INSTITUTE OF CHEMICAL BIOLOGY
UNIVERSITY OF SAN FRANCISCO
San Francisco, California 94117

SEMI-ANNUAL REPORT

Project

Brain Amino Acids and Biogenic Amines

Under Various Atmospheric Mixtures

Control: SC-NGR-05-029-001

Project Period*

Starting Date: 1 March 1965

Termination Date: 30 April 1966

Date: 9 May 1966

Submitted by:

Arthur Furst, Ph.D., Director
Institute of Chemical Biology

Personnel:
Joseph H. Gast, Sc.D.
Senior Investigator
H. B. Chermside III
M. A. Kelly

* Renewal request submitted.

No restriction requested for this report.

INTRODUCTION AND SUMMARY

The second semiannual report covers the period from 1 October 1965 through 30 April 1966, with some reference to the period from the initiation of the project through 30 September 1965.

We are studying the possible effects of exposure to different gaseous atmospheric mixtures for various period of time upon the free amino acids and on the biogenic amines, especially serotonin (5-hydroxytryptamine), present in rat brains.

The first year has been essentially devoted to development of methodology. These efforts may be divided into five categories:

I. Experimental Design

The experiment has been designed to gain as much information as possible from a small number of experimental animals. This has required development and application of sensitive and sophisticated experimental techniques and data processing.

II. Instrumentation

The Beckman 120-C Amino Acid Analyzer as delivered proved to be unsatisfactory for our analyses of rat brain tissues. We have modified it to increase its utility. Considerable time has been spent standardizing the 120-C. Standardization of the Aminco Bowman Spectrofluorophotometer for serotonin analysis has been routine.

III. Exposure and Tissue Preparation Procedures

Due to the sensitivity of the 120-C, we have been forced to modify the recommended procedures for tissue preparation to be used for amino acid analysis in order that we may work accurately with smaller tissue samples. In so doing, we have developed special glassware. The procedures of tissue preparation for biogenic amine analysis also needed modification. We have constructed animal exposure chambers in our laboratory which are satisfactory for exposures up to approximately 24 hours. We have obtained brain material from animals exposed for long terms at NASA Ames Laboratories.

IV. Results and Discussion

Experimental results thus far are primarily qualitative rather than quantitative. They have been principally concerned with developing details of experimental methodology and are discussed in this context. We have found fifty different ninhydrin-positive substances in rat brain extracts. Of these, we have positively identified twenty-seven and tentatively identified several others. Quantitative baselines for normal, or control, animals are being established for amino acids and amines.

V. Data Analysis

We have been fortunate in obtaining the use of an IBM 1620 computer for handling massive amounts of data. We have developed programs for analysis of some of our data and are writing other programs.

Some funds have not yet been expended and are being carried over. These funds will be used to finance the research on a continuous basis until the second year's funds are released after the beginning of the fiscal year.

The details of experimental design have been formulated on the basis of this year's pilot studies. During the next year, all efforts will be channeled into implementation of this design.

I - EXPERIMENTAL DESIGN

Changes in behavior of mammals are correlated with changes in certain biogenic amines and amino acids, particularly serotonin, epinephrine, nor-epinephrine, and GABA (gamma-aminobutyric acid. [References: Eiduson, S., Geller, E., Yuwiler, A., and Eiduson, B. T., Biochemistry and Behavior, Van Nostrand, New York, 1964; de Ropp, R. S., and Furst, A., "Biochemical and Behavior Effects of Some Substituted Tryptophans," To be published.]

The biochemical pathways for formation of these compounds are known. Their precursors are certain amino acids. These pathways are elucidated in Appendix I.

Should changes in the brain-levels of these amines be altered by varying the atmosphere the animal breathes, the manifestations will be different behavior patterns. Further, it is possible that the first chemically detectable changes on thesebiogenic amines will be noted in precursor amino acids.

An overall experimental design was adopted which allows an exploratory investigation of the effect of atmospheric conditions on both brain-amine and brain-amino acid levels. In addition, behavioral studies are possible.

Rats are exposed to varying gaseous mixtures for different lengths of time; their brains are removed and analyzed for biogenic amine and amino acid content. The data are subjected to statistical analysis. The animals may be subjected to classical behavioral measures of random and purposive behavior before and after exposure to the experimental atmosphere.

Male rats, of Long-Evans and Sprague-Dawley strains, are used. The Long-Evans animals tend to be more hardy and more amenable to behavioral procedures, but Sprague-Dawley animals are available which have been exposed to conditions beyond the capabilities of this laboratory. The data will be examined to determine the effects of strain difference.

Five animals constitute the sample for each exposure condition. The conditions can be replicated if larger samples are required for any reason.

Initial exposures in 100% O_2 will be made in our laboratory for periods of 6, 12, 18 and 24 hours. Data from these exposures and from chronic exposures made at NASA Ames will be analyzed for effects of exposure length on amine and amino acid quantities and balances. A further analysis may be made, if the data so indicates, on possible acclimatization effects in chronic exposures. Other exposures will be made with other gaseous mixtures, including volatile anesthetics and O_2 + inert gases. The lengths of these exposures will be decided after preliminary studies. The results will be compared to those of 100% O_2 exposures and the data examined for the effects of varying gaseous mixtures. All data will be analyzed for relations between amines and their precursor amino acids.

Behavioral studies presently contemplated are comparisons of total activity and purposeful activity, and the effects upon those of the experimental conditions. Total activity will be measured by a simple integrating activity cage. Purposeful activity will be measured by training the animal to perform a simple task which can be measured by the number of performances or by the activity expended for performance. The latter measure is preferred, as a direct comparison can then be made between general and specific activity. These activity measures will then be related to the exposure condition and the chemical measures. We do not expect that this design will need any further modification, as all preliminary studies and training of personnel are essentially completed.

II - INSTRUMENTATION

The time factor in the installation of the Beckman 120-C Amino Acid Analyzer was discussed in the previous semiannual report. Immediately following the writing of that report, it was discovered that the instrument needed modification in order to be of use to us. Rat brain tissues contain amino acids whose quantitative ratios may be on the order of 100:1 or greater. We found it necessary to change the sensitivity of the attenuated channel of the colorimeter in order that high and low concentration amino acids could be measured simultaneously. At the same time we made several

other modifications which reduce the time that an operator must be in attendance during an analysis. We are preparing a discussion of these changes for submission for publication; a draft of this discussion is included as Appendix II. Copies will be submitted in the usual manner when the manuscript is in final form.

Recently, mold contamination has been a problem. Mold in the buffers blocks the ion exchange resin column, leading to prohibitive pressures. The instrument must then be turned off, the resin cleaned, and the new standardization established. We have tried several mold inhibitors in search of one which allows continuous operation. Caprylic acid is presently being used and has given the best results to date.

Slight problems with the reliability of the Aminco Bowman Spectrofluorophotometer were solved by substituting more modern solid state rectifiers in the photomultiplier power supply.

