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FM V. VINOGRAD M.D.

TO ZZB/MR. HARRY SMITH

OPN

RX: T LARGE DOSE OF S Y M P A T H Y FOR MODERATE SUNBURN
OBTAINED WHILE ON DUTY SKIN DIVING IN ZANZIBAR

12/1231Z MAY MCC

POTENTIAL OPERATIONAL VALUE OF SPACECRAFT ROTATION VS.

ON-BOARD HUMAN CENTRIFUGE

	<u>Spacecraft Rotation</u>	<u>On-Board Centrifuge</u>
1. Prevent physiological deconditioning of zero g	+	+
2. May enable broader segment of population to participate in space flight	+	0
3. Housekeeping; particulate contamination	+	0
4. Waste management	+	+
5. Handling of materials	+	0
6. Up-down familiarity for mission tasks	+	0
7. Reduce complexity of restraint design (for mission tasks)	+	0
8. Traction for mobility	+	0
9. Scientific investigation capability	+(greater)	+(lesser)

V. SUMMARY AND CONCLUSIONS

1. Artificial g will probably not be required operationally for long duration flights of the future. Other countermeasures against physiological deconditioning, habitability problems, and performance disadvantages will probably prove to be sufficiently effective without introducing the vestibular, cost, and other (navigational, spacecraft design, etc.) impacts of spacecraft rotation.
2. An artificial g experiment is needed to determine how to apply artificial g optimally in case it is required or desired, i.e., there is sufficient probability to warrant inflight artificial g experimentation.
3. The determination of how to apply artificial g requires a rotated spacecraft experiment and the evaluation of flight crew responses.
4. This experiment should be accomplished relatively early in the series of prospective flight programs, preferably Skylab II (or Shuttle Module), for operationally oriented human and design data. This should be followed by experiments aboard the rotating space station for more definitive human response data and information of purely scientific interest. The space station should include a non-rotating hub for continuing studies of long term zero g.
5. The initial spacecraft artificial g experiment should be designed to yield physiological, performance, habitability, and human engineering information on:
 - a) The effects of various rotation rates and transitions from one radius to another (rate durations of one to two days each).
 - b) The effects of at least two sustained subgravity g levels, such as 0.5g and 0.2g.



SPACE SHUTTLE ORBITER ENTERPRISE ON LAUNCH PAD 39A

SPACE SHUTTLE ENTERPRISE FACILITY VERIFICATION VEHICLE ON LAUNCH PAD 39A AT THE KENNEDY SPACE CENTER, FLA. The Space Shuttle Orbiter Enterprise was rolled out of the Vehicle Assembly Building at 8:24 a.m. EDT May 1, 1979, for the three and a half-mile journey to Launch Complex 39's Pad A. The move proceeded smoothly and was successfully concluded at 7:20 p.m. when the Mobile Launcher Platform was hard-down on its mounts at the pad. This marks the first time that the complete Space Shuttle configuration has been assembled in the VAB and moved to the pad. The Enterprise will remain for fit and function checks which will clear the way for the launch of its sister ship, Columbia, on its first mission into space. It will be moved back to the VAB later this summer.



National Aeronautics and
Space Administration

Lyndon B. Johnson Space Center

☆ U.S. GOVERNMENT PRINTING OFFICE: 1979-674-065

JSCL 201



View of Human Problems to be Addressed for Long-Duration Space Flights

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BERRY, C. A. *View of human problems to be addressed for long-duration space flights.* Aerospace Med. 44(10):1136-1146, 1973.

After 9,501 man-hours in space, no physiological problems serious enough to cause lasting medical concern have been seen among U. S. astronauts. Heart rates tend to stabilize at lower levels in zero gravity and electrical activity, except for occasional bigeminy and premature auricular and ventricular contractions (Apollo 15), is normal. Postflight, cardiac silhouette size is decreased. Reduced orthostatic tolerance is consistently found. Slight loss of bone mass has been observed as has decreased leg size and, in the 2-week Gemini flight, slightly negative nitrogen balance. Electrolyte changes parallel the time course of body weight loss and gain. Hormone changes are consistent with electrolyte changes. Growth of "opportunistic" organisms is favored, with a resistance to antibiotics demonstrated by some. The number and distribution of microorganisms also change. Vestibular problems are minimal; adaptation appears to occur rapidly. Countermeasures to zero-g effects are being explored. The use of lower body negative pressure is under investigation as a means of delaying, reducing, or preventing redistribution of the circulating blood volume in zero g. The use of medications is under investigation for, for example, cardiovascular and anti-radiation protection. Vestibular preadaptation programs are being considered, particularly for non-test pilot space travelers. In future, longer-duration space flights, psychological variables may become increasingly significant. The effects of isolation, estrangement, and reduced privacy on crew stability are being studied. Crew composition is being reassessed with a view toward including female crew members. Improved sleeping accommodations, entertainment facilities, and food will be provided. Waste management and personal hygiene facilities, while still "basic" by earth-based standards, are being steadily improved. In the opinion of the author, 6 months of biomedical, psychological, and human factors data of the type that the Skylab program will begin to collect will be sufficient basis for predicting man's safe commitment to a Mars mission with appropriate understanding of the range of adaptational changes and use of countermeasures where they are found necessary.

AN AMERICAN EDITOR, who was thunderstruck by the difference in the sizes of early American space capsules and the Skylab workshop, said, "It's like stepping from a racing car cockpit into your house." This commodious (by former standards) orbital habitat is designed to accommodate three men for nearly 2 months in earth orbit. The size of the Skylab, about 3,600 cubic feet of living and working volume, will permit us to look much more closely and systematically at the effects of the space flight environment on those who venture into it while they are maintained in an atmos-

phere of comfort unprecedented in space activities. No longer are astronauts required to sleep in their couches. Private sleeping quarters are provided. A wardroom arrangement permits the occupants to dine rather than merely eat. And the food is better than it has been in the past. Defecation is no longer the irksome public affair it once was, and cleanliness has taken a quantum leap. In this atmosphere, the space crew are both subjects and principal investigators in a series of experiments that will begin to tell us whether man can be safely committed to space missions that may one day take him to the planet Mars.

Space flight is an experiment; undoubtedly man's most successful, but essentially experimental all the same. Knowledge of man's response to space flight stresses is limited, and it can be reasonably expected that space flight missions for a long time to come will be dedicated in large measure to investigating and clarifying the nature of physiological changes that have been seen or may be seen in the future. Because this is the case, certain compromises must be made. For example, the mechanisms involved in calcium metabolism in space cannot be defined unless the diet is strictly controlled. While the space crewman would ideally like to have a diet that is as varied and tasty as any he might have in an earth-bound restaurant this may, at least for the immediate future, represent an unreasonable demand.

