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HUMAN PATHOLOGY

May 5, 1975

Dr. Robert McConnell
Director, Toxicology and
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Searle Laboratories
Box 5110
Chicago, Illinois 60680

Dear Doctor McConnell:

I have reviewed your attempt to tabulate, correlate and report on the late Dr. Harry Waisman's data as regards the administration of SC-18862 to mated and pregnant Rhesus monkeys. It is necessary for me to assume that the laboratory staff gave you all of the data available. However, I must conclude that the data reflect an exploratory effort perhaps to ascertain important aspects for a more complete and detailed study. In its present form, the Waisman data can be considered as fragmentary, very preliminary and of questionable scientific value.

While I realize your desire to collect all biological data concerning SC-18862 and disseminate this information to appropriate individuals, I am afraid that the incompleteness of the data severely limits its usefulness. Let us consider some of the serious defects in the collected data.

Post-mating data on Eight Female Rhesus Monkeys

Animal 879 - delivered an infant, condition and disposition unknown
Animal 863 - not pregnant, not re-mated
Animal 830) - presumed pregnant, no information on SC-18862 ingestion, no
Animal 831) - information on delivery or disposition of newborn
Animal A23 - delivered "stillborn" infant, condition and disposition of
fetus unknown
Animal A39 - delivered live infant, due to inadequate care, died three
days post partum
Animal B24) - spontaneous "abortions;" condition and disposition of
Animal 836 - abortus unknown

There are no answers available for the following pertinent questions:

1. What criteria were used to select the animals?
2. What were the reproductive histories of these animals?
3. What was the status of their menstrual cycle at time of mating?
4. Who were the mates? How were they selected?
5. Why was there no daily evaluation of the maternal monkey?
6. How was pregnancy actually established?

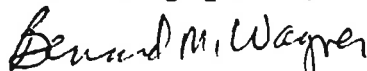
- 2 -

The term "stillbirth" used in the record is decidedly unclear and fails to prove that the newborn never lived. There is a striking lack of observations concerning this infant. Likewise, I must reject the use of the term abortion since prior pregnancy was not established and the expelled uterine contents never examined.

The summary presented on page 33 of the report is fully supported by the information made available. Briefly this is: SC-18862 does not alter maternal appetite or weight gain, there are no recognized malformations in the term fetus, serum phenylalanine levels in pregnant monkeys increase to predictable levels and if there were 2 abortions they were obviously spontaneous.

As an Editor of a scientific journal, I would find this report unacceptable for publication. It would be returned to the authors stating that the experimental design and evaluation of data are incomplete and not suitable for the scientific community. I trust these brief remarks will be of some value to you.

Sincerely yours,



Bernard M. Wagner, M.D.

BMW/mz

cc: Dr. P. Klimstra

SC-18862: EXPERIMENTS IN MATED AND PREGNANT RHESUS MONKEYS

A Compilation of Available Fragmentary Data

91v

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SC-18862: EXPERIMENTS IN MATED AND PREGNANT RHESUS MONKEYS

INTRODUCTION

This report has been compiled in an effort to make available to the Food and Drug Administration all information known to us regarding the experimental use of SC-18862 in animals. It is a compilation of fragmentary data on individual monkeys made available to Searle Laboratories by the laboratory staff of the late Dr. Harry A. Waisman, Professor of Pediatrics, University of Wisconsin Medical School, Madison, Wisconsin, regarding the administration of SC-18862 to mated and/or pregnant Rhesus monkeys. These data apparently reflect an exploratory effort perhaps to ascertain information for future studies. It is readily apparent that these data failed to establish or refute any given scientific hypothesis. All raw data records available to the authors are reproduced in this report.

Dr. Waisman's death appears to have necessitated abrupt termination of this exploratory research, irrespective of the pregnancy status of each monkey. We have conferred with the late Dr. Waisman's laboratory staff, with the intent of obtaining all data available from these experiments. We were informed on 6 February 1975 that these data constituted the entire data package; no additional data exists.

These experiments were presumably performed by the staff of the late Dr. Waisman's group at the Primate Research Center, Madison, Wisconsin, concurrent with other research on SC-18862 and newborn monkeys. The data presented in this report may constitute some exploratory work conceived and conducted unilaterally by Dr. Waisman or one or more of his staff, during this period when he was retained by Searle Laboratories as a consultant. Searle, however, had no knowledge of the purpose for which any of these animals were studied.

Conducting a series of tightly controlled experiments in the pregnant monkey of sufficient precision to confirm or refute a scientific hypothesis is a difficult task. Nonetheless, a failure to assess and control the critical variables renders the resulting data meaningless. Data on the following variables are considered critical in primate reproduction studies involving evaluation of a chemical agent; the presence or absence of such data in this series of experiments is indicated below:

<u>VARIABLE</u>	<u>WAS DATA INCLUDED?</u>
1) Age; maternal	NO
2) Reproductive history; maternal	NO
3) Menstrual cycle status prior to/at mating	NO
4) Identity of male parent	NO
5) Breeding record; paternal	NO
6) Data on prior progeny	NO
7) Evidence of established pregnancy (rectal palpat., etc.)	NO
8) Periodic assessment of gestational status	NO
9) Observations at parturition	NO
10) Physical description of aborted uterine contents	NO
11) Histopathologic examination of each conceptus/stillbirth	NO
12) Physical examination of each stillbirth/infant	NO
13) Postmortem examination of each stillbirth	NO
14) Previous experimental use of each animal	NO
15) Experimental conditions during the study (e.g., location, temperature, unexpected stress, diseases, drugs, etc.)	NO

For the above and other reasons, the data reported are not sufficient to establish the effects of huge quantities of SC-18362 in the pregnant Rhesus monkey. This report is submitted simply to comply with the obligation of providing all information known to us regarding this agent.

Because of the paucity and fragmentary nature of the data available, it is not apparent whether the procedures followed for each of the eight animals were the same or if they differed significantly from time to time or from animal to animal. The information available and the manner in which it was recorded does differ from animal to animal. Therefore, the experimental conditions and/or environment may also have differed, and comparison of data from animal to animal may not be scientifically meaningful. Certain aspects of the data generated do however deserve comment.

Procedure employed for determination of pregnancy.

Not known.

Compound administration.

The record for some animals indicates that SC-18862 was mixed with Similac liquid formula and administered orally, presumably ad libitum. Where such data are available the records show that the concentration of SC-18862 in Similac, the total daily intake volume, and duration of administration of SC-18862 varied markedly among monkeys. One monkey received unmodified Similac liquid formula only.

Physical examinations and observations.

No information is available regarding the procedures employed and the frequency of such examinations.

Body weight.

Body weight data are available on selected monkeys.

Clinical laboratory procedures.

The following parameters were evaluated in selected monkeys, employing SMA-12/60 analyses of serum specimens:

- | | |
|------------------------|---------------------------------------|
| 1. Total protein | 7. Blood urea nitrogen |
| 2. Albumin | 8. Uric acid |
| 3. Calcium | 9. Creatinine |
| 4. Inorganic phosphate | 10. Total bilirubin |
| 5. Cholesterol | 11. Alkaline phosphatase |
| 6. Glucose | 12. Glutamic oxaloacetic transaminase |

In addition, serum phenylalanine, tyrosine and tryptophan levels were monitored at various times in selected monkeys. Blood specimens were collected 3-4 hours following a routine dietary intake, often of varying volume.

RESULTS

The nature and volume of data available on each monkey varies considerably depending upon the duration of treatment. Hence, each monkey must be considered individually in the presentation of data. It is important to note that a concurrent control group of mated monkeys was not employed in this study. As a general guide, the body weight data from 31 historical control pregnant monkeys on an unidentified diet are superimposed in the graphs. Parturition was expected 168 ± 4 days post-conception.

The raw data received are as follows.