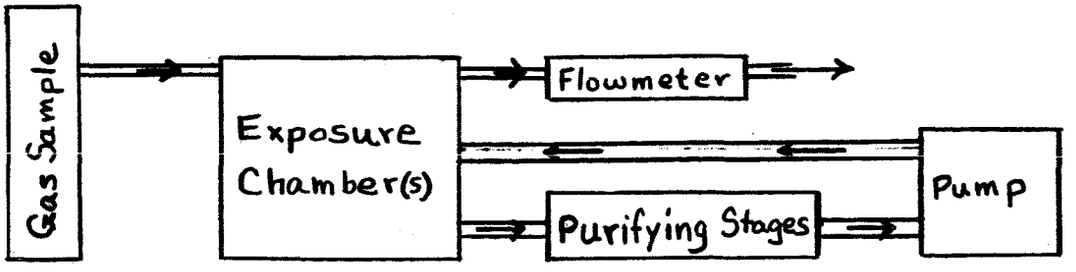
III - EXPOSURE AND TISSUE PREPARATION PROCEDURE

Animals are exposed in our laboratory in chambers constructed from 250 mm desiccators. Two desiccator exposure chambers are connected in parallel to a single gas input, and exhaust through separate gas flowmeters. An additional 200 mm desiccator houses an aquarium aerator pump which circulates the contents of the two exposure desiccators through a closed loop containing NaOH to absorb excess CO_2, concentrated H_2SO_4 to absorb excess H_2O, and CaO to absorb H_2SO_4 fumes.

Diagram 1

EXPOSURE APPARATUS

gas flow Schematic



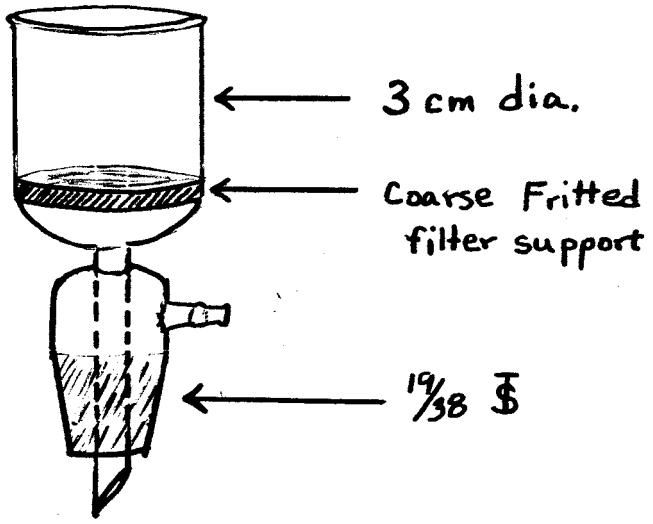
The animal is supported on a screen which allows urine and feces to fall to the floor. Food is placed in the chamber at the beginning of the exposure unless the animal is to be fasted. Drinking water is available ad libitum from a stainless steel drinking tube from an external container. This equipment can expose two 350 g rats per desiccator for period up to 12 hours and one per desiccator for period up to 24 hours.

The tissue preparations for biogenic amines were reported in the first semiannual report. Essentially, this procedure extracts the amines from the tissue in aqueous HCl solution from which they are removed by differential solubility using varying solutions for the various amines. The only modification of the earlier reported technique is that we homogenize the tissues with an ultrasonic probe (Biosonik II, Bronwill Scientific Co.).

We have modified the picric acid method of SPACKMAN, STEIN AND MOORE for extraction of free amino acids from brain tissue. [Reference: Spackman, D. H., Stein, W. H., and Moore, S., Anal. Chem. 30, 1190 (1958); Agrawal, H. C., Davis, Jimmie M., and Himwich, Williamina, A., to be published.]

We have designed two pieces of glassware for this procedure to reduce the chance of error and speed the process. Our procedure is as follows:

1. Use one-half rat brain frozen in LN and stored on dry ice.
2. In 15 ml centrifuge tube, add to tissue picric acid to 13 ml.
3. Homogenize ultrasonically using 5/32 in "needle" probe 1 min.
4. Centrifuge 30 sec, rehomogenize 30 sec.
5. Centrifuge 5 min at 1500 RPM.
6. Remove supernatant and save.
7. Add H_2O to centrifugate in tube to 10 ml.
8. Homogenize 30 sec, centrifuge 5 min as before.
9. Remove supernatant and add to previously collected supernatant in beaker.
10. Add about 5 g Dowex 2X8 of 2X10 to remove yellow impurities.
11. Filter slurry in special filter through E&D #613 filter paper directly into Virtis lyophilization jar. Wash 5 times with 4 ml 0.02 N HCl.

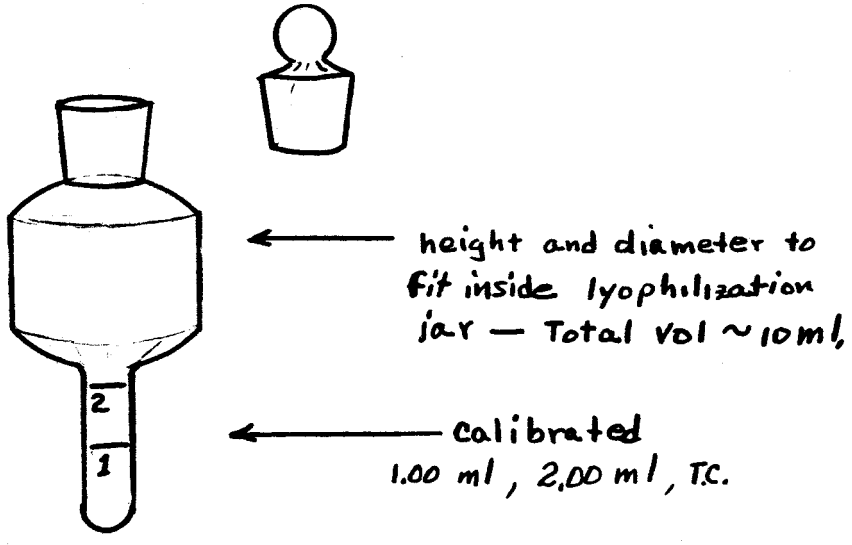


Vacuum Filter for use with Lyophilization Jar

(This filter saves one transfer of sample for each use; this reduces volume to be lyophilized by negating need for washing out a transfer container.)

Lyophilize to a volume of about 5 ml.

12. Add 4 g Celite to remove suspended solids, filter again into special flask.



Calibrated Flask for Lyophilization

(This flask eliminates one transfer of sample and allows a small sample to be made relatively undiluted by washings.)

13. Lyophilize with special flask in lyophilization jar to about 1 ml.
14. Add about 0.5 ml sample diluting buffer (pH 2.2, Beckman 120-C operating manual).

15. Shake on mechanized shaker 5 min with top on flask.
16. Centrifuge 5 min at 500 RPM.
17. Bring volume to 2.00 ml.
18. Aliquots of this sample may be applied directly to the column of the 120-C.

IV - EXPERIMENTAL RESULTS

We have determined that there is no difference in amino acid content of tissue subjected to LN and stored on dry ice from that of tissue prepared immediately after sacrifice. This allows greater flexibility of schedules. For procedural sophistication, however, all tissues will be LN frozen before preparation.

Preliminary studies were made exposing rats to 100% O_2 at 760 mm Hg. With our equipment the maximum replicable exposure for each of two chambers which does not produce noticeable lung edema or other physiological malfunction is 24 hours for one animal. With two rats per chamber, 12 hours is the maximum. In longer exposures the chambers become befouled with urine, feces, and metabolic H_2O. Long exposures require multiple chambers for each animal with air locks for transferring the animal between chambers. Such complex exposure equipment is in use at NASA Ames Laboratories.