At the same time, every effort must be made to make the environment of spacecraft and space laboratories as habitable as possible within the framework of constraints imposed by the objectives of any given mission. If habitability considerations are disregarded or inadequately addressed, the goals of missions, particularly very-long-term ones, could be compromised. If, for example, hygiene facilities provided a level of personal cleanliness that crews considered substandard, psychological as well as physiological well-being could easily be affected on a mission of long duration. Indeed, the lack of adequate provisions for personal hygiene maintenance has been the source of significant irritation to the crews of missions lasting for less than 2 weeks.

We must begin now, based on our current experience, to take the long-term view of the problems man will face in long duration space flight. Short-term flights have produced physiologic changes which must be evaluated

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as to progression and the need for countermeasures. In addition, other problems, principally those related to habitability of the spacecraft environment, have not been directly addressed so far. It has been presumed that inadequacies in systems, such as the waste management system, would be viewed by crews as little more than inconveniences during brief space flights. The same supposition has been made about aspects of the environment that might have psychologically negative impact. However, things which are tolerable in the short run can become problems of major proportion during long-duration space missions. The effects of confinement could, theoretically, become more acute, and small group interactions more difficult. Additional data are needed in all of these areas. Nothing can be left to chance.

This article will briefly review the principal physiological changes seen in space flight and discuss various countermeasures which may prove to be useful in combating these changes in long-term space flight, if such intervention is required. The second half of the discussion will focus on the psychological aspects of living and working in space for long periods of time and describe those aspects of the environment which may be critical to maintaining psychological fitness.

PHYSIOLOGICAL INTEGRITY OF SPACE CREWS

American astronauts by the close of the Apollo program had logged 9501 manhours in space with no physiological changes seen postflight that were serious enough to cause lasting concern. Certain transient changes have, however, been seen. These may be summarized as follows:

Principal Body Systems Responsive to Weightlessness

- Cardiovascular/hemodynamic
- Musculoskeletal
- Endocrine/electrolyte
- Microfloral
- Vestibular

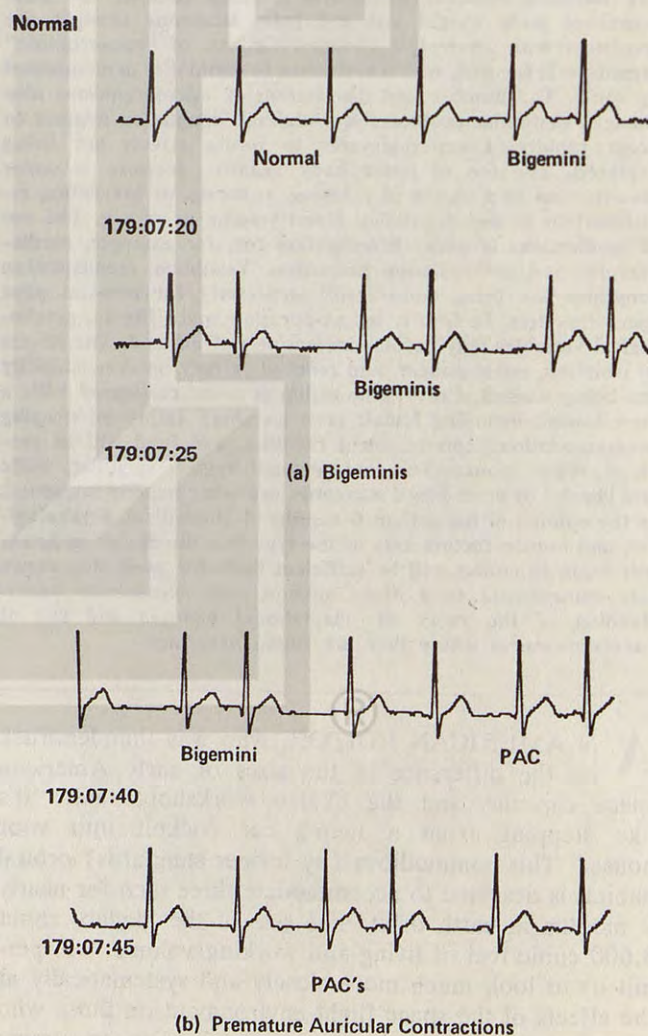
Cardiovascular and Hemodynamic Responses to Weightlessness: Cardiovascular responses in space crews have been examined extensively and closely since the cardiovascular system was the first to show a dramatic response to the space flight environment. Table I summarizes the principal cardiovascular and related hemodynamic responses thus far consistently observed. Heart rates, after initial elevation during the launch period, tend, on the whole, to stabilize at lower than preflight levels during the period of weightlessness. Postflight

TABLE I. CARDIOVASCULAR/HEMODYNAMIC RESPONSES.

Measure	Finding
Heart rate	Stabilized at lower levels in zero g
Electrical activity	Normal except for bigemini, PAC's, PVC's in Apollo 15
Cardiac silhouette	Decrease in size postflight
Blood pressure	Normal inflight—Labile postflight
Orthostatic tolerance	Decreased postflight

heart rates have been elevated in most astronauts, and normalization has been inhibited. Return to preflight baselines has required as much as two days in Apollo crews.

ECG's, recorded during all U.S. missions, had revealed nothing about the electrical activity of the heart more dramatic than rare extrasystoles and an occasional arrhythmia until the Apollo 15 mission. In this crew, irregular heart beats were observed both during lunar surface activity and during the return flight to Earth. Twelve bigemini were recorded, as were premature auricular and ventricular contractions. In one instance, the arrhythmia was accompanied by a heart rate of 28 beats per minute. Fig. 1 (a, b) illustrates the arrhythmias experienced over a 25-sec period, 179 hrs into the flight of Apollo 15. The normal beat shown at the top of Fig. 1a converted to a nodal bigemini rhythm which lasted for 14 secs. One-half minute before the bigemini, a heart rate of 120 beats per minute was recorded and this dropped to 95 beats per minute just before the appearance of the irregular beat. In Fig. 1b the bigemini rhythm can be seen converting to a series of premature auricular contractions which did not appear as coupled beats. These



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irregularities in heart beat were thought to be linked to potassium deficits, and a program was initiated to preclude these deficits in the Apollo 16 crew. High preflight potassium levels were maintained with potassium-rich diets. Inflight, potassium supplements were added. These measures prevented negative potassium balance in the Apollo 16 crew but not in the Apollo 17 crew. In both crews, however, cardiac electrical activity was entirely normal.