Figure 1

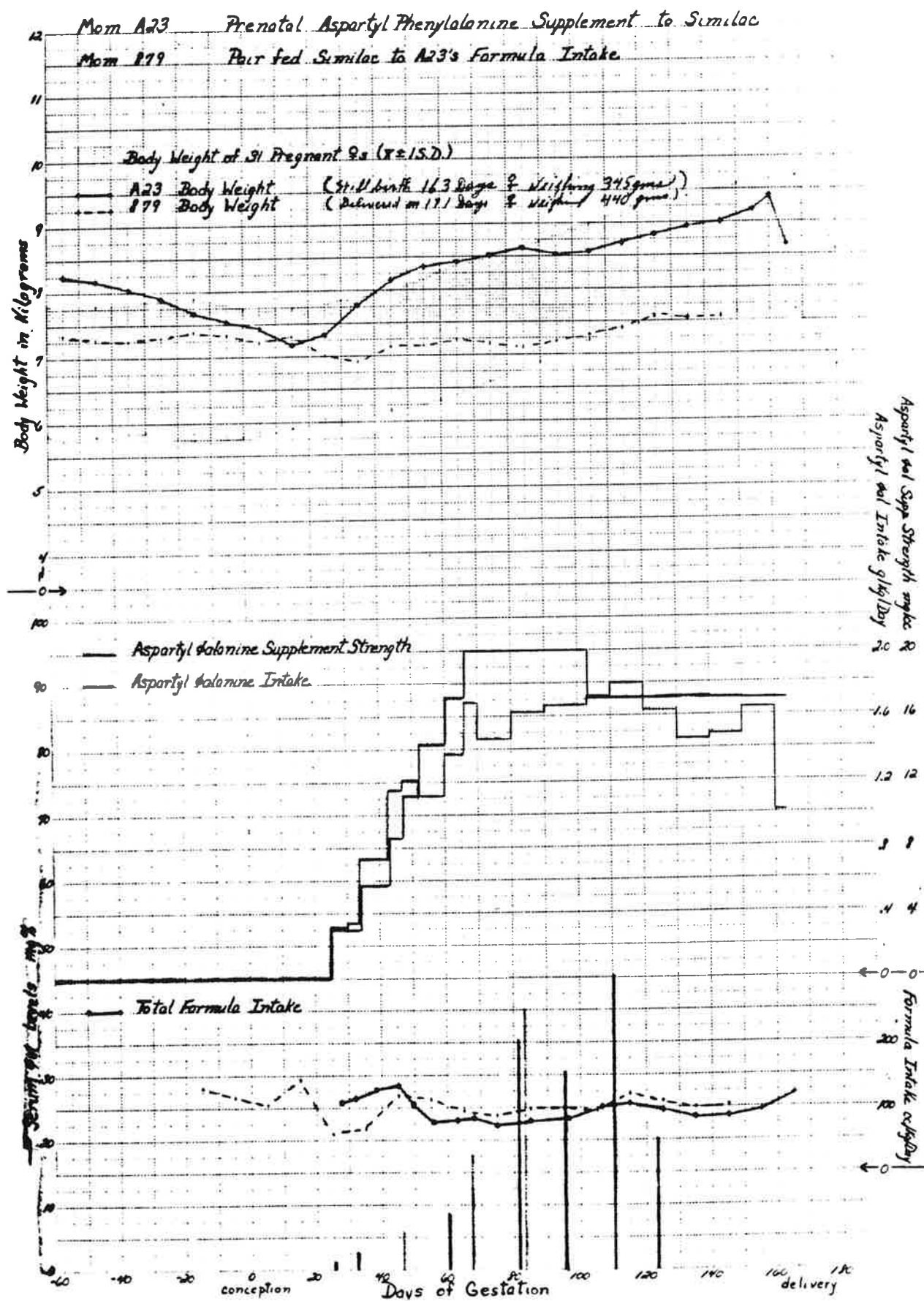
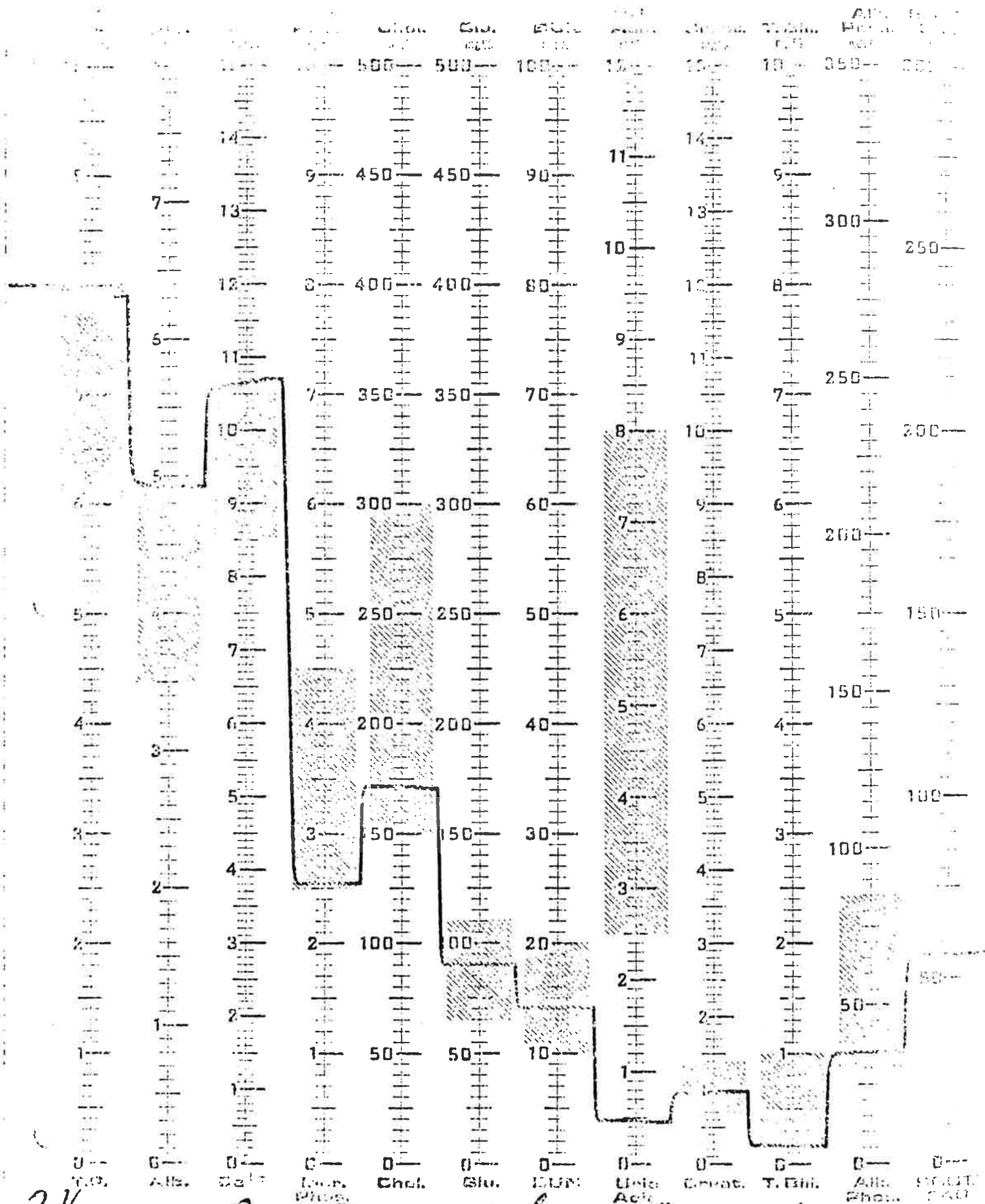