Through the cooperation of Dr. H. P. Klein, Dr. H. Leon, and Mr. Gerald Brooksby at the NASA Ames Laboratories we are able to obtain brain material from animals subjected to chronic exposures to 100% O_2 and other gaseous mixtures. These animals are used for other purposes in their laboratory, so they provide a cost-free bonus to this project. The brain is removed at Ames by our technician immediately after sacrifice, frozen on LN and stored on dry ice for further preparation in our laboratory. We hope at some later date to correlate their findings on these animals with ours.

The rat brains we have used contain between 1.5 g and 1.9 g of tissue. A midventral division of the brain into approximately equal portions therefore produces a minimum of 0.70 g of tissue. This quantity of material has been found sufficient for one analysis for amines (two determinations), or for two analyses for amino acids. Thus, five animals make a sufficiently large sample for exploratory research of this type. Yet, by identifying the samples, direct comparisons can be made between the amine and amino acid content of the brain of any individual animal. This will allow direct comparison of the behavior and the chemistry of each animal.

The first exposure of animals to 100% O_2 at 760 mm Hg has been made. The procedure was as previously discussed. Ten Sprague-Dawley males were exposed at Ames. Only three survived the full 72 hours. There were ten controls in

similar exposure chambers, breathing ambient atmosphere. The analyses of tissue from these thirteen animals is in progress.

Superficial examination of the chromatograms from the Beckman 120-C Analyzer for several of these animals does, however, give some useful information. All of the analyses thus far have been performed on control animals. The chromatograms are extremely similar, indicating little variation among animals. There is a noticeable correlation observable in superficial scanning of the chromatograms between the amounts of the most easily estimated amino acids and the weight of brain tissue analyzed. This indicates that, although the instrument has been modified and the resin has needed constant cleaning, the instrumental reliability is good for brain tissue analyses. It was determined and previously reported that instrumental reliability between analyses was excellent for an artificial standardizing mixture.

Since modification of the 120-C, we have made complete analyses of the amino acid content of the brain tissue of twelve rats of all types. These include four Sprague-Dawley male controls, six Long-Evans male controls, and two Sprague-Dawley surplus animals. Of the last eight mentioned, six analyses cannot be quantitated because of unsatisfactory constants for data reduction. We can, however, use these data for qualitative work, including calculation of relative concentrations of amino acids for each animal. Quantitative estimates indicate that the results parallel those of other workers. [Shaw, R. K., and Heine, J. D., J. Neurochem., 12, 151 (1965); In Holden, J. T., ed., Amino Acid Pools, Elsevier Publishing Co., New York, 1962; Mussine, E., and Marcucci, F., "Free Amino Acids After Treatment with Psychotropic Drugs," p. 486; Tallan, H. H., "Free Amino Acids in Brain After Administration of Imipramine, Chloropromazine, and Other Psychotropic Drugs," p. 465; Agrawal, Davis, & Himwich, personal communication.]

From these analyses we have noted as peaks on chromatograms, 50 ninhydrin-positive substances (listed on the following page). This is a considerably greater number than reported by others except in the SHAW AND HEINE literature. This may be due to the fact that the Beckman 120-C, being a newer instrument than those available to other researchers, is more sensitive. We doubt that it is due to the method of tissue preparation. Our method is essentially similar to that reported by others with the exception that we have largely eliminated destructive enzyme action through LN freezing of fresh tissue.

Of the 50 substances we have noted, we have identified 27 by comparing their elution times with those of known standards. We find that these identification do not

contradict those in other researchers' publications. We have made tentative identifications of 7 other substances, but these will have to be confirmed in the same manner.

Of the 16 unknown peaks there are 3 which have appeared onat least two but not all chromatograms. These tend to be the chromatograms on which peaks are best defined and differentiated. These substances do not appear in sufficient amount to allow the possibility of quantification from a single brain sample. They may be identifiable if they prove not to be artifacts.

There are 4 more peaks which are strain-specific in the data we have gathered thus far, 2 appearing in the analysis of tissue from Long-Evans animals and 2 from Sprague-Dawley. Although these 4 substances also are presently unquantifiable, we will attempt to identify them if they continue to prove strain-specific.

NINHYDRIN-POSITIVE SUBSTANCES IN RAT BRAIN

Name in CAPS indicates positive identification.
Name in lower case indicates tentative identification.

1. unknown
2. phosphoserine
3. glycerophosphoethanolamine
4. PHOSPHOETHANOLAMINE
5. unknown (440 mμ only - phosphohydroxyproline?)
6. TAURINE
7. UREA
8. unknown (Long-Evans strain only)
9. unknown
10. unknown
11. unknown
12. unknown, uncertain (Sprague-Dawley strain only
13. ASPARTIC ACID
14. THREONINE
15. SERINE
16. glutamine and/or asparagine (may be two undifferentiated peaks)
17. GLUTATHIONE (occasionally broad, underlying 13-20)
18. unknown (may be second glutathione peak)
19. PROLINE (440 mμ only)
20. GLUTAMIC ACID
21. GLYCINE
22. ALANINE
23. unknown
24. unknown
25. unknown, uncertain
26. VALINE
27. CYSTEINE
28. Buffer change peak, may be fused with unknown trace
29. CYSTATHIONINE
30. unknown
31. METHIONINE
32. unknown
33. ISOLEUCINE
34. LEUCINE
35. unknown (Long-Evans strain only)
36. TYROSINE
37. PHENYLALANINE
38. α-alanine
39. α-aminoisobutyric acid
40. GABA
41. ORITHINE
42. ethanolamine
43. AMMONIA (often contaminated from atmosphere)
44. unknown, uncertain (Sprague-Dawley only)
46. HISTIDINE
47. unknown, uncertain
48. CARNOSINE
49. TRYPTOPHAN
50. ARGININE

V - DATA PROCESSING

At present, fifty amino acid and related compounds have been noted on chromatograms of rat brain tissue. The calculation of the amounts of these compounds alone would require considerable effort if done on an office calculator. We have been fortunate in gaining access to an IBM 1620 Computer for reduction of this and other data. We have written and tested four programs for analyzing the data from these chromatograms. These are presented in Appendix III. Further programs are being written to analyze the raw data from the amine determinations in the same manner. Others will be written or obtained from the program library to analyze the significance of differences which may be found to be correlated with such different experimental parameters such as atmosphere, lenght of exposure, and strain of animal.

Program ICBAA1 calculates the instrumental constant for each amino acid from raw data taken from chromatograms and the amount of sample in each standardizing analysis. The output from this program is a deck of punched cards containing the constants for each amino acid suitable for inclusion as input to Program ICBAA3. The recovery for each amino acid will be recorded on these cards.

Program ICBAA2 compiles data for Program ICBAA3 in a form which greatly reduces running time for the latter program. The two programs may be combined if it is found that the storage capacity of the 1620 to which we have access is great enough.

Program ICBAA3 calculates the amount in μM/g brain tissue of each amino acid in each tissue sample in an experimental group. It also calculates the mean and standard deviation of each such amount over the experimental group and prints out the results in a table, identifying each value. The input to this program is the raw data from the chromatogram, the animal number, the brain weight, the fraction of total tissue extract analyzed for each chromatogram, the recovery value for each amino acid, and a table of instrumental constants for each amino acid. The program as now written assumes 100% recovery of all amino acids in the preparation procedure. This assumption will be made except where recovery values can be calculated. The program will be modified to utilize these recoveries as soon as they are available for any amino acids.

Program ICBAA4 calculates the cross-product ratio between each amino acid and other amino acids across each animal and across the average for an exposure and prints this out in a table. The input to this program is the output of Program ICBAA3. Values for amines can be included as input.