Recent studies have also revealed a decrease in the cardiac silhouette. Examination of pre- and postflight posterior and anterior chest films revealed a decreased heart size in U.S. space crews postflight. These data were confirmed by X-ray studies in the Apollo 16 and 17 crews. Correlation of X-ray views taken at the same time in the cardiac cycle, systole or diastole, also revealed an apparent decrease in the cardiac silhouette. Chest X-rays revealed a definite decrease in cardiac size for only one Apollo 17 crewman.*

Blood pressure has been normal inflight for U.S. crews and labile for up to 3 days postflight.

A certain degree of deconditioning of the cardiovascular system was expected as a consequence of reduction in hydrostatic pressure gradients in zero gravity. Consistent postflight reduction in orthostatic tolerance indicates that this probably occurs. Deconditioning is further evidenced by decreased exercise tolerance postflight.

Musculoskeletal Changes: Small but detectable changes have been noted in the skeletal system. These have been seen in U.S. and Soviet crews in the form of slightly reduced bone optical density. The causes of these changes are not clear but they do not appear to be progressive. Functional underloading of the skeletal muscles and a decrease in the energy expended during muscular effort in the weightless environment could certainly be linked with the changes which have been observed, but there are many other possibilities. Calcium metabolic mechanisms and endocrine changes could well be implicated. It is possible, for example, that calcium ions might be mobilized from the bone cation pool to correct electrolyte imbalances in the weightless state.²

No gross neuromuscular changes have been observed in U.S. space crews. Slightly negative nitrogen balances which persisted postflight had been observed after 2 weeks of exposure to zero gravity by the Gemini 7 crew. Measurements of leg size were made in the Apollo 16 crew. These revealed postflight decreases of the size of both the calf and the thigh. Limb volumes showed consistent postflight decrements from 100 to 800 milliliters. These decrements persisted beyond 7 days postflight.

The musculoskeletal findings for U.S. space crews were as follows:

- Slight loss of bone mass

*The Apollo 17 Commander showed a definite decrease in cardiac size. Little change could be seen for the Lunar Module Pilot. The Command Module Pilot's cardiac silhouette was somewhat larger postflight. It should be noted that the CMP wore an anti-hypertension garment inflight.

TABLE II. CHANGE IN TOTAL BODY EXCHANGEABLE POTASSIUM DETERMINED BY K⁴² STUDIES IN APOLLO 15, 16, AND 17 (PREFLIGHT VS 24 HR POSTFLIGHT).

	(meq/kg Body Weight)
Apollo 15	-12.7
Apollo 16	+ 7.7
Apollo 17	-13.5

- Decreased leg size (Apollo 16)
- Decreased cardiac silhouette size
- Slightly negative N₂ balance (Gemini 7)

In contrast to U.S. findings, the Soviet Soyuz 9 crew, after exposure to 18 days of weightlessness, exhibited severe problems in the motor sphere. Muscular pain was reported as were measurable alterations in gait. Chekirda and coworkers reported that cyclogravimetric analysis revealed abnormalities in motor skills.³ No such changes have been seen in any U.S. crews.

Fluid and Electrolyte Balance: Of all changes seen in physiological systems during and after space flight, probably the most reliable clues to the mechanisms involved are offered by an examination of electrolyte response. Postflight retention of electrolytes has been an absolutely typical response. Total body gamma spectrometry has indicated significant decreases in total body potassium. Total exchangeable potassium studies using the isotope K⁴² indicated a significant loss in total exchangeable potassium in the Apollo 15 crew but no loss in the Apollo 16 crew. Potassium deficits were again noted in the Apollo 17 crew despite the use of dietary potassium supplements. The reason for the disparity of results remains unclear.

Electrolyte changes have paralleled the time course of body weight loss and gain. Hormone changes, specifically postflight increase in antidiuretic hormone and aldosterone levels, consistent with electrolyte retention have been observed, and these paralleled the pattern of rapid recovery of inflight weight loss in the immediate postflight period.

Electrolyte response provides the basis for an hypothesis concerning the nature of man's adaptation to zero gravity. This hypothesis, if correct, might also explain changes in the muscles, including cardiac muscle. Table III presents this hypothesis. Briefly summarized, electrolyte data appear to indicate that the redistribution of circulating blood volume upon entry into zero gravity is interpreted by the body as an increase in total circulating blood volume which the body attempts to reduce by a decrease in the production of ADH and aldosterone. This causes a decrease in plasma volume, which may then produce a secondary aldosteronism. The extracellular alkalosis which would result from such a response would produce an intracellular exchange of potassium and hydrogen ions. This loss of potassium, verifiable postflight, could produce loss of muscle cell potassium. If the heart muscle were also affected, and there is no reason to presume that it would not be, cardiac arrhythmias could also be explained. It is apparently at this stage in the

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TABLE III. OVERVIEW OF CURRENT HYPOTHESIS CONCERNING PROCESSES INVOLVED IN MAN'S ADAPTATION TO ZERO GRAVITY.

Event	Response of Body
Entry into zero gravity. Redistribution of circulating blood volume.	→ Body attempts to reduce volume. ADH decreases, aldosterone production decreases.
Loss of water, sodium, potassium (loss of body weight).	→ Decrease in plasma volume. Aldosterone increases (secondary aldosteronism).
Increased sodium retention. Potassium loss continues. Cell: acidotic — extracellular fluid: alkalotic.	→ Intracellular exchange of potassium and hydrogen ions. Decrease in bone density, muscle cell potassium, and muscle mass; possibly including cardiac muscle.
Respiratory and renal compensation. Halt to weight loss trend.	→ Stabilizes with new effective circulating blood volume. New body fluid and electrolyte balance or "set."

postulated adaptation process that we find man after about 2 weeks of space flight. Possibly, longer residence in space might result in a completion of the adaptation process rather than a continuation of negative electrolyte balance trends. Given a sufficiently long stay in weightlessness—how long at this point it is not possible to say—respiratory and renal compensation could halt the negative balance trend to produce a physiological system which is stabilized with a new effective circulating blood volume and fluid and electrolyte balance. On the other hand, since it is not possible to say whether this reversal will, indeed, occur and, if so, how long a period of time will be required, countermeasures to these changes are being investigated so that man can safely return from space to earth at any point in the adaptation process without dangerous results.