Figure 2

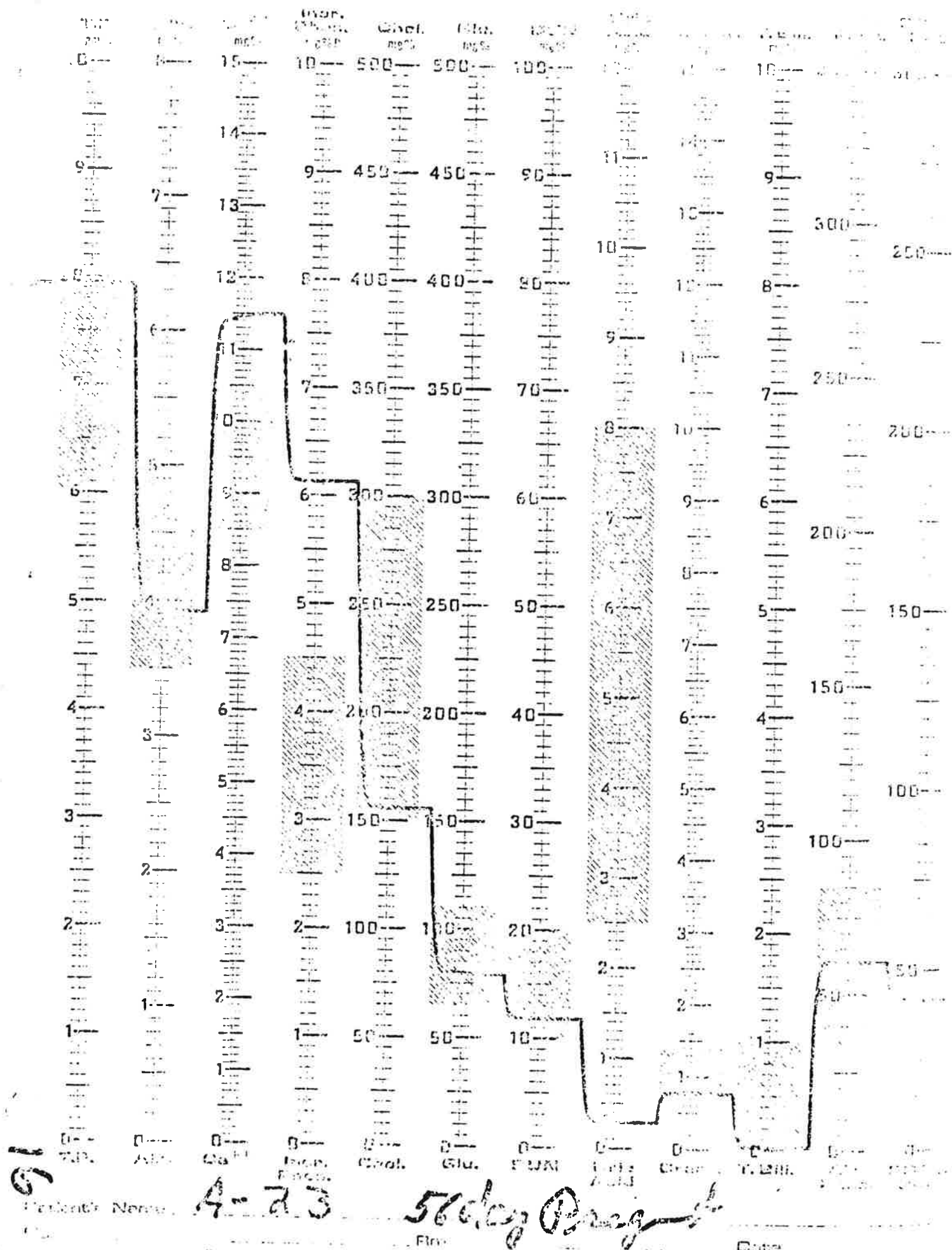


34

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1-53

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8
Figure 4

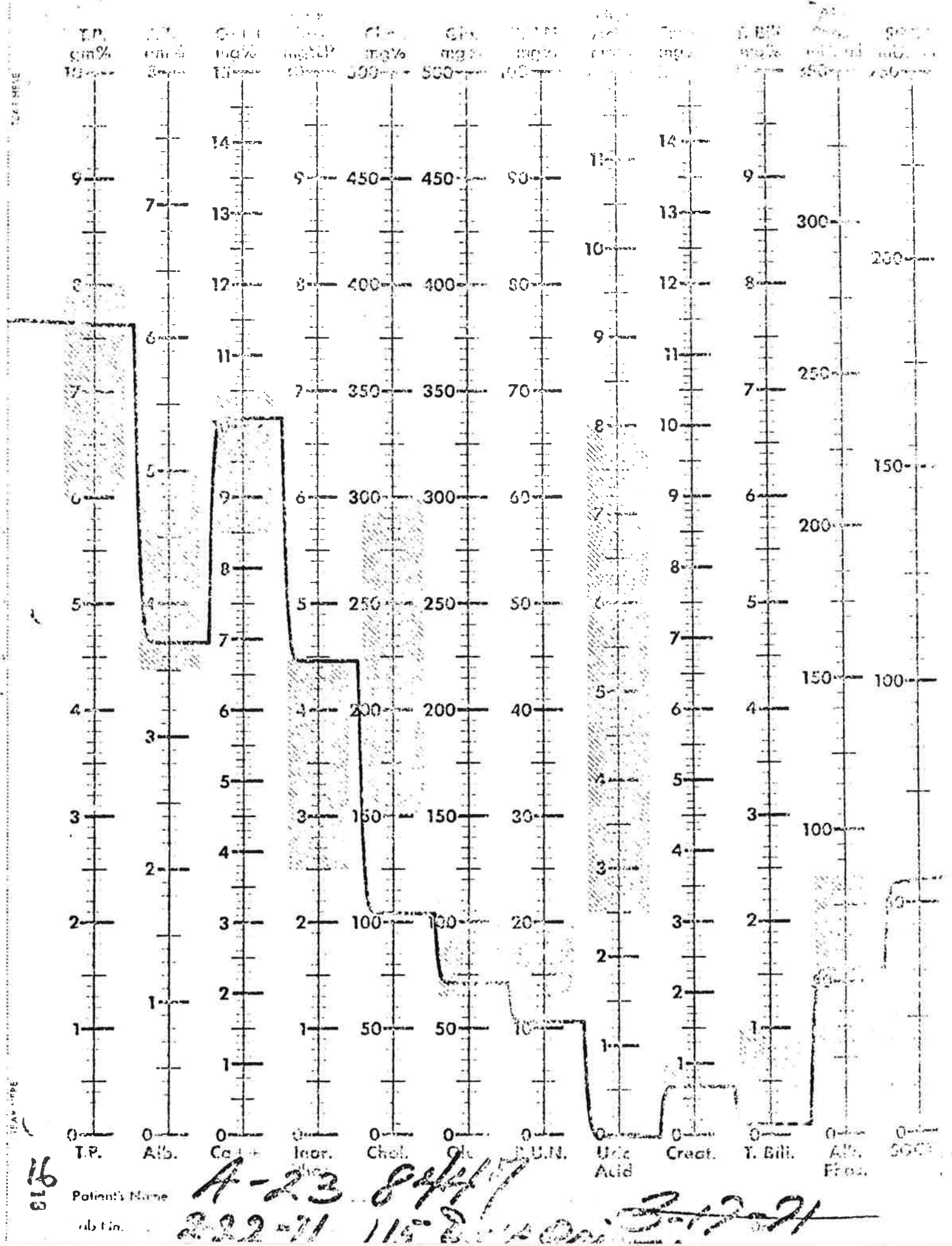
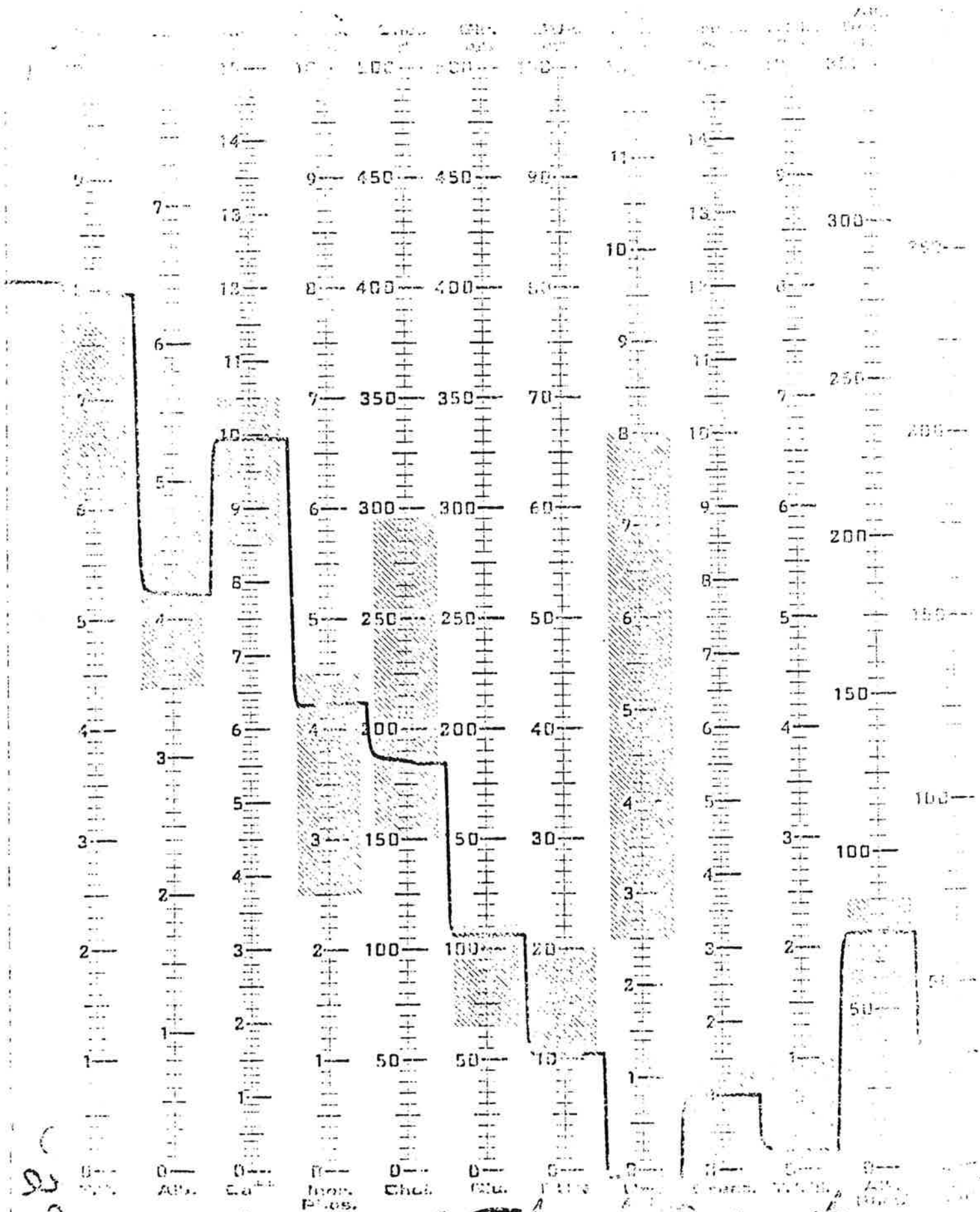