All input data is recorded on mark-sense cards by student help and is machine-punched: Thus keypunch is needed only occasionally, for information conclusion to be placed on print-out. All outputs for human consumption are arranged in tabular form, eliminating further data recording.

BRIEF FINANCIAL REPORT

The following report is correct as of 12 April 1966:

Award:		$37,022.00
Personnel, expended through 1 April	$ 1,884.34	
Fringe benefits, " " "	650.79	
Personnel, encumbered through 1 May	976.87	
Fringe benefits, " " "	81.75	
Consumable supplies	2,784.51	
Overhead on above	2,875.63	
Beckman 120-C (no O/H)	15,588.00	
TOTAL EXPENSES		$32,841.79
BALANCE		$ 4,180.21

Of the balance noted above, approximately $230 has been encumbered for supplies since receipt of that report.

Mr. Ray H. Sutton at Ames has informed us that a balance of this magnitude may be carried over into an additional year's accounting. As we do not expect the funds requested for the second year's support to be available until after 1 July 1966, we plan to utilize the balance of the funds on hand to insure continuity of the research. This will provide us with approximately $3,250, not including overhead, for two months' research. This will restrict us only in that, until after receipt of funds for the second year's research, we cannot stock large amounts of supplies and cannot purchase standards to be used in identifying the brain constituents presently unidentified.

In the coming year we contemplate considerably greater expenses in supplies, as preliminary studies are essentially completed and the volume of exposures and analyses will be much greater than in the past year. No large equipment expenses will occur.

ENSUING RESEARCH

The second year's research will produce detailed information of the effects of atmospheric conditions on the biogenic amines and amino acids of rat brains. The details of this research have been covered in the previous discussions of experimental design and techniques developed during the past year.

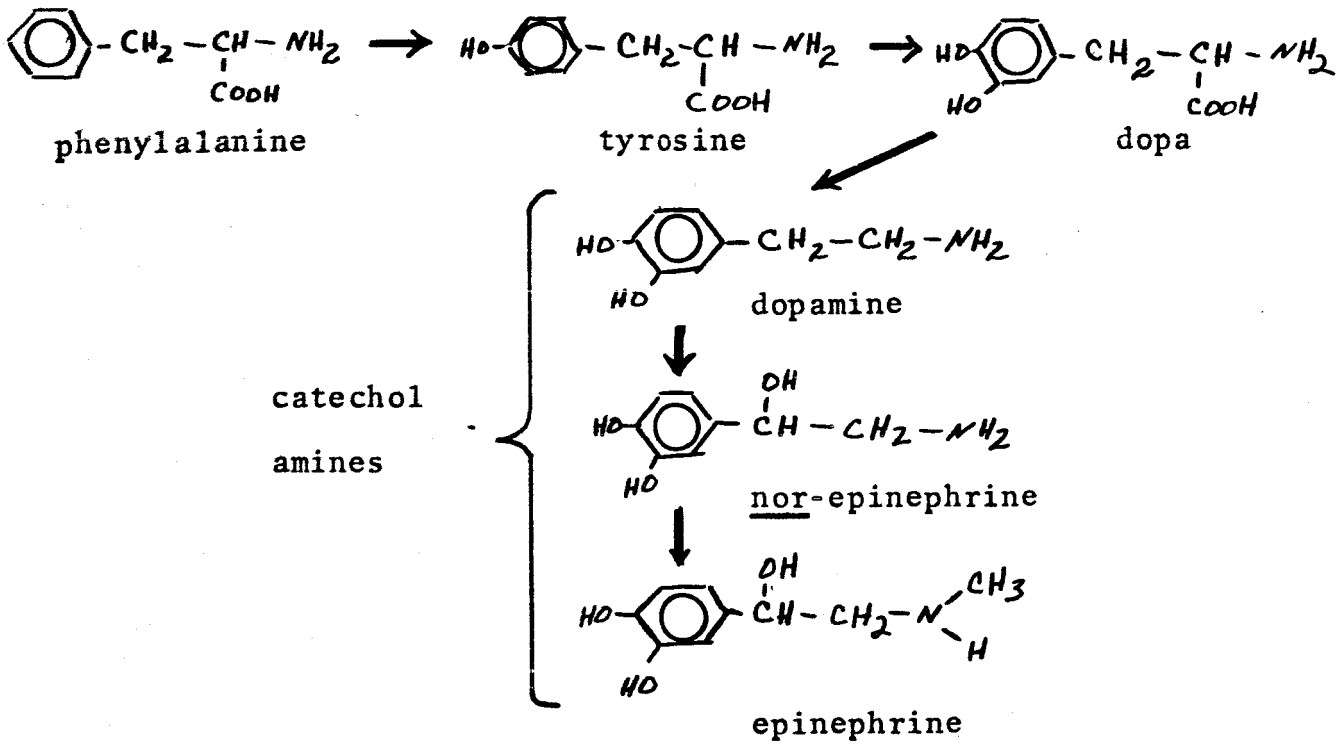
The opportunity to make the behavioral studies mentioned earlier depends upon the availability of personnel from the Psychology Department. This work will not be financed from funds on this grant.

We will be analyzing brain tissue for amino acids on a full-time basis. The speed of these analyses will determine the frequency of exposures of experimental groups. The data analysis is so designed that the baseline of the chemistry of the brains of control animals is gathered simultaneously with the data on experimental animals. An initial group of ten control animals of each strain will provide baseline data for interim decisions regarding the effects of experimental conditions on the experimental groups of five animals each. There will be one or two control animals provided for each experimental group in order that the baseline group be enlarged systematically.

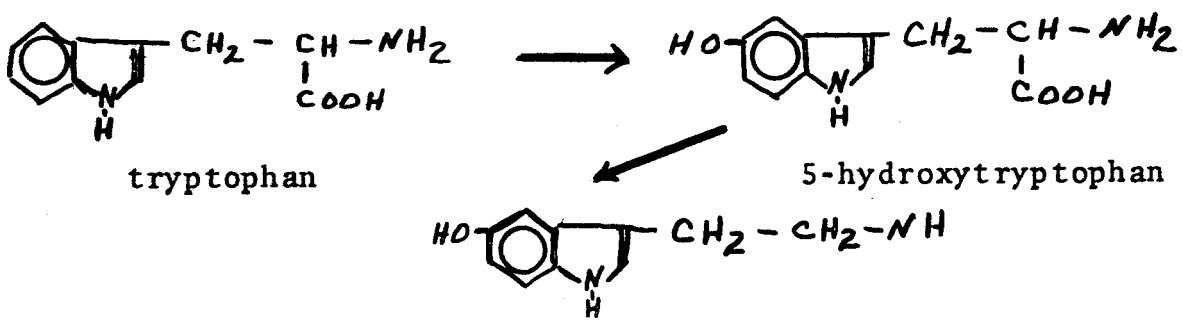
As previously mentioned, the selection of experimental conditions will be somewhat flexible, allowing the selection of those which will most clearly elucidate the biochemical effects of the exposures, or most strongly suggest that such effects are negligible, the primary end of exploratory research of this type.

Formation of Biogenic Amines

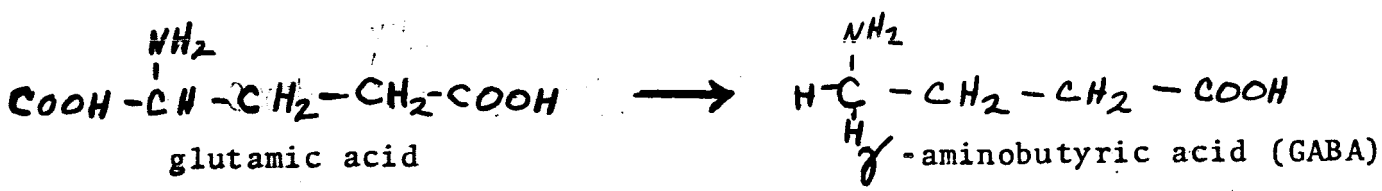
1.