Microbiological Changes: Certain changes in the character and distribution of the microflora have been noted in both U.S. and Soviet space crews. The growth of opportunistic organisms appears to be favored in space flight and certain organisms become less resistant to antibiotics postflight.¹ The etiology of these changes is not yet certain but they are not surprising against the background in which they occur. Space crews undergo a period of semi-isolation prior to space flight and are provided with diets which restrict bacterial intake inflight. The atmosphere control system in the spacecraft cabin also limits the number of microbes to which the crew is exposed. These factors, coupled with the change in distribution of microbe-bearing particles in an environment in which there are no gravity gradients to cause them to settle out and the unusually small size of the human population, cannot be expected to be without consequence in the microbiological sphere.

The clinically significant micro-organisms isolated in the postflight period during the Apollo mission series are as follows:

TABLE IV. VESTIBULAR-RELATED RESPONSES: APOLLO.

	No. Cases
Tumbling illusions	3
Stomach awareness	10
Nausea	4
Vomiting	3

TABLE V. RADIATION EXPOSURE FOR APOLLO 7-17.

	Rad		Rad
Apollo 7	.16	Apollo 12	.58
8	.16	13	.24
9	.20	14	1.14
10	.48	15	0.30
11	.18	16	0.53
		17	0.6

Microflora of Possible Medical Importance Identified Postflight in Apollo Crews

Staphylococcus— <i>aureus</i>	Moraxella— <i>species</i>
epidermis	Corynebacterium— <i>species</i>
faecalis	Enterobacter— <i>aerogenes</i>
Klebsiella— <i>aerobacter</i>	Haemophilus— <i>parahaemolyticus</i>
enterobacter	Herella— <i>vaginicola</i>
pneumoniae	E. coli (Throat)
Proteus— <i>mirabilis</i>	β — Streptococcus
Pseudomonas— <i>aeruginosa</i>	Mycoplasma
Serratia— <i>species</i>	Candida— <i>albicans</i>
Mima— <i>polymorpha</i>	

Overall changes from the pre- to postflight period may be summarized in the following way:

- Anaerobic bacteria decreased in number
- Aerobic bacteria increased in number and type
- Microorganisms isolated at more body sites
- Organisms tend to spread across crewmembers (especially *Staphylococcus Aureus*)
- Fungal isolates decreased in number
- Higher carrier states for mycoplasma indicated

Vestibular Effects: U.S. crews have not reported vestibular-related difficulties of the severity or with the consistency reported by Soviet cosmonauts. Gemini crewmembers reported no vestibular-related difficulties. Some Apollo crewmen have reported problems that might be related to the vestibular system but these were in all cases reversible, subsiding after 2 to 5 days into any given flight. Table IV lists the incidence of vestibular problems. It should be pointed out that this listing represents the number of cases, not the number of individuals, experiencing any particular difficulty, and that in at least one individual the symptoms were related to clinical illness in flight.

That vestibular problems have been minimal during U. S. missions is very possibly attributable to the fact that U.S. astronauts have had test pilot experience.

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rience, and test pilots as a group suffer from a lower incidence of motion sickness than the general population. In future missions, when space crews will be drawn from a broader population including many persons who have no aviation experience whatsoever, the incidence of vestibular symptomatology may increase.

Radiation-Related Effects: Thus far, the radiation doses received by space crews have been well below those which might be expected to produce biologically significant effects. Table V lists the average radiation dose received by Apollo crewmembers for missions 7 through 17. Because the Apollo 14 mission occurred at a time of solar minimum, the cosmic ray flux was relatively higher for this crew than for those involved in previous missions. No demonstrable problems have been related to these radiation exposures. Because it has been suggested that a synergism might exist between radiation and other space flight factors, leukocyte samples have been examined for chromosomal aberrations for Gemini and Apollo astronauts. A small, but not statistically significant, number of aberrations of the chromatid type have been observable. There does not however, appear to be a correlation between increase in chromosomal aberrations and mission duration.⁶

The much publicized light flash phenomenon may also be associated with radiation. The most likely explanation for the light flashes first reported by the Apollo 11 crew is that they result from high-energy, high atomic particle radiation generated in the ocular media or the retina. Results of inflight experiments directed toward establishing the cause of flashes and their biological significance are presently being analyzed.

Countermeasures to the Effects of Space Flight on Man: The effect of space flight exposure on U.S. astronauts has not so far indicated a clear-cut need for countermeasures. Nevertheless, because the effects of longer term exposure to zero g cannot be predicted for the cardiovascular system, or indeed for any other body system, evaluation of potential countermeasures to weightlessness continues. The following list summarizes the techniques which have been investigated.¹²

Exercise	Tumbling
Medication	Electrical Stimulation of Muscles
Diet	Exercise and LBNP
LBNP	Exercise and Venous Occlusion Cuffs
Gradient Positive Pressure	Exercise and Positive Pressure Breathing
G—Suit	Exercise and Bone Stress
Venous Occlusion Cuffs	Exercise and Hypoxia
Positive Pressure Breathing	Venous Occlusion Cuffs and Medication
Valsalva Maneuver	Venous Occlusion Cuffs and Leotards
Bone Stress	Hypoxia, LBNP and Exercise
Double Trampoline	Centrifugation

Cardiovascular System Protection: If it proves necessary to alter the cardiovascular response to weightlessness, the application of several techniques may prove to

be useful. The most promising of these are the application of lower body negative pressure or its opposite number, gradient positive pressure.

Gradient positive pressure employs a reverse pressure garment which acts to simulate gravity by applying greater pressure in the upper body with a decreasing gradient down the body. The blood volume is effectively distributed in the way in which it is under the 1 G force. The net effect is the same as that produced by lower body negative pressure, but the results are produced in the opposite manner, since lower body negative pressure causes blood to be drawn into the lower part of the body by reducing pressure in that part of the body. The one outstanding difference between the two techniques, besides the manner of application, is the application of a gradient in the one case and not in the other: lower body negative pressure applies the same negative pressure to the entire lower part of the body.

Lower body negative pressure shows substantial promise as a therapeutic technique against the effects of zero gravity on the cardiovascular system. The technique can be used to "train" the body to respond appropriately to the new environment by being applied intermittently as a stressor. This develops longitudinal G tolerance—that is, the ability to withstand blood shifts—and insures that there will be an adequate venous return to the heart.

Ongoing studies are investigating the potential efficacy of lower body negative pressure applied for perhaps the week prior to reentry for spacecrews involved in long-term missions. Preliminary results are promising. In one study the intermittent application of -70 mm Hg restored orthostatic tolerance in subjects who had been subjected to 5 days of water immersion and bedrest. Final LBNP endpoints after 5 days were reported to have risen to 165% of control values.⁴ In a further study now in process (at the Public Health Service Hospital, San Francisco), a negative pressure of -30 mm Hg applied for 4 hrs a day appears to be sufficient to (1) produce a post-bedrest tilt response equal to the pre-bedrest response, and to (2) restore the drop in plasma volume caused by bedrest. It should be stressed again, these results are preliminary and the establishment of an LBNP profile suitable for space flight application awaits further data.