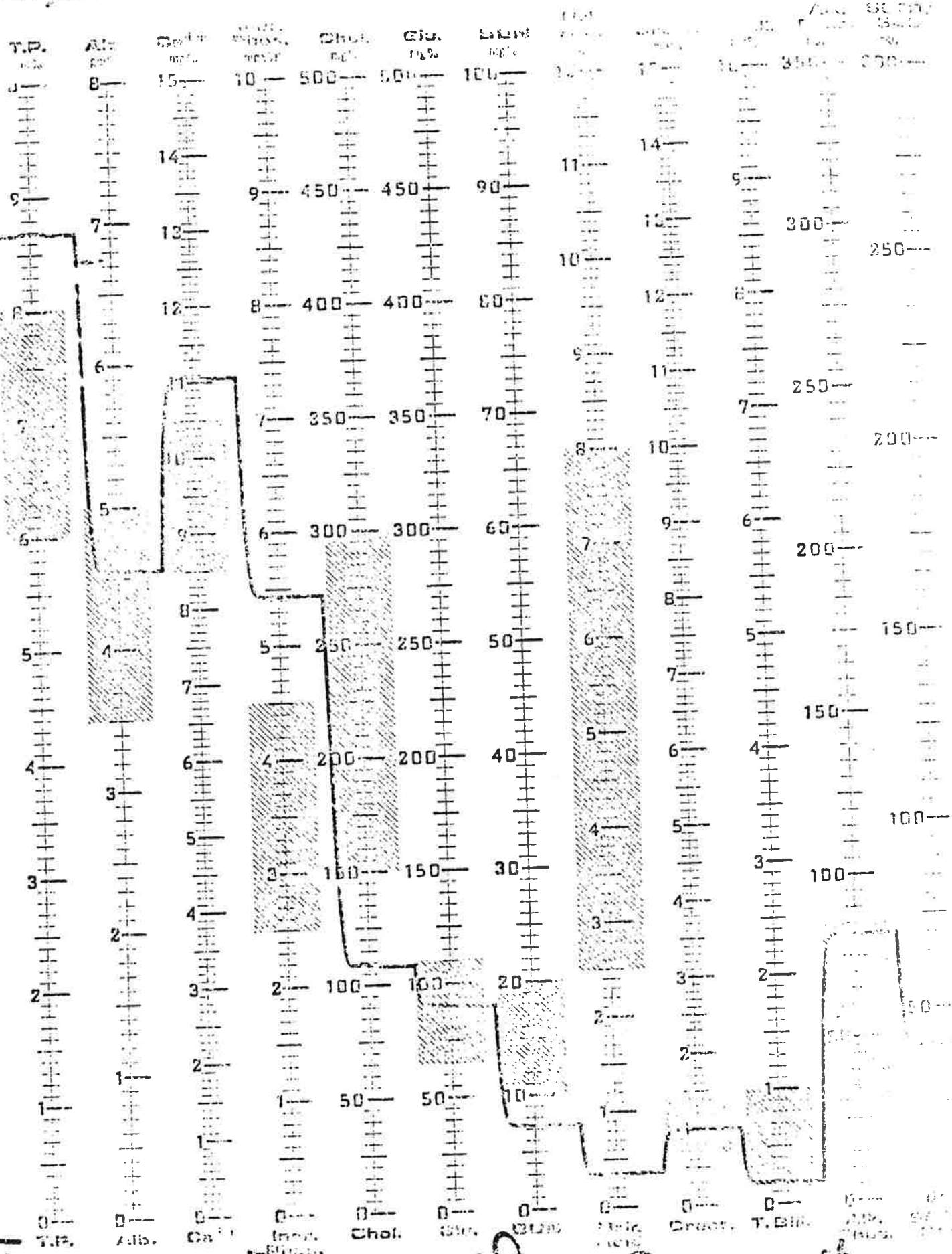
Figure 5



879 25

10
Figure 6

12/1/60



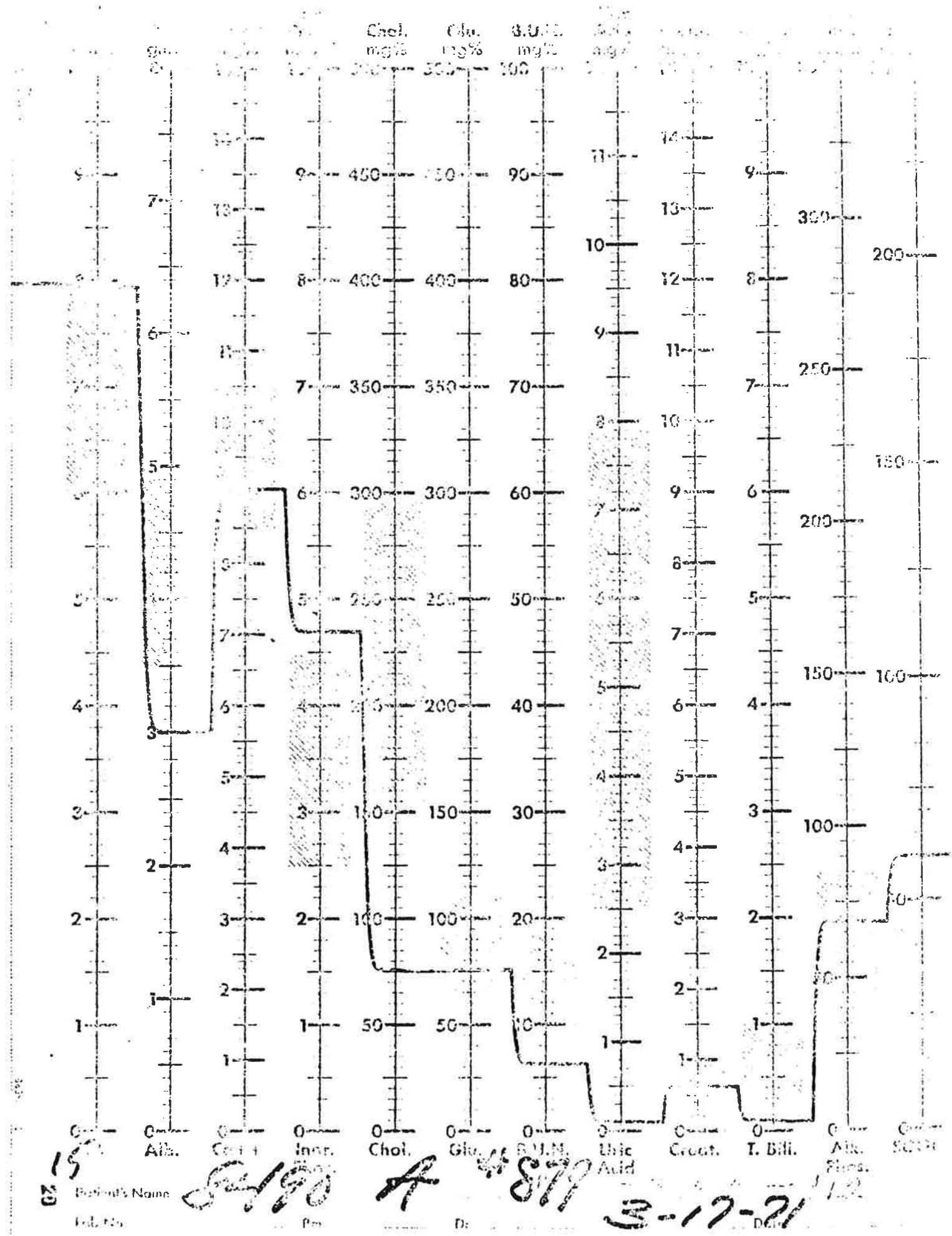
51

879

56 days Pregnant

Date

Figure 7

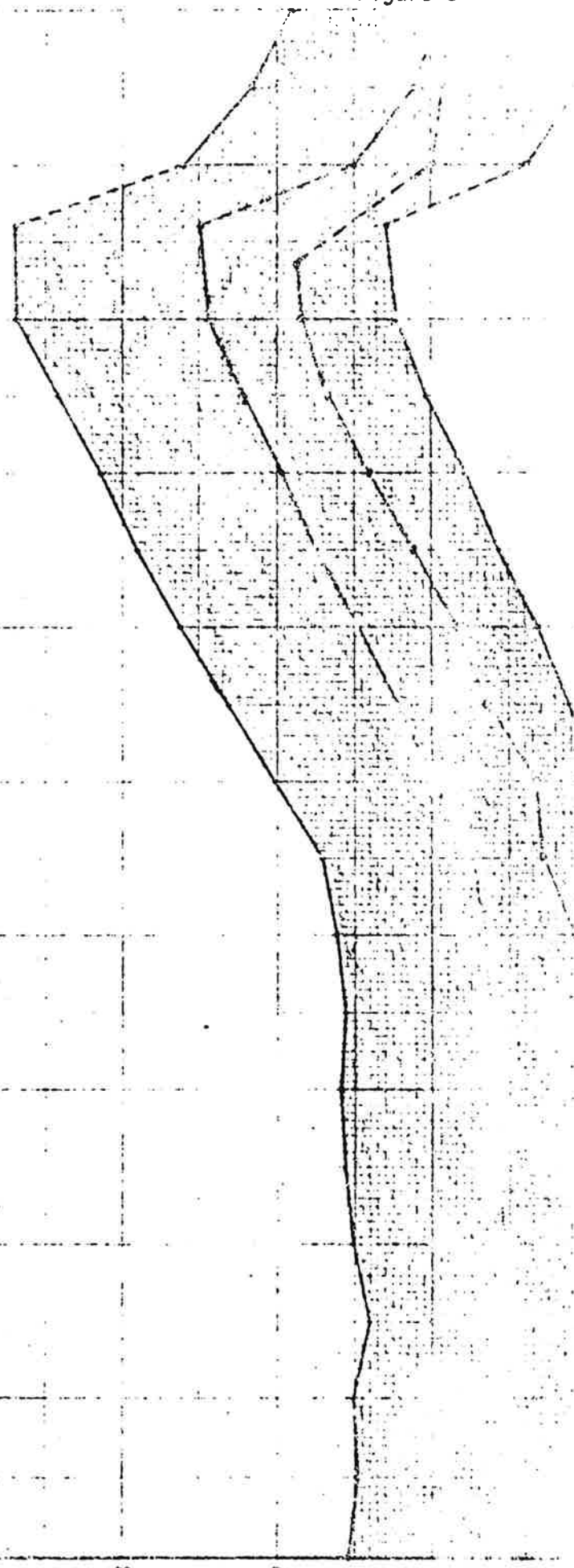