2.



3.



APPENDIX II

INCREASED AUTOMATION OF AN AMINO ACID ANALYZER FOR PHYSIOLOGICAL FLUID ANALYSIS

The new Beckman Amino Acid Analyzer (Spinco Division of Beckman Instruments, Inc., Palo Alto, California) is designed to accelerate semiautomatic amino acid analysis by ion exchange chromatography. The principle of operation is that developed by SPACKMAN, STEIN AND MOORE (1) and produced as the Model 120-B. The newer Model 120-C incorporates a different cuvette assembly (2) for increased sensitivity and special ion exchange resins (3) to decrease analysis time. These modifications may be applied to the Model 120-B also. The operation procedures suggested for these instruments (4,5), in conjunction with the capabilities of the programming equipment, are especially applicable to automated analysis of protein hydrolysates.

The automation of the analysis for free amino acids associated with physiological fluids is less complete. The instrument requires almost constant attendance during the first several hours of an analysis. Further, in analysis of samples such as free amino acids associated with rodent brain, two independent determinations must be made. Such compounds as phenylalanine and tyrosine are present in quite low amounts, others such as glutamic acid and gamma-aminobutyric acid are present in relatively high amounts (6,7). If the concentrations of the former in the sample are sufficient for accurate calculation, the peak heights of the latter are recorded beyond the accurate range of the chart. This necessitates analysis of the sample in two dilutions.

By minor modifications of the programming equipment, fluid flow and cuvette assembly, it is possible to record all data simultaneously in only one analysis, with much less operator time.

Analysis of physiological fluids by the method of SPACKMAN, STEIN AND MOORE requires timed buffer change for each of two chromatographic columns, whereas analysis of protein hydrolysates requires timed buffer change for one column only. The 120-C provides automatic timed buffer change for one column and manual buffer change for the other. Thus, an operator is required to make the buffer change at the correct time, which may be up to four hours after initiation of analysis. By installation of an additional motorized valve, this buffer change can be automated, relieving the need of an operator and avoiding the possibility of error. The motorized valve added (Beckman part No. 313373) replaces the manual buffer change valve in the fluid flow, as shown in Figure 1. In the 120-C programming section, it is wired according to Figure 2. A four-pole double throw switch, SX1, is employed to select the valve, and thus the buffer system, to be controlled by the buffer change timer provided with the instrument. Two normally open, spring-loaded switches, SX2 and SX3, are provided for manual cycling of either motor valve from Buffer B to Buffer A. These are wired so that the valve under timed control cannot accidentally be cycled manually during an analysis under automatic control.

The instrument as supplied uses nitrogen pressure to drive the regenerating fluid through the ion exchange column. With the resins for accelerated analysis, regeneration should be continued until NaOH has been detected at the bottom of the

column (8). This regeneration may extend as long as two hours and an additional hour is needed for equilibration. These operations are not automated, thus requiring an operator's presence for four hours after initiation of an analysis. Regeneration and equilibration time can be shortened to only one hour by pumping the regenerating fluid, NaOH. By installing two manually operated valves, the NaOH can be routed to the pumps supplying the columns, as shown in Figure 1. These valves may be mounted conveniently on the selector valve panel. Valves on the inlet side of the pumps should be of a larger bore than valves on the outlet side. Small bore valves can be modified by replacing the sliding piston with Beckman part No. 312247. Alternatively, complete large bore valve assemblies (part No. 313370) may be installed.

The Model 120-C is supplied with three cuvettes for colorimetric analysis. The two which analyze at 570 mμ have optical path lengths of 6.6 mm and 2.2 mm. These provide two-level sensitivity with peak height ratios of approximately 3. This ratio provides insufficient range to record both high- and low-concentration compounds accurately in one sample. This ratio can be increased at least twofold by another modification, bringing peak height of high concentration compounds within the accurate range of the chart.

The tubular glass cuvette of the Model 120-B has a section with an optical path length of 0.7 mm. This cuvette also uses an external masking slit between the cuvette and the lamp of the colorimeter. The 0.7 mm section was removed from such a tubular cuvette and fitted with 12 mm spherical ground glass joints. This

assembly with its masking slit has approximately the same dimensions as the 2.2 mm cuvette of the 120-C and was installed in its place. The masking slit was mounted with a single screw in order that it might be removed easily for modifying its dimensions as needed. With this modified 0.7 mm cuvette and a masking slit size of 8 x 1 mm, a peak height ratio of approximately 6 is achieved. Table 1 gives comparative data.

Research requirements often exceed the capabilities of production instrumentation. With these modifications, the Beckman 120-C Amino Acid Analyzer is made more suitable for automated physiological fluid analysis.

ACKNOWLEDGMENT

This work was supported in part by a grant from the National Aeronautics and Space Administration, No. 05-029-001.

REFERENCES

1. Spackman, D. H., Stein, W. H., and Moore, S., Anal. Chem. 30, 1190 (1958).
2. Hubbard, R. W., and Kremen, D. M., Anal. Biochem. 12, 593 (1965).
3. Benzon, J. V., and Patterson, J. A., Anal. Biochem. 13, 265 (1965).
4. Beckman Instruction Manual AIM-2.
5. Beckman Instruction Manual AIM-3.
6. Shaw, R. K., and Heine, J. D., J. Neurochem. 12, 151 (1965)
7. Furst, A., and Chermside, H. B., unpublished data.
8. Slocum, R., and Topp, D., of Beckman Instruments, Personal Communication.

Table 1

Heights and ratios of peak heights for 570 mu cuvettes of representative amino acids.

Sample	Amount uM	Before Modification Height[a] Normal	Attenuated	Ratio Norm/Att	After Modification Height[a] Normal	Attenuated	Ratio Norm/Att
Glutamic Acid	0.10	244	80	3.05	254	41	6.19
	0.10	250	83	3.01	237	39	6.08
Aspartic Acid	0.10	336	113	2.97	380	63	6.04
	0.10	380	128	2.97	-	-	-
Tyrosine	0.10	232	77	3.01	246	40	6.15
	0.10	240	79	3.04	244	40	6.10
Phenylalanine	0.10	222	73	3.04	252	38	6.64
	0.10	233	77	3.03	236	40	5.90
gamma-Aminobutyric Acid	0.50	-	-	-	1150[b]	196	5.86
	0.25	655	213	3.07	-	-	-
			Ave Ratio	3.02		Ave Ratio	6.12

a. Height in chart units. Chart on log scale 0 - ∞, range of acceptable readings 0 - 1000.