Provision of positive pressure, by the application of an anti-hypotension garment, is also under consideration as a means of supporting the individual in the immediate postflight period. This approach was investigated in conjunction with the Apollo 17 mission and in bedrest studies with promising results.

Efforts were made to counteract the effects of zero gravity on the circulatory system in the Soyuz 11 crew by a complex of techniques which included the use of a treadmill device, a "gravity" suit, and medications. Inflight testing indicated adequate adaptive capability of the cardiovascular system, although a possible decline in orthostatic tolerance was indicated by LBNP. It was, nevertheless, believed that readaptation for the Soyuz/Salyut crew could have been less difficult than it had been for the Soyuz 9 cosmonauts, possibly as a result of the preventive measures taken.⁷

Inflight exercise regimes have been employed to safe-

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guard the overall fitness of space crews. Exercise alone, however, is not effective in combating cardiovascular deconditioning. It may be helpful as an adjunctive therapy provided the technique employed raises the heart rate above 120 beats per minute. A bicycle ergometer device, such as the one which will be used on all Skylab missions, or a treadmill, as was used in the Salyut mission, will accomplish this. Exercise has also been examined as a means of preventing musculoskeletal disturbances but the results have not been impressive. While cardiac arrhythmias did not occur in the two space crews receiving dietary potassium supplements, only partial success was achieved in preventing negative potassium balances with this approach. Many other approaches have been examined with mixed findings.

Musculoskeletal Protection: Recent data from bedrest studies suggest that orally administered calcium and phosphate are more effective than exercise (no effect) or longitudinal compression (limited effect) for preventing the loss of minerals from the bones. The period of efficacy was, however, limited to 10 weeks in a 4-month study (Hulley *et al.*, unpublished data). Mineral loss was principally from weight-bearing bones, and was recoverable in the post-test period. It is possible that in periods of prolonged weightlessness stabilization of mineral balance may occur. Calcium balance does, for example, appear to normalize after some years in paralyzed patients.⁸ Nevertheless, these data indicate the requirement for high calcium and phosphate intake during long-duration spaceflight.

Disease Prevention: Protection for the individual whose microflora have undergone an alteration after exposure to the spaceflight environment should pose no real problem provided he is gradually re-exposed to the microflora of Earth upon return from prolonged spaceflight.

Radiation: Thus far, exposure to the radiations of the space environment appear not to pose a problem of any magnitude. However, radiation could pose a more serious hazard on longer term missions, particularly if they occur during periods of solar flare activity. Medications have been examined as a means of providing protection against radiation but, unfortunately, those which offer some degree of protection have proved to be too toxic to be safely prescribed.

Vestibular Preadaptation: Future crews may be more prone to vestibular disturbances than past crews have been. As a consequence, the possibility of preadapting the vestibular responses of individuals to the effects of zero gravity is being studied. Studies conducted in slow-rotation rooms are expected to demonstrate the feasibility of preadaptation.

Artificial Gravity: A final approach to counteract the effects of zero gravity on man is to provide some system of artificial gravity in space vehicles. Two approaches are possible: rotation of the entire space vehicle or inclusion of an onboard centrifuge. It should however be pointed out that medical and performance data available at the present time do not support a requirement for artificial gravity systems in spacecraft. In the view of space crews, artificial gravity systems are unnecessary

for task performance. Crews have learned to live in a zero gravity environment and feel confident in this state. Many have, in fact, expressed a preference for zero gravity since the absence of the G force increases the effective volume of what would otherwise be rather confined work spaces. While the provision of artificial gravity would undoubtedly increase the habitability of spacecraft, the engineering tasks associated with designing an adequate artificial gravity system are formidable and could be very costly.

Clinical Medical Care: The need for clinical medical care inflight becomes more important as mission durations increase. The longer the duration, the more pressing is the need for an onboard diagnostic and treatment capability. Partly to this end, an integrated medical/behavioral laboratory measurement system, known by the acronym IMBLMS, is being developed. This sophisticated laboratory system will have among its capabilities supply of medical support for the crew. It will, for example, present the results of hematological studies on video tape.

For very-long-duration missions, it may be best for one crewmember to be a physician. It is now a well-known fact that the first U.S. Skylab mission included a physician in its crew. This approach has numerous advantages in addition to the obvious one of insuring that medical treatment is readily available. The astronaut-physician will understand the purpose as well as the mechanics of any experiments which may be performed inflight and will, therefore, be able to assure that procedures employed safeguard their aims. In short, he can distinguish between trivial procedural variations and serious ones that might create a bias in results.¹¹ He will, in the course of the mission, be alert for signs of changes which may not have been expected and recognize patterns of response. On this basis, he may suggest modifications to experimental protocols as missions progress. It is conceivable that a mission could be salvaged through intervention of an onboard physician should any serious problem of a clinical nature arise.

PSYCHOLOGICAL FITNESS

Now that it is clear that the human organism can withstand the rigors of space flight, attention must properly turn from man as an organism to man as a psychological entity. We know man can live safely in space, but can he live productively hundreds of thousands of miles away from everything to which he is accustomed, in a microcosm, in a void, for 6 months or a year or possibly three? Principal emphasis has been placed on understanding the mechanisms of man's body in the space-flight environment and, while we know much more than was known a decade ago about bodily reactions, we still know very little about the effects of the space environmental complex on personality and psychic well-being. These aspects of the human could prove to be the factors which limit the duration of space flight if they do not now receive the attention required.

There is a data base upon which certain predictions can be made concerning the relative importance of psychogenic variables in long-term space missions. But this base is admittedly small. Two- and three-month

space cabin simulation tests, which were principally oriented toward equipment verification in the past several years, have also included some habitability assessments. The Skylab Program will look even more extensively at characteristics of the environment of a space habitat which make it more or less acceptable. These data should be invaluable for planning longer term missions, as should data from Salyut missions. This information, coupled with observations of and reports from astronauts and cosmonauts who have engaged in space flights to date, should provide the basis upon which engineers and designers may alter the aspects of the spacecraft environment which it is possible to alter so that those aspects which cannot be altered will be that much more tolerable.