19 *5/90* *A 4877* 3-17-21
 Patient's Name *5/90* Date *3-17-21*
 Initials *A*

Weight Gain of Females Pregnant for the Test Time N=51

Control Diet Available - 100% Diet

A39' Aspartylol supp Day 112 to Delivery



Infant Wt.
430.7 ± 47.9
30 Males: 499.2
23 Females: 400.0

Number of Days for Calculation

Figure 8

Figure 9

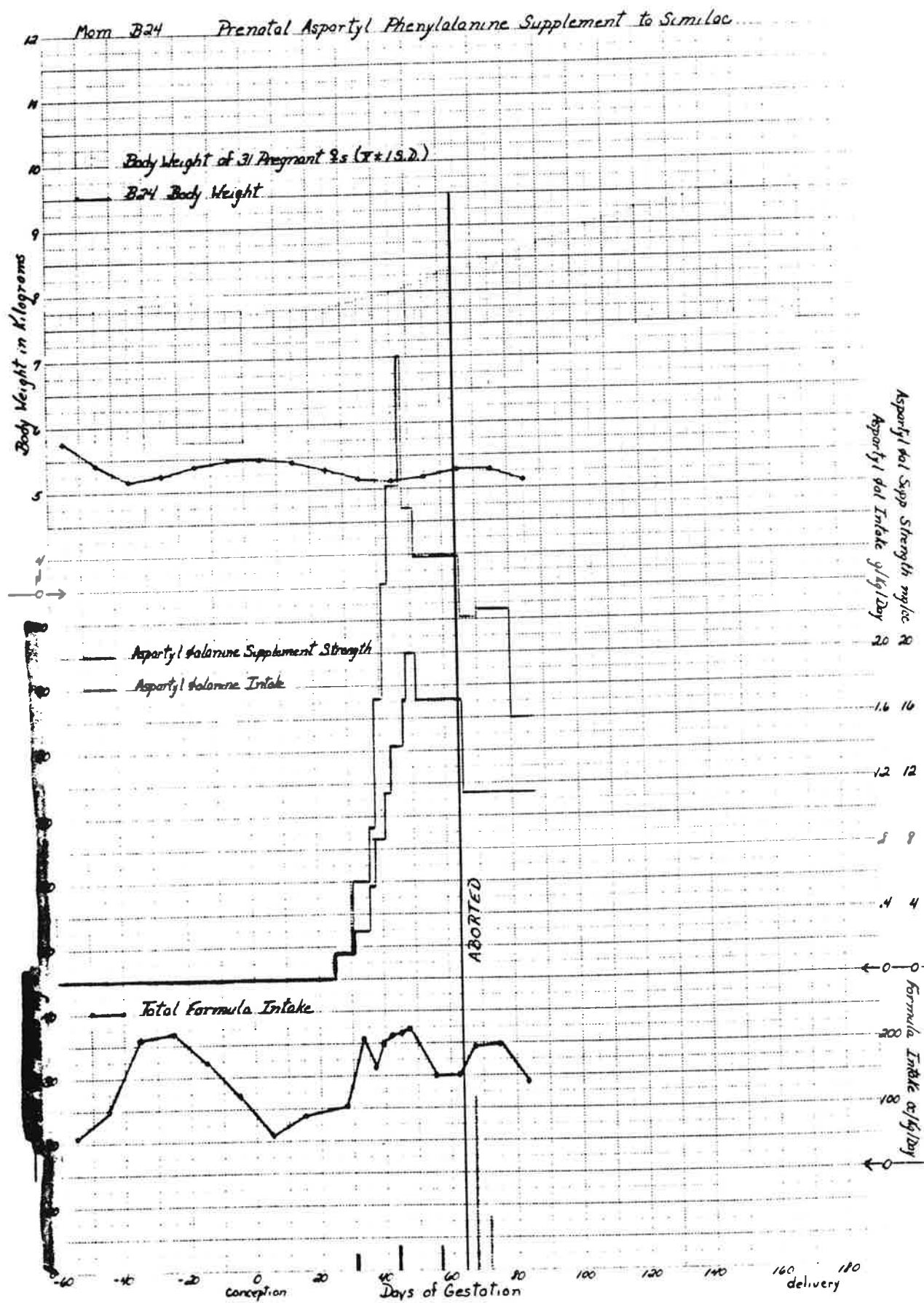
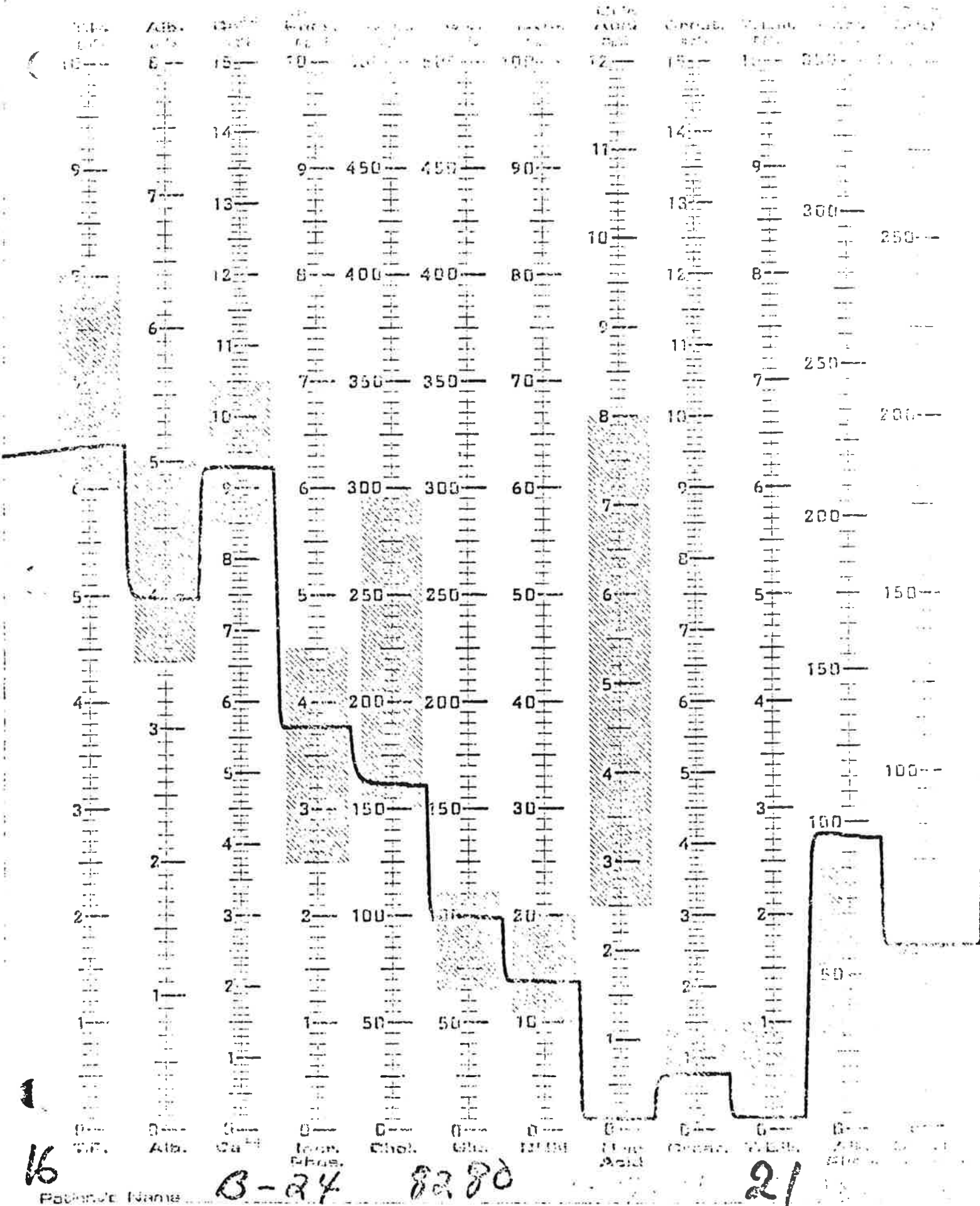
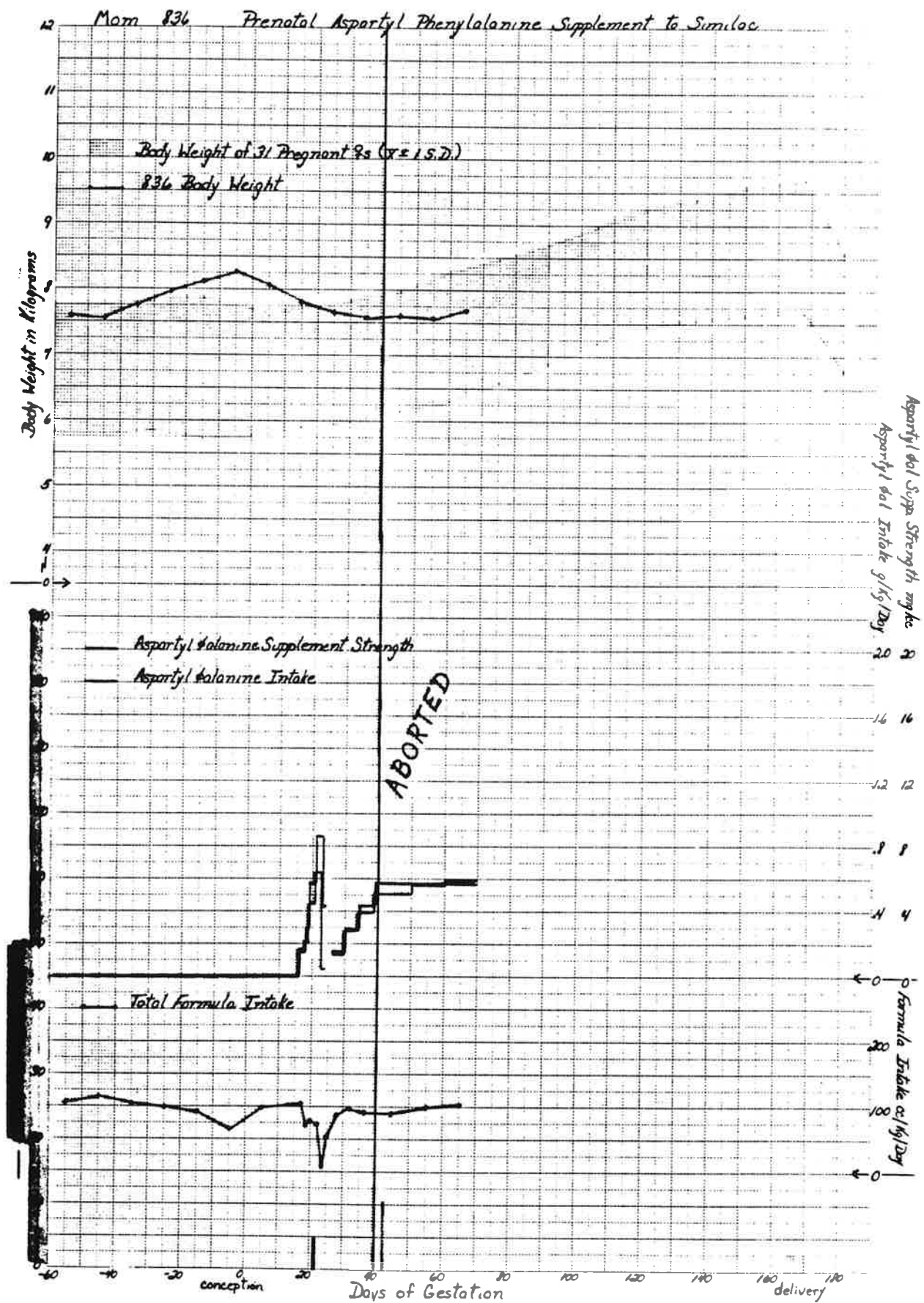