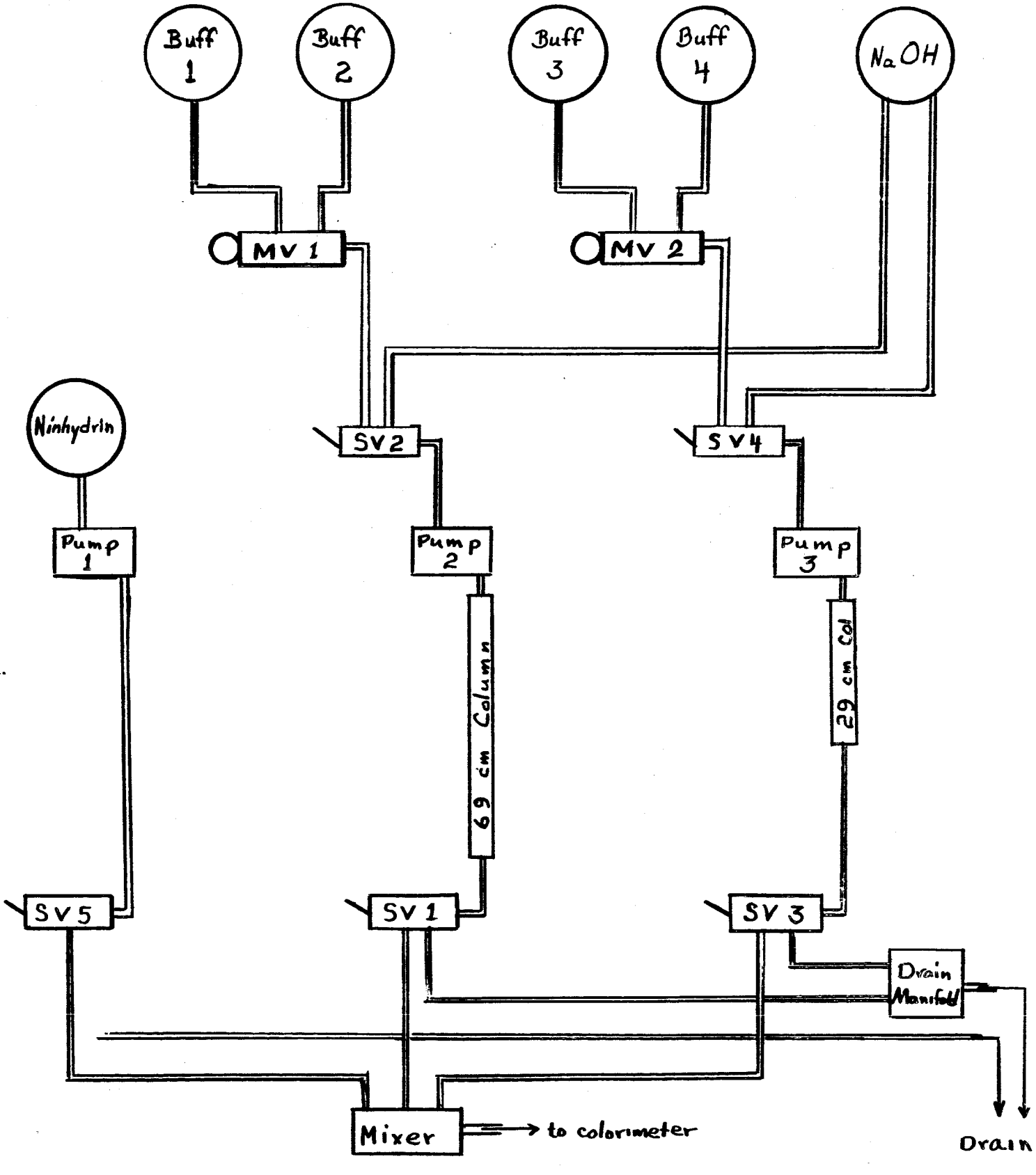
b. This value approximate lies beyond range of accurate reading of the chart.

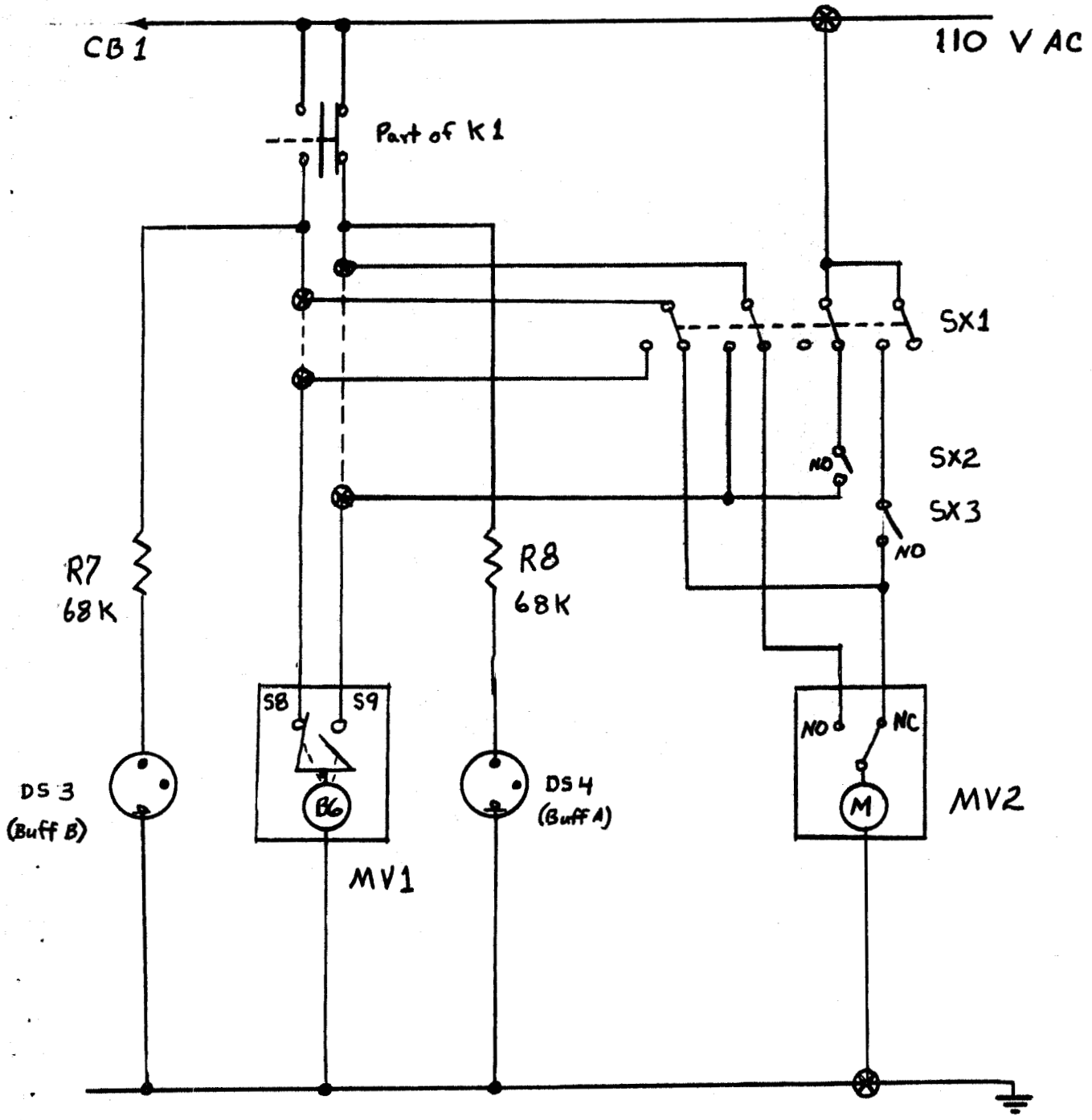
LEGENDS FOR FIGURES

Fig. 1--Schematic drawing of fluid flow.

Fig. 2--Electrical circuit for valve control. Section A is installed in the 120-C as delivered. Section B is the added circuitry. Dashed lines indicate original circuitry. ⊠ indicates connection of added circuitry to original.

