Tyrosine	59±5	64±6	87±11	88±17	60±6
Taurine	361±68	255±35	271±33	237±89	196±35
Catecholamines (pg/ml):					
Epinephrine	154±35	361±102	708±206	726±367	224±81
Norepinephrine	276±119	520±215	423±118	488±100	268±126



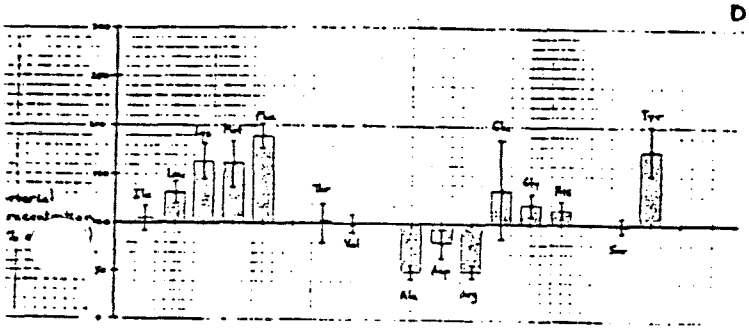


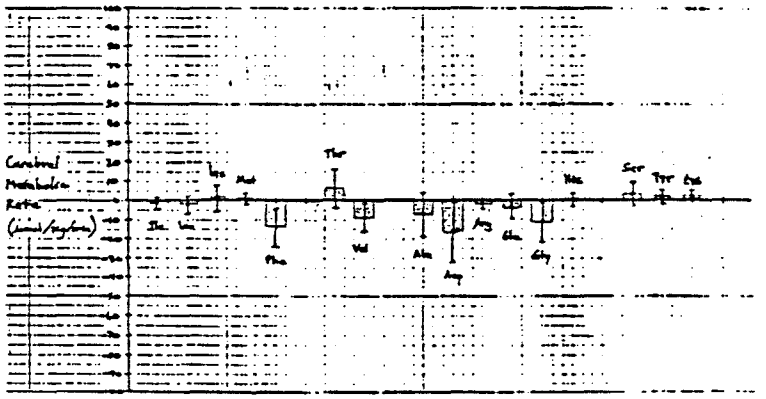
Day 2



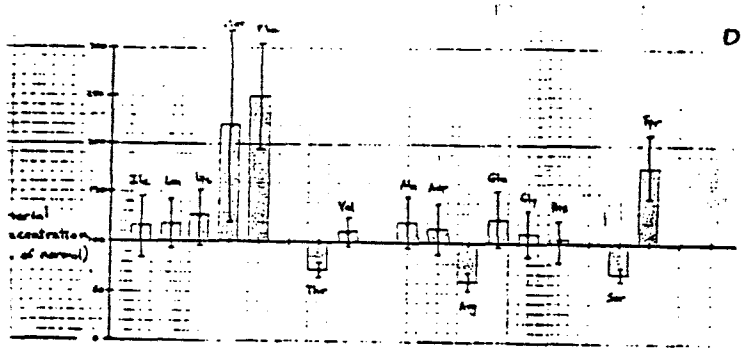


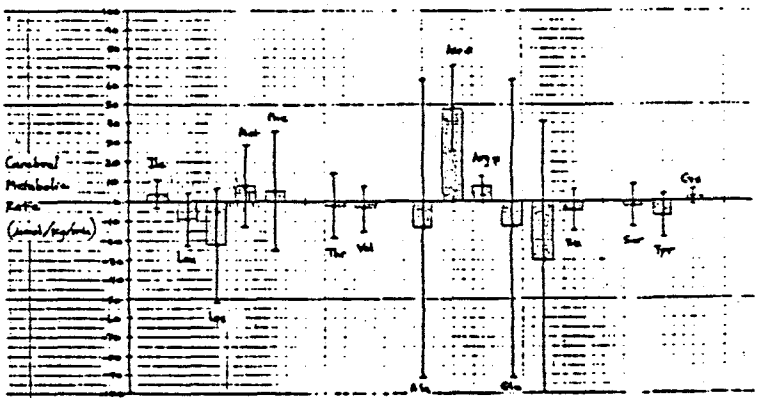
Day 3



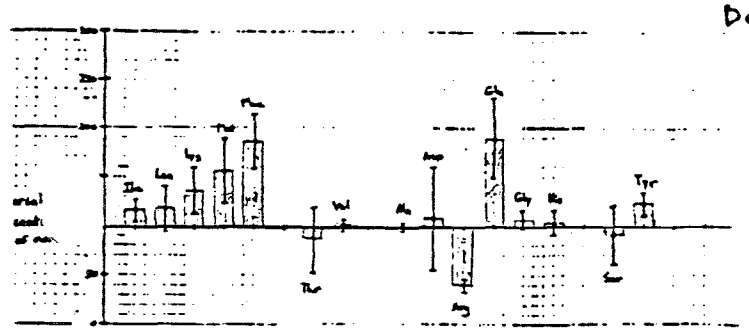


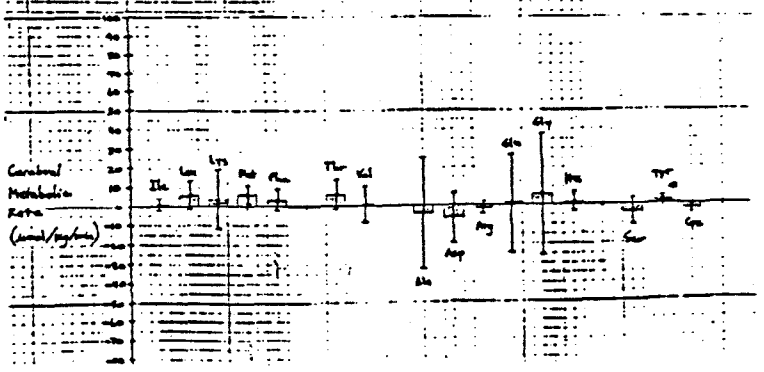
Day 4





Day 5





The Moran Foundation
DEPARTMENT OF PATHOLOGY
BAYLOR COLLEGE OF MEDICINE
TEXAS MEDICAL CENTER
HOUSTON, TEXAS 77030

June 5, 1986

Clay Goodman, M.D.
Assistant Professor of Pathology
Department of Pathology
Baylor College of Medicine
Houston, Texas 77030

Dear Dr. Goodman,

Following review of the latest revision of the addendum to your study, "Clinical Application of Rapid Amino Acid Analysis" (1-85-0013), the Scientific Advisory Committee has unanimously recommended that additional funds, amounting to $5,304.00, be made available to allow you to continue your studies. Requests for use of these funds should continue to be made through Mr. Wes Moreland in the Department of Pathology office (X 4661). We appreciate your patience and willingness to work with us during the past few months during the review process. As a reminder, annual progress reports are due by December 1 of each year.

Sincerely yours,

Philip J. Migliore, M.D.
Research Director
The Moran Foundation

c: Jack L. Titus, M.D., Ph.D.
Mr. John Moran
Gregory Buffone, Ph.D.
Matthew Noall, Ph.D.
David Yawn, M.D.
Mr. Wes Moreland

PJM/ms