Confinement: Because booster technology had restricted payload, early space vehicles were extremely confining. The Apollo vehicle, while still small, did not prove to be as confining as it might have been expected to be. Earth based studies of volumetric requirements have tended to present a distorted picture of man's need for space because of the impossibility of simulating the critical dimension of zero gravity. While the volume of the Apollo command module was only 3,600 cubic feet, this volume was felt by many astronauts to be adequate because of the enhanced facility for movement in the weightless state. Whereas a doorway of less than standard height is obstructive on earth, the ability to move in a swimming manner in zero gravity would make even a 3-foot hatch more than adequate in a space cabin. By these standards, the 10,000 cubic foot living area of the Skylab with its two-story, or two-compartment depending upon one's orientation, arrangement would seem exceptionally unconfining.

Isolation, Estrangement, and Privacy: The notions of isolation and sensory deprivation once expected to characterize space flight have thus far proved to be virtual non-issues. If anything, man is over-stimulated by the numerous tasks he must perform and the unique visual experiences of his new view of the cosmos. The "silence of the void" is replaced by the sound of machinery which makes the spacecraft cabin at least as noisy as any typical office and sometimes noisier. Audiovisual monitoring prevents man from being isolated in the strict sense, even to the point of denying him a modicum of privacy. If isolation is at all a problem in space it is an isolation that might better be termed estrangement. It is only natural for one to feel very much alone over a quarter of a million miles from all that is familiar. This response on the part of space crews was expected and, as early as the Gemini 7 mission, efforts were made to combat the potentially demoralizing effects this estrangement might produce by supplying crewmen with news of events on Earth and arranging for them to talk with their families. These steps should be continued in future space flight missions, with all possible efforts being made to ensure that personal communications can be conducted privately.

The issue of private or privileged communications arises also in connection with discussions between space crews and physicians monitoring their flight. It is essential that these communications be as private across the

void of space as they are in an Earth-based physician's office. While it is critical that telecommunications between Earth-based monitoring stations and space vehicles be maintained, it is still possible to afford space crews a certain degree of privacy. In a space vehicle with room for separate compartments, it may be perfectly adequate for only one member of the crew to be observed at any given time while others go about their activities in privacy.

Where the need for one crewmember to remain private from another is concerned, Earth-based studies have indicated that a feeling of privacy is not necessarily predicated on the provision of a specific amount of physical space. The subjects who participated in the NASA sponsored 90-day manned test of a regenerative life support system were confined to a chamber which provided only 90 square feet of floor space per man, yet the crew indicated that their privacy needs had been satisfied. They came to view privacy as the ability to separate one's self from others, if not in a physical sense, then in a psychological sense. Privacy was thought to be satisfied when all four men were located in the same area but were engaged in individual activities which did not require interaction.⁹

Crew Roles: Another element in the stability of the individuals in a group rests on the stability of the group itself. In order to insure that crew interaction is orderly and does not become a source of friction, roles must be strictly defined. The assignment of specific roles has been a feature of past space flight missions and will be a feature of future ones. As space crews grow larger and mission length increases, organizational structure will become even more important.

Crew Composition: Crew composition is as important as crew structure. For long-duration missions, crews will have to be even more carefully selected than they are now. It will be necessary to be psychologically and physiologically selective and, once adequate techniques have identified the most suitable individuals, to train those selected in psychodynamics so that they will better understand what they will face in long term space flight. Training in group dynamics, for example, should give the crewmember a better understanding of himself and a greater facility for solving his own problems and understanding the problems of his fellow crewmen. Such an approach, it is hoped, would contribute substantially to harmony and cooperation during long-term space missions. Psychological selectivity in training will be even more important when crews are no longer composed of highly motivated, robust test pilots, but include scientists and other civilian personnel.

Soviet scientists suggest that psychological compatibility of crews be given very careful attention and ample time be allowed for crews to adjust to each other. In a 1 year test of a manned regenerative life support system conducted in the Soviet Union in 1967-68, the three-man crew was selected both on the basis of somatic and psychological compatibility. They underwent a series of group compatibility tests for an entire month prior to the start of the experiment. By the end of the experiment, no serious troubles had erupted among the subjects. This

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was attributed to the careful attention given to psychological selection and group preadjustment.[†]

It is quite possible that the lack of normal heterosexual relationships could cause a significant buildup of emotional tension during missions lasting for a year or more. The resulting unsettling effects could well undo all other efforts directed toward making environments habitable. For missions, such as the Mars type, mixed crews must be seriously considered. The issue of mixing of sexes in space crews in the future may not be the delicate one it has been traditionally expected to be. Sexual mores have changed significantly in the U.S. and throughout the world. As a consequence, living in close proximity with persons of the opposite sex may seem to future space crews a comfortable and natural thing. The population from which astronauts will be drawn in future years will more than likely have spent their years in university training, studying and working in mixed groups and living in sexually unsegregated dormitories. Indeed, many universities throughout the U.S. now feature such arrangements.

HABITABILITY FACTORS WITH IMPACT ON SPACE MISSION SUCCESS

In addition to the factors just described which are related to the individual himself and his relationship to other individuals involved in space missions, factors external to the space crewman can have a tremendous impact upon his psychological state and ultimately upon his performance. Again, it must be borne in mind that any factor which has the potential to degrade morale in the short run has the potential to deteriorate performance in long-term space flight.

The Space Cabin Environment: Several aspects of the space cabin environment are particularly relevant to the psychic state of the inhabitant. Decor and lighting can be surprisingly important. A habitat designed for efficient living, if it is not also appealingly decorated and cheerful, will do little to buoy the morale of those who must live in it. Astronauts engaged in even the relatively brief Apollo missions have been critical of the lack of warmth and variety in their environment. On long-term missions, sleeping accommodations and entertainment facilities will also play a more important role. Early space flights and, particularly, long-term ground-based studies, have also shown that food becomes a critical morale building or degrading factor in isolated microsocieties. Finally, personal hygiene maintenance and body waste elimination provisions, which have received far too little emphasis in the past, could become a major problem area for very-long-term missions unless they are at last acknowledged to play a serious and key role in habitability. The problems of defecation in space must be dealt with directly and without a trace of embarrassment by those responsible for the procuring, design, and development of life support systems before the physiological and, particularly, psychological difficulties they create become any more irksome to crews than they are now.

[†]Translated by Boris Mandrovsky, Library of Congress, from articles which appeared in the Soviet press.

Decor and Lighting: Sensory deprivation in the ordinary sense, as has been mentioned, is not a problem in space flight. On the other hand, sensory invariance can be. Innovative design in the environment of space vehicles for long-term habitation can contribute much to providing sensory enrichment. Apollo 12 astronaut Alan Bean undoubtedly spoke for other astronauts as well when he indicated that variety in spacecraft decor was important for keeping up morale. He described the look of the interior of the Apollo spacecraft, which is conspicuously lacking in any decorations, as a "turnoff."