Fig 1


Buff 1
Buff 2
Buff 3
Buff 4
NaOH
MV 1
MV 2
Ninhydrin
SV2
SV4
Pump 1
Pump 2
Pump 3
69 cm Column
29 cm Col
SV 5
SV 1
SV 3
Drain Manifold
Mixer
to colorimeter
Drain



Sx1 = 4pdt

Sx2 = spst, NO

Sx3 = spst, NO

MV2 = Beckman
pt# 313373

Fig 2

```
3400032007013600032007024902402511963611300102
ZZJOB                                  MICHAEL A KELLY
ZZDUP
*DELETMAKAAM
ZZJOB
ZZDUP
*DELETMAKAA1
ZZJOB
ZZDUP
*DELETMAKAA2
ZZJOB
ZZDUP
*DELETMAKAA3
ZZJOB
ZZDUP
*DELETMAKAA4
```

```
ZZJOB
ZZFOR
*LDISKMAKAAM
*FANDK0808
C       MAKAAM
C       PROGRAMMER          MICHAEL A KELLY
C                           INSTITUTE OF CHEMICAL BIOLOGY
C                           UNIVERSITY OF SAN FRANCISCO
        DIMENSION  KALL(5),JB(5)
        GOTO530
500     PRINT505
505     FORMAT(    28H-CLEAR PUNCH BY REMOVING ANY /
       1           28H CARDS FROM THE PUNCH HOPPER /
       2           28H AND PRESS -NON PROC RUNOUT- /
       3           25H ON THE PUNCH SIDE OF THE /
       4           10H 1622 UNIT //)
        PRINT510
510     FORMAT(    29H-RELOAD THE PUNCH FEED HOPPER /
       1           17H WITH BLANK CARDS //
       2           29H-PLACE DATA DECK IN READ FEED /
       3            7H HOPPER //
       4           20H-PRESS CONSOLE START //)
        PRINT520
520     FORMAT(    31H-PRESS READER START ON THE 1622 //
       1           30H-PRESS READER START TO FEED IN /
       2           20H THE LAST DATA CARDS //
       3           30H-PRESS PUNCH START ON THE 1622 //)
        JC=JB(I)
        GOTO(640,680,720,760),JC
530     DO535L=1,5
        KALL(L)=0
535     JB(L)=0
        PRINT540
540     FORMAT(    31H-LOAD ICBAA- PROGRAM CALL CARDS /
       1           20H PLUS ONE BLANK CARD //
       2           30H-PLACE CALL CARDS IN READ FEED /
       3           30H HOPPER AND PRESS READER START /
       4           17H ON THE 1622 UNIT //)
        PRINT550
550     FORMAT(    30H-PRESS READER START TO FEED IN /
       1           20H THE LAST CALL CARDS  //)
        I=1
```

```
560   READ575,KALL(I)
575   FORMAT(5XI1)
      IF(KALL(I))580,580,570
570   JB(I)=KALL(I)
      I=I+1
      GOTO560
580   I=1
590   IF(JB(I))600,600,610
600   I=I+1
      GOTO590
610   JC=JB(I)
      GOTO(620,660,700,740),JC
620   PRINT630
630   FORMAT(   31H-AMINO ACID CONSTANTS AVERAGING /
     1          16H PROGRAM  ICBAA1  //)
      GOTO500
640   CALLMAKAA1
      PRINT650
650   FORMAT(   12H-CLEAR PUNCH //
     1          25H-SET CONSTANTS DECK ASIDE //)
      I=2
      GOTO590
660   PRINT670
670   FORMAT(   26H-AMINO ACIDS DATA ASSEMBLY /
     1          16H PROGRAM  ICBAA2  //)
      GOTO500
680   CALLMAKAA2
      PRINT690
690   FORMAT(   12H-CLEAR PUNCH  //
     1          20H-SET DATA DECK ASIDE //)
      I=3
      GOTO590
700   PRINT710
710   FORMAT(   33H-AMINO ACID CONCENTRATION PROGRAM /
     1           7H ICBAA3 //)
      GOTO500
720   CALLMAKAA3
      PRINT730
730   FORMAT(   12H-CLEAR PUNCH //)
      I=4
      GOTO590
740   PRINT750
750   FORMAT(   29H-CONCENTRATIONS RATIO PROGRAM /
```

```
      1           7H 1CBAA4 //)
        GOTO500
  760   CALLMAKAA4
        PRINT770
  770   FORMAT(    12H-CLEAR PUNCH //)
        CALLEXIT
        END
```

```
ZZJOB
ZZFOR
*FANDKO808
*LDISK
      SUBROUTINE MAKAA1
C     MAKAA1
C     AMINO ACID CONSTANT AVERAGING PROGRAM
C     PROGRAMMER          MICHAEL A KELLY
C                         INSTITUTE OF CHEMICAL BIOLOGY
C                         UNIVERSITY OF SAN FRANCISCO
1     FORMAT(4A4,1XF5.2,1XF5.2,1XF4.0,1XF4.0,39X13)
2     FORMAT(I3,1XF4.3,1XF3.1,3XF4.3,1XF3.1,3XF6.3)
3     FORMAT(25HDATA IN INCORRECT ORDER     )
4     FORMAT(4A4,2(1XI5)2(1XI4)2(1XF8.3)2(1XI3)
99    READ1,AA,AB,AC,AD,CN,CA,UN,UA,K
      IF(K)10,10,20
10    RETURN
20    READ2,ID,HN,WN,HA,WA,AM
      IF(K-ID)30,40,30
30    PRINT3
      RETURN
40    IF(HN+WN)50,60,50
50    CTN=(HN*WN)/AM
      KCN=((CN*UN+CTN)/(UN+1.))*100.
      JN=UN+1.
      GOTO65
60    CTN=0.
      KCN=CN
      JN=UN
65    IF(HA+WA)80, 70,80
80    CTA=(HA*WA)/AM
      KCA=((CA*UA+CTA)/(UA+1.))*100.
      JA=UA+1.
      GOTO90
70    CTA=0.
      KCA=CA
      JA=UA
      GOTO90
90    PUNCH4,AA,AB,AC,AD,KCN,KCA,JN,JA,CTN,CTA,K
      GOTO99
      END
```

```
ZZJOB
ZZFOR
*FANDK0808
*LDISK
      SUBROUTINE MAKAA2
C     MAKAA2
C     AMINO ACID DATA ASSEMBLY PROGRAM
C     PROGRAMMER          MICHAEL A KELLY
C                         INSTITUTE OF CHEMICAL BIOLOGY
C                         UNIVERSITY OF SAN FRANCISCO
      DIMENSIONKD1(56),KD2(56),KD3(56)
      K1=0
      K2=0
      K3=0
      K4=0
      DO10I=1,56
      KD1(I)=0
      KD2(I)=0
10    KD3(I)=0
1     READ2,NO,KUE,NO1,NO2,NACID
2     FORMAT(I3,I1,I5,I4,I2)
      IF(NO)6,6,3