Figure 10



16
Figure 12



17
Figure 13

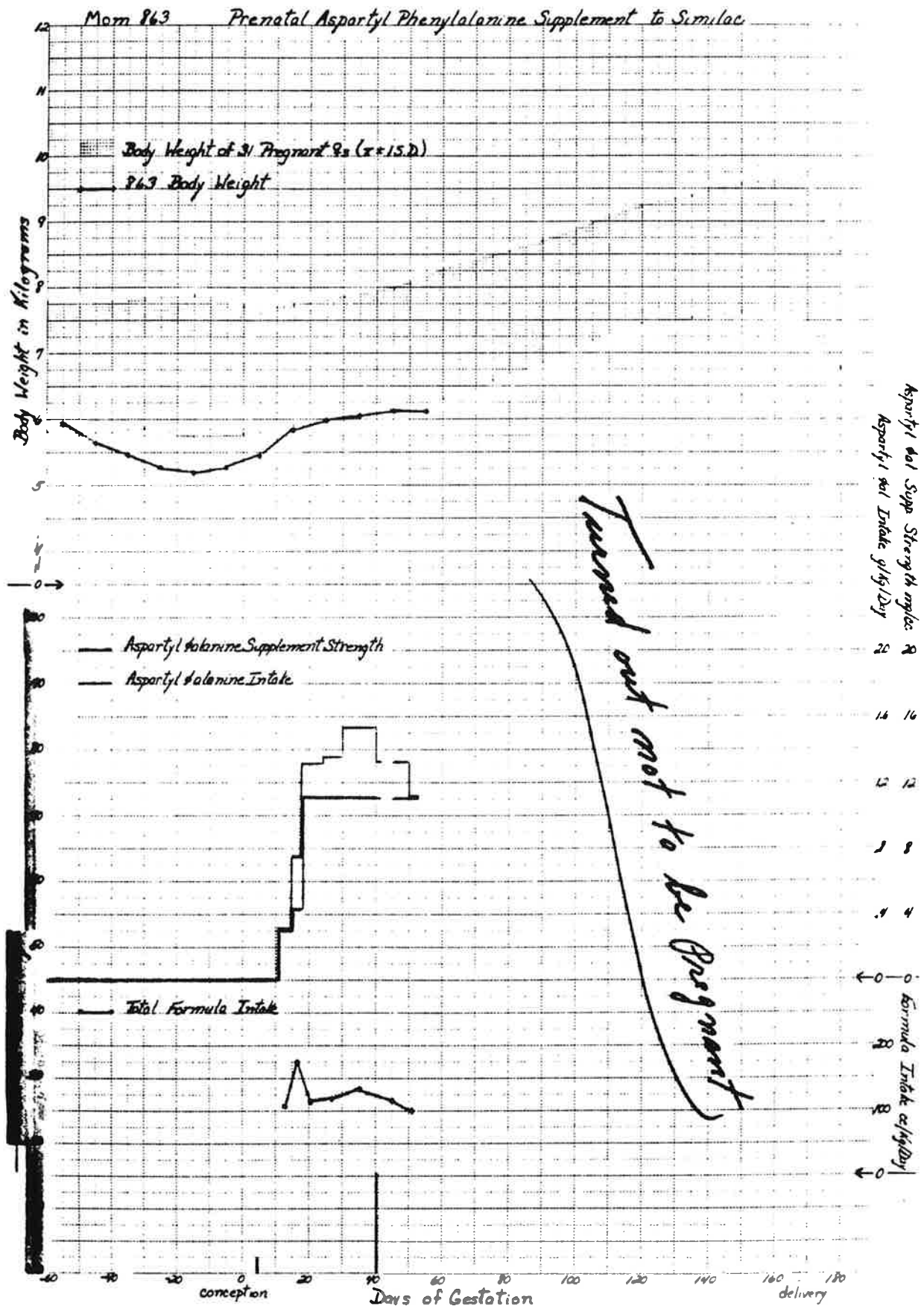
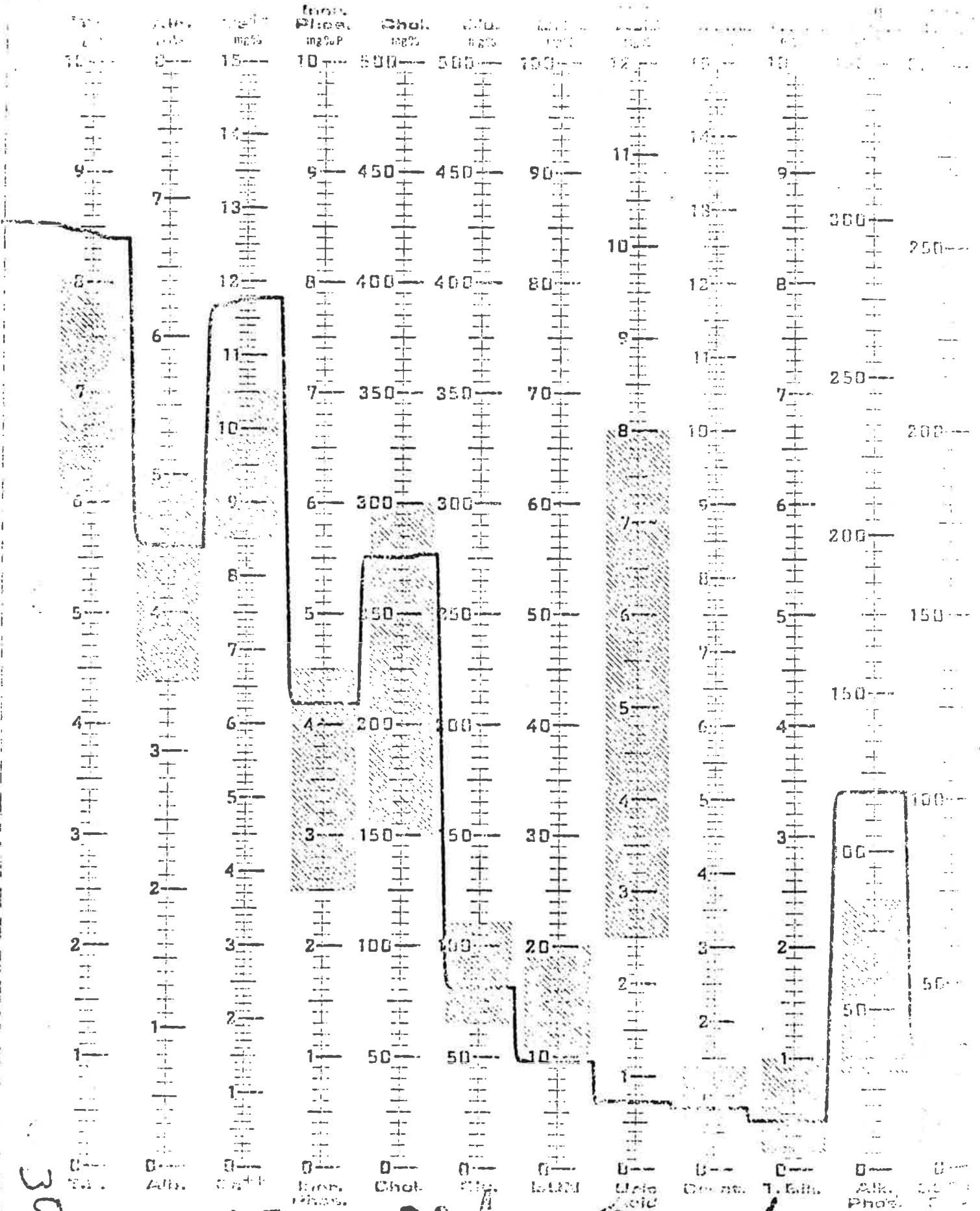


Figure 14



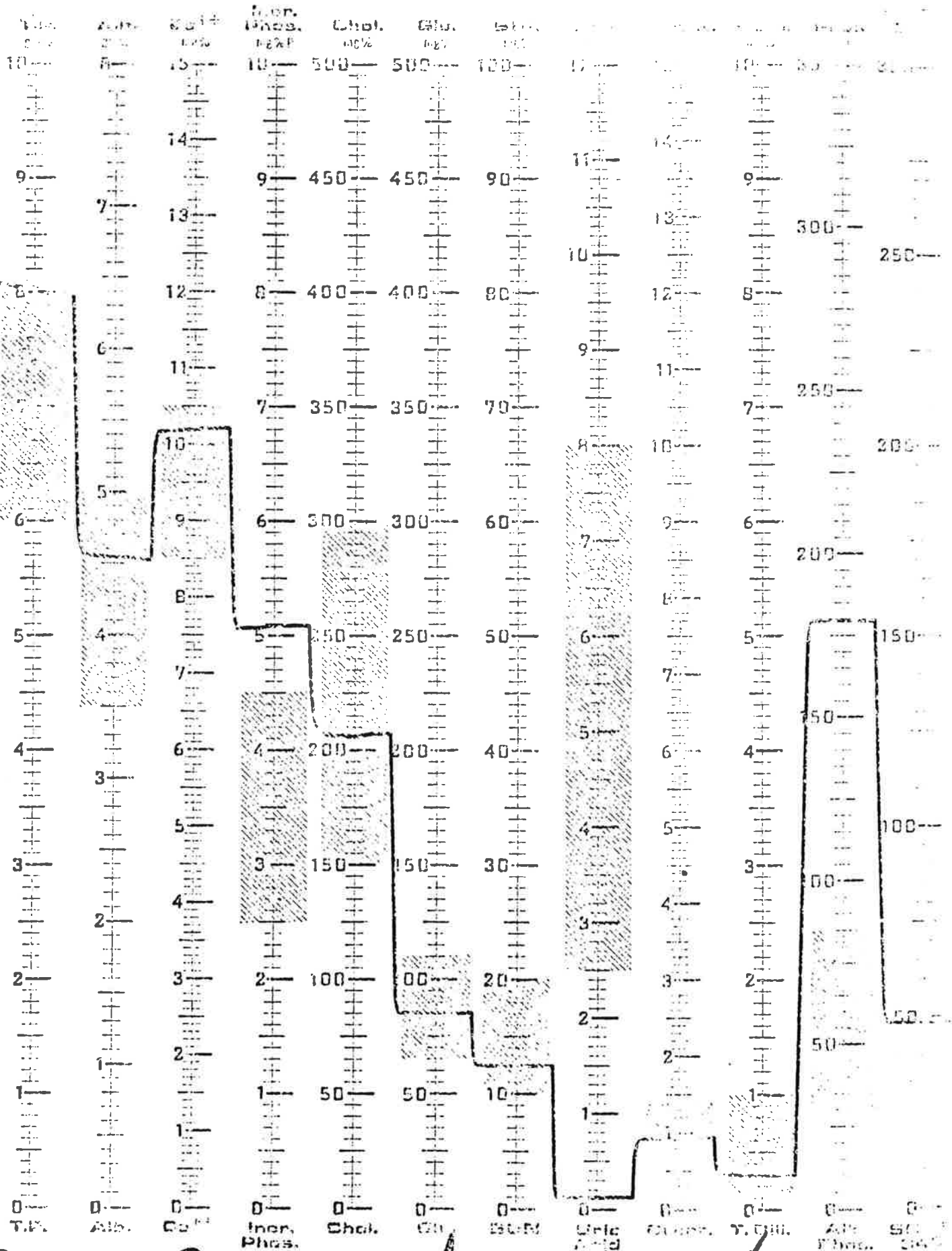
Patient's Name

4820

30 days Reg.