Participants in a 3-month manned space cabin simulator test evaluated features of the interior decor. The results of this test, which was the longest one of its type in the U.S., indicated that interior decor should be enhanced by the inclusion of more materials which have more and diverse surface textural gradients. The subject also recommended that living and working quarters be sharply discernible from one another by virtue of design features such as lighting, furniture, and acoustics.⁹

Of all these factors, illumination may be the most important determinant of the visual environment. Here, the details of the illumination system are more appropriately discussed by engineers. Suffice it to say, however, that a level of illumination which is adequate for work performance may be unsatisfactory from a point of view of personal preference. In the 3-month study, although the Skylab illumination of 5- to 9-foot candles was found to be perfectly adequate for work performance, subjects chose to employ a light level about four or five times as bright.⁹ Provisions must also be made to exclude illumination where it is not desired, for example in sleeping quarters. Rheostatic control might also be desirable.

There are many solutions to these problems which are better discussed by designers than scientists. But the point is this: while technological problems of a more pressing nature and economic issues dictated that the area of spacecraft decor would receive little or no attention in past and near future missions, for longer term missions this element of the environment must receive higher priority.

Sleeping Accommodations: Many space crewmen have experienced difficulty sleeping in space. Others have slept in the weightless environment with minimal difficulty or none at all. In several cases, during both U.S. and Soviet missions, sleeping medications have been prescribed. While lack of sleep or the taking of sleeping medications for short periods of time is acceptable during relatively brief missions, these will be unacceptable conditions for long-term occupants of spacecraft. Because sleeping in an environment in which one floats is so radically different from sleeping in a 1 g environment, this problem cannot be adequately studied on the ground. Sleep experiments included in the Skylab program may help to explain more about the nature of sleep in the weightless environment. Meanwhile, to insure that more restful sleep is obtained, special sleeping quarters will be provided. The sleeping compartment in the orbital workshop is illustrated in Fig. 2. Each individual is provided with an isolated sleep area. Partitions and curtains insure privacy and a sleep restraint which hangs on the

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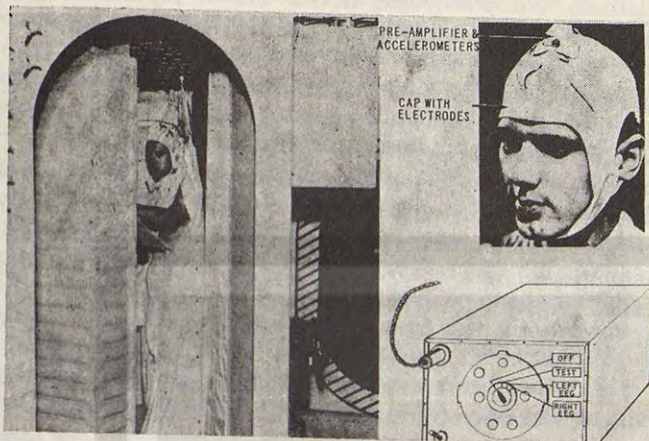


Fig. 2. Sleep monitoring, Skylab Experiment, M133.

wall provides a space "bed." At the right side of the figure, the occupant of the sleep compartment can be seen wearing a cap which contains EEG electrodes for sleep data collection.

Entertainment Facilities: Leisure time activities must be provided for long-term space flight, and those entertainment facilities selected must, of course, suit the preference of the individual crewman and not disturb those who do not choose to participate. On the Skylab missions, personally selected off-duty equipment are stored in the wardroom. Surprisingly, however, leisure time activities in space seem to take on less significance than they do on Earth. In one study, which asked 30 astronauts to rank order their preference of off-duty activities,⁵ job related activities were ranked first. Reading was ranked second and physical exercises third. Amusements, as we commonly think of them, did not begin to appear in the listing of preferences until fifth place. The need to exercise and the desire of astronauts for this activity is reflected in the inclusion of a bicycle ergometer exercise device on the Skylab mission. This device replaces an earlier, less-effective exercise device which many astronauts found unsatisfactory.

Missions to date have been extremely active with experimental tasks. As missions become longer with the possibility of increased leisure time, the astronauts will undoubtedly place leisure time activity higher on their preference lists. To this end, off-duty activities equipment has been provided. Some of this equipment is illustrated in Fig. 3.

Food and Meal-Taking: Space cabin simulator projects and underwater missions, notably the 60-day Tektite mission, have clearly indicated that ever-increasing importance is attached to mealtime activities in environments such as would be typified by long-term space stations. Dining became a major social event of the day and a time for communication and problem-solving. Food preparation itself was often viewed as recreation. This contrasts sharply with the experience of crews during short-term space missions. Crews to date have in general complained about the amount of time required for prepar-



Fig. 3. Skylab off-duty activities equipment.

ing food. This is undoubtedly because crewmembers were required to perform numerous and important duties, and food preparation, by comparison, appeared mundane. However, based on the importance attached to food during long-term tests of groups in isolation, efforts have been made and will continue to be made to improve the quality and variety of the foods for long-duration flights.

The scientific nature of space flights will preclude an *ad libitum* approach to food for a long time to come. Nevertheless, the foods provided can and should be attractive. To this end, the Skylab mission features, in addition to the freeze-dried foods served on earlier space missions, a greater variety of intermediate moisture foods that can be heated very simply in a heating tray, and frozen foods made possible by the inclusion of onboard freezers. Fig. 4 permits comparison of Apollo foods with the more natural Skylab diet. Meals which include such popular foods as steaks will be eaten with utensils. A separate dining facility, or wardroom, has been provided.

Personal Hygiene and Waste Management Facilities: Only the most basic provisions have been made for cleansing of the body during space missions to date and, in many instances, not even these. The provisions made for urine and fecal collection have been completely unacceptable from a psychological point of view and inadequate from the point of view of good sanitation. The difficulty experienced in collecting fecal matter in weight-

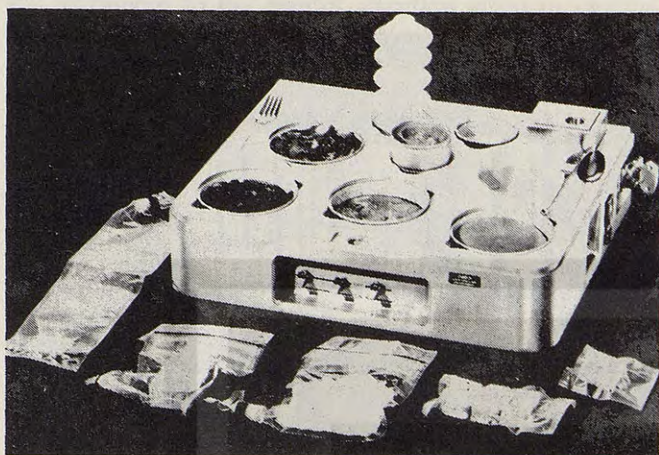


Fig. 4. Skylab and Apollo food.