3     IF(NACID)4,4,5
4     K1=NO
      K2=NO1
      K3=NO2
      GOTO1
5     I=NACID
      KD1(I)=KUE
      KD2(I)=NO1
      KD3(I)=NO2
      GOTO1
6     IF(NO)9,9,11
11    PUNCH7,K1,K2,K3
7     FORMAT(I3,2XI5,1XI4)
      PUNCH8,(KD1(I),KD2(I),KD3(I),I=1,56)
8     FORMAT(3XI1,I5,1XI4,I1,I5,1XI4,I1,I5,1XI4,I1,I5,1XI4,I1,I5,1XI4,
     1   I1,I5,1XI4,I1,I5,1XI4)
      GOTO1
9     RETURN
      END
```

```
ZZJOB
ZZFOR
*FANDK0808
*LDISK
      SUBROUTINE MAKAA3
C     MAKAA3
C     AMINO ACID BRAIN CONCENTRATION PROGRAM
C     PROGRAMMER          MICHAEL A KELLY
C                         INSTITUTE OF CHEMICAL BIOLOGY
C                         UNIVERSITY OF SAN FRANCISCO
      DIMENSIONA(37),NAME(56,4),CA(56),CB(56),SUM(56),SS(56),FN(56),
     1  ID(25),WT(25),AF(25),IG(56),HT(56),NN(56),CONC(56),AMEAN(56),
     2  SD(56),WD(56)
C  FORMAT STATMENTS
C  INPUT FORMAT
1     FORMAT(3X13,37A2)
2     FORMAT(4A4,1XF5.2,1XF5.2,50X12)
3     FORMAT(13,2XF5.4,1XF4.4)
4     FORMAT(3X1X11,1XF4.3,1XF3.1,
     11X11,1XF4.3,1XF3.1,1X11,1XF4.3,1XF3.1,1X11,1XF4.3,1XF3.1,
     21X11,1XF4.3,1XF3.1,1X11,1XF4.3,1XF3.1,1X11,1XF4.3,1XF3.1)
C  OUTPUT FORMAT
5     FORMAT (2X,13,1X37A2)
6     FORMAT(/8X,14HANIMAL NUMBER ,I4)
7     FORMAT(10X,31H MICROMOLES/GRAM OF BRAIN WT.  )
8     FORMAT(8X13,1X4A4, 9XF8.3)
9     FORMAT(/9X,36HSUMMARY TABLE FOR ANIMALS NUMBERED  )
11    FORMAT(12X,17(1X,I3))
12    FORMAT(8X,4HNAME,20X,1HN,7X,4HMEAN,10X,18HSTANDARD DEVIATION)
13    FORMAT(5X13,1X4A4,6X15,F9.3,11XF8.4)
C  READ SEQUENCE
99    READ1,EXT,(A(I),I=1,37)
      N=56
      L=0
      DO100I=1,N
      CA(I)=0.
      CB(I)=0.
      NN(I)=0.
      SUM(I)=0
      SS(I)=0
100   FN(I)=0
      IF(N)10,10,20
```

```
20      DO105J=1,56
        READ2,NA,NB,NC,ND,DA,DB,NACID
        IF(NACID)107,107,106
106     I=NACID
        NAME(I,1)=NA
        NAME(I,2)=NB
        NAME(I,3)=NC
        NAME(I,4)=ND
        CA(I)=DA
105     CB(I)=DB
107     M=0
66      M=M+1
        READ3,ID(M),WT(M),AF(M)
        IF(ID(M))80,80,30
30      READ4,(IQ(I),HT(I),WD(I),I=1,N)
C   CALCULATIONS SEQUENCE
        DO110I=1,N
        IF(IQ(I))40,40,50
40      IF(CA(I))110,110,45
45      AMT=(HT(I)*WD(I))/CA(I)
        GOTO60
50      IF(CB(I))110,110,55
55      AMT=(HT(I)*WD(I))/CB(I)
60      CONC(I)=AMT/(WT(M)*AF(M))
        IF(CONC(I))110,110,70
70      FN(I)=FN(I)+1.
        SUM(I)=SUM(I)+CONC(I)
        SS(I)=SS(I)+CONC(I)*CONC(I)
110     CONTINUE
C   OUTPUT SEQUENCE
        IF(L)90,90,95
90      PUNCH5,EXT,(A(I),I=1,37)
        L=L+1
95      PUNCH6,ID(M)
        PUNCH7
        DO125 I=1,N
        IF(CA(I))126,126,125
125     PUNCH8,ID(M), (NAME(I,J),J=1,4),CONC(I)
126     GOTO66
80      DO130I=1,N
        IF(FN(I))130,130,96
96      AMEAN(I)=(SUM(I)/FN(I))+0.0005
        SD(I)=SQRT ((FN(I)*SS(I)-(SUM(I)*SUM(I)))/(FN(I)*(FN(I)-1.)))
```

```
      NN(I)=FN(I)
  130 CONTINUE
      PUNCH9
      M=M-1
      PUNCH11,(ID(J),J=1,M)
      PUNCH12
      DO135I=1,N
      IF(CA(I))135,135,134
  134 PUNCH13, EXT,(NAME(I,J),J=1,4),NN(I),AMEAN(I),SD(I)
  135 CONTINUE
      GOTO99
   10 PRINT14
   14 FORMAT(      31H-CLEARPUNCH - SET OUTPUT ASIDE  //
     1             34H-USE NEXT OUTPUT FOR RATIO PROGRAM   //
     2            12H-PRESS START  //)
      PAUSE
      DO129I=1,N
      IF(CA(I))129,129,128
  128 PUNCH8,ID(M),(NAME(I,J),J=1,4),CONC(I)
  129 CONTINUE
      RETURN
      END
```

```
ZZJOB
ZZFOR
*FANDK0808
*LDISK
      SUBROUTINE MAKAA4
C     MAKAA4
C     AMINO ACIDS RATIO OF CONCENTRATIONS PROGRAM
C     PROGRAMMER          MICHAEL A KELLY
C                         INSTITUTE OF CHEMICAL BIOLOGY
C                         UNIVERSITY OF SAN FRANCISCO
1     FORMAT (5A4,19X,F6.3,35X)
4     FORMAT(8X20HRATIO TABLE                )
5     FORMAT( 8X1X1H(I2,1H/I2,1H)F8.3,
     1          1X1H(I2,1H/I2,1H)F8.3,
     2          1X1H(I2,1H/I2,1H)F8.3,
     3          1X1H(I2,1H/I2,1H)F8.3)
6     FORMAT(8X13HSUMMARY TABLE   8X15HSAMPLE SIZE IS   I2//18X8H  NAME
     1   10X3HKEY2X8HMEAN    8X18HSTANDARD DEVIATION  //)
7     FORMAT(8X5A4,5XI3,1XF10.3,10XF6.2,18X)
      DIMENSION NAME(35,5),CONC(35),RATIO(35,35),SUM(35),SS(35),SD(35),
     1 AMEAN(35)
50    I=1
60    READ1,(NAME(I,J),J=1,5),CONC(I)
      IF(CONC(I))100,100,80
80    I=I+1
      GOTO60
100   N=I-1
      IF(N)120,120,130
120   RETURN
130   DO125I=1,N
      SUM(I)=0.
125   SS(I)=0.
      DO170I=1,N
      DO170J=1,N
      RATIO(I,J)=CONC(J)/CONC(I)
      SUM(I)=SUM(I)+RATIO(I,J)
170   SS(I)=SS(I)+RATIO(I,J)*RATIO(I,J)
      FN=N
      DO175I=1,N
      AMEAN(I)=(SUM(I)/FN)+0.0005
```

```
      WRITH7,((NAME(I,J),J=1,5),I,AMEAN(I),SD(I),I=1,N)
      FORMA
      PUNCH5,((I,J,RATIO(I,J),J=1,N),I=1,N)
      GOTO50
      END
```