Date 12-3-17

Figure 15

Patient's Name: *821*Age: *51 days*Sex: *Male*Date: *12-2-78*

SUMMARY

Incomplete post-mating data are presented on eight female Rhesus monkeys. No information of the established reproductive capabilities, the reproductive status at the time of mating, the identity and reproductive history of the mate, evidence of actual establishment of pregnancy, evidence of periodic assessment of the gestational status of the pregnant animal, or experimental conditions during the period of feeding was provided for any animal.

One monkey (No. 879) functioned as a pair-fed control and delivered a 440 g infant on gestation day 171. The condition and disposition of the infant are not known. One monkey (No. 863) was not pregnant, and apparently was not re-mated. Two monkeys (Nos. 830 and 831) were presumed pregnant, but information on neither SC-18862 ingestion nor presence and disposition of a conceptus were available.

Thus, four allegedly pregnant monkeys were treated with SC-18862. In the first instance the infant was reported as being a "stillbirth"; however, the parturition status/performance of the maternal animal was not assessed, nor was a general physical description or postmortem evaluation of the infant provided. The total evaluation of the maternal-fetal system is one word --- "stillbirth". At delivery, there was no evidence that the event was observed and thus whether parturition proceeded normally or otherwise. The record fails to establish that the "stillbirth" was in fact stillborn, or whether it may have died shortly after birth, since there is no record of when it was initially observed.

In the second instance, the pregnant female delivered a live infant of normal weight at term, but the neonate was neglected and died within a few days. Since the maternal animal was stated to be "socially maladjusted", her appropriateness for this experiment is questionable, recognizing that adequate maternal-fetal care was not available. In the third and fourth instances neither objective evidence of pregnancy having been achieved nor physical evidence of abortion having occurred are provided. The gestational status of the maternal animal and a description of the conceptus are provided in one word --- "aborted". No physical evidence of abortion having occurred is provided. A statement of "abortion" in early pregnancy cannot be evaluated unless substantiated by prior evidence of the existence of pregnancy and/or a precise description of the nature of the uterine contents expelled.

It was apparent that feeding SC-18862 throughout the entire gestation period (monkey No. A23) resulted in an elevation of the maternal serum L-phenylalanine level comparable to the increases observed by Kerr, et al.² when feeding approximately equimolar quantities of L-phenylalanine. Also, the birth weight of the neonates (A23 and B24) are comparable with those obtained by Kerr, et al. when feeding L-phenylalanine. Thus, it would appear that in monkeys exposed to SC-18862 in large amounts throughout the entire gestation period and proceeding to term delivery, no adverse effects other than those produced by feeding L-phenylalanine only were observed.

CONCLUSIONS

The following generalizations appear to be consistent with the data presented:

1. Administration of SC-18862 to pregnant monkeys at dose levels up to 3.8 g/kg/day did not adversely affect the maternal appetite (formula intake) or body weight, nor did it induce seizures.
2. Administration of SC-18862 to pregnant monkeys during a major portion of the gestation period did not produce obvious anatomical malformations in the term fetus.
3. Administration of huge doses of SC-18862 to pregnant monkeys did produce significant elevation of maternal serum phenylalanine levels, roughly comparable to those produced by administering approximately equimolar quantities of L-phenylalanine.
4. The alleged premature termination of pregnancy (abortion) in the SC-18862 treated monkey was not associated with notable elevations of maternal serum phenylalanine levels. The two alleged abortions reported involved monkeys with serum phenylalanine levels within the normal range. This infers that the abortions may be incidental and not directly related to SC-18862 administration.

The above report is a compilation of data made available to Searle Laboratories by the associates of the late Dr. Harry A. Waisman, University of Wisconsin Medical School. It was compiled, interpreted and discussed by Drs. K. S. Rao and R. G. McConnell, Department of Pathology-Toxicology, Searle Laboratories, Box 5110, Chicago, Illinois.

K. S. Rao
K. S. Rao

R. G. McConnell
R. G. McConnell

REFERENCES

1. Oppermann, J. A., Muldoon, E. and Ranney, R. E. (1973). J. Nutr. 103;1454.
2. Kerr, G. E., Chamode, A. S., Harlow, H. F. and Waismann, H. A. (1968). Pediatrics 42;27.

24a

APPENDIX

Autopsy 70:53

Animal No. N 38

Investigator: Kerr

Macaca mulatta

Date: 5/13/70

Sex: Male *100% (see Macaca mulatta)*

0 hours postmortem

Weight: 385 g.

Prosector: Paik

Age: 3 days

*KSP.
3/17/75*PREVIOUS HISTORY.

Mother of this subject received an artificial sweetener during pregnancy and she gave birth to this monkey 10 days early. The infant was ice cold when found about four hours later, after delivery. The infant received fluids prior to death.

GROSS DESCRIPTION.

At the time of autopsy, the subject is an emaciated male infant monkey. The head, scalp, eyes and nose are not remarkable. The extremities are free of any detectable abnormalities. Inspection of the abdomen reveals the peritoneum to be smooth and glistening with no adhesions present. The mesentery, omentum, and diaphragm are unremarkable. Examination of the thorax reveals it to be unremarkable.

Heart. 3.2 g. The pericardium is of normal thickness and is smooth without adhesions. The myocardium is of normal color and consistency. All chambers of the heart are of normal size. The valves are thin, delicate and freely moveable.

Lungs. 4.0 g. There is no sign of edema, congestion or consolidation. The tracheobronchial tree contains moderate amounts of serous fluid. There are no adhesions of the visceral and parietal pleura.

Liver. 18.1 g. The capsular surface is smooth. The cut surface of the liver appears to be normal in its color, lobular pattern, and consistency.

Spleen. 0.5 g. It appears to be of normal consistency and has a smooth, intact capsule and dark red coloration on cut surface. The malpighian corpuscles and trabeculae are distinct.

Kidneys. 1.5 g. and 1.4 g. (right and left respectively).

Autopsy 70:53

Page 2

These are free of any detectable abnormalities.

Thyroid glands. 0.3 g. Normal in size, color and general appearance.

Adrenals. 0.2 g. (right and left respectively). Not remarkable.

Gastrointestinal tract. Practically no food materials are found in the gastrointestinal tract. The stomach is unremarkable, without evidence of hyperemia or ulceration. The small and large intestine is within normal limit.

Pancreas. Not remarkable.

Brain. 63.9 g. Not remarkable.

PROVISIONAL DIAGNOSIS.

No abnormal gross findings were obtained.

Microscopic Report.

Autopsy 70:53

Small intestine.(1). Not remarkable.

Thymus. (1). Not remarkable.

Adrenal. (1). Not remarkable.

Ovary (2). Not remarkable.

Spleen (3). Not remarkable.

Cerebrum. (4). Not remarkable.

Heart (5). Minimal myocardial hemorrhage is present.

Lung. (6). There are areas of unexpanded alveolar tissue present.

Kidney. (7). Not remarkable.

Large intestine (8). Not remarkable.

Lymph node (9). Not remarkable.

Liver (10). There are large numbers of liver cells which have a intracytoplasmic vacuolization.

In summary, except for minimal myocardial hemorrhage and changes of the liver which appear to be attributable to toxic characteristics, there were no prominent pathological abnormalities presented. It is postulated that this infant may have been receiving no proper care after birth, leading to inanition and terminal coma.

FINAL DIAGNOSIS.

1. Inanition.

N38's mother, A39, is a socially maladjusted, lab-reared female, who did not take care of her infant. Infant was born 8 days early, on a Sunday morning, while we were working with a skeleton crew who did not perform the normal hourly check. When found at the next feeding, the infant was more dead than alive - - although it lived for the next 3 days in an incubator receiving O₂. We were unable to get any blood from the infant.

Amino Acid Composition of SIMILAC Liquid Formula*

[Reproduced from the paper of Kerr, et al. (Pediatrics 42, 27, 1968)]

	<u>mg/100 ml of Reconstituted Formula</u>
Tryptophan	25
Tyrosine	94
Cystine	16
Glutamic acid	337
Lysine	130
Serine	79
Phenylalanine	87
Arginine	63
Glycine	6
Isoleucine	107
Histidine	40
Valine	115
Leucine	180
Proline	124
Threonine	77
Methionine	44
Alanine	38
Aspartic acid	82

* Presumably similar formula was used in the present study.

-29-

FROM DAY - - DIED: / /

$P \cap F = \text{Finite}$