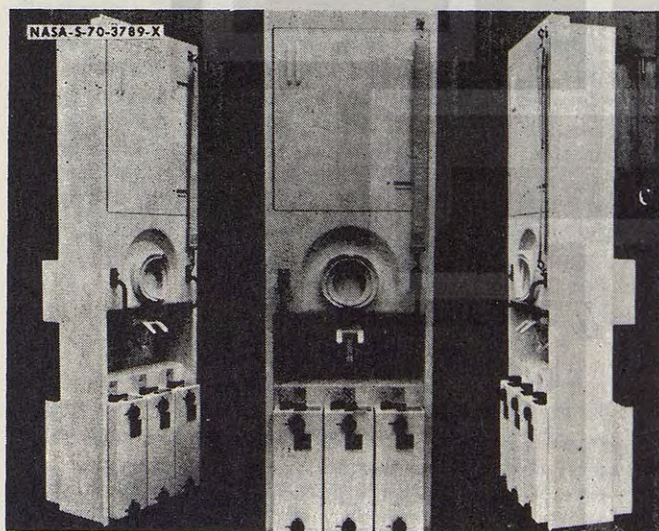


Fig. 5. Skylab hygiene facilities.

lessness with a plastic bag which must then be handled by the crewman is apparent and need not be belabored. The potential for contaminating one's clothing and the atmosphere with such a "system" is also self evident. Nothing more elaborate than small wipes has been provided for washing the body. In both these areas, personal hygiene and body waste elimination, present approaches must be scrapped entirely and new ones substituted which are easy to use, automatic, and psychologically unobjectionable.

The Skylab hygiene facilities (Fig. 5) represent a significant improvement over the archaic methods employed on previous missions. They still, however, are not optimal. For the first time, a separate compartment has been provided for personal hygiene and waste management. The compartment contains running hot water which will be used to moisten towels for general body cleansing. Provisions have also been made for a "shower" to be taken once a week. The shower is admittedly a

relatively unsophisticated device but it represents the first step toward whole body cleansing in space and it is the cleansing method of choice for most crewmen. The shower consists of a hand-held showerhead which permits the astronaut to spray his body with water. No vacuum drawing method is provided to remove the water from the body so that the individual must brush the water droplets off by hand and then remove the remaining moisture with a towel. The water remaining in the shower compartment is removed by a vacuum cleaner intended primarily for other purposes.

CONCLUSIONS

This paper has reviewed the human problems which must be addressed for long-duration space flights and has discussed solutions which appear to be promising for the most pressing of these problems. Long-term bedrest data offer some clues to the potential effects of weightlessness upon physiological systems, but there is still a great deal to learn about these effects. There are, however, some indications that some zero gravity effects may be self-limiting. Only flight data can provide definitive information. The collection of good inflight data for longer periods, coupled with ground based experiments is vital in any planning for longer duration space flight.

Countermeasures which have been explored have been needed in the U.S. space program only for the prevention of the cardiac arrhythmias noted in the Apollo 15 crew and, in this case, consisted of potassium supplements. While countermeasures have not yet been needed to reverse the effects of weightlessness on other physiological responses, cardiovascular system responses appear to be the most likely to require control. Fortunately, these responses also appear to be the most amenable to regulation by the application of inflight measures, such as the use of lower body negative pressure. If such techniques are employed, it may not be necessary to apply them for the entire duration of a space mission; a brief period of application prior to reentry may be sufficient.

Medications, in our experience, have not proved to be of value in treating either the cardiovascular deconditioning or the loss of exercise capacity noted in crewmen postflight. There is, however, some early indication from bedrest studies that combinations of calcium and phosphate help to forestall the loss of calcium from the bones. Oral administration of calcium and phosphate would seem, therefore, to be a suitable approach for space flight use.

The incidence of vestibular problems has not been great enough thus far for U.S. crews to require a pre-adaptation program. Such a program must, however, be considered as the number of potential crewmen is increased, particularly as scientists with no test pilot experience are added to crews. However, I do not at the present time see any physiological requirement for the addition of artificial gravity for long-duration space flight.

Areas that have not posed problems thus far because mission durations have been short and crews have been very active may well pose problems on longer duration flights. In the future, efforts must be exerted towards selecting and training crews in the psychological sphere, for long-duration space flight imposes many different

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types of stresses than those encountered in the flight durations thus far. Waste management and body hygiene provisions for all missions except Skylab have been, frankly, archaic. Such conditions cannot be tolerated any longer, particularly as we look to long-duration flights. Serious attention must be paid to planning and design of these systems so that they are completely acceptable to crews. In addition, attention must be given to providing a virtually automatic capability for sampling urine and feces.

By the end of the U.S. Skylab program, a substantial amount of data will have been collected that will provide an invaluable basis for planning future missions. Further problems may also emerge that will require study. Ultimately, we envision a manned mission to Mars. Obviously, the relatively brief Skylab missions will not provide a sufficiently sound basis upon which to predict that man can tolerate space flight habitation for 2½ years, but they represent a significant start toward this goal. I personally believe that 6 months of inflight data would provide an adequate basis for safe projection. In this period of time, all physical changes of a progressive nature could be identified. Certainly, within this period, any aspects of the environment having a deleterious psychological effect would become obvious. It is information of this degree of reliability toward which we must strive.

I believe that man can be supported for long-duration space flight if we exert our energies to learn what is happening in that environment and then to define the responsible mechanisms. Once mechanisms are clearly understood, the proper countermeasures can be applied if necessary. I think we can gain this information and support man on a journey to the planets.

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MEDICAL PROGRESS

HEPATIC BILE FORMATION (First of Two Parts)

NORMAN B. JAVITT, M.D.

EXCEPT that hepatic excretory function is frequently disturbed during the course of many illnesses, attempts by investigators to understand the physiologic mechanisms governing bile formation might better be delayed until more fundamental knowledge has accumulated in the basic disciplines that must be applied to the problem. These disciplines include the structure of water,¹ water transport across biologic membranes,² solute transport across biologic membranes³ and the structure of membranes.⁴ In these reports studies of transport across a variety of tissues are described, but in none does one find a study of transport function of the liver. Investigators usually try to limit the number of variables with which they must contend, and therefore choose to study isolated cells or thin-walled epithelial structures, where there is ready access to both sides of the membrane for the measurement of both chemical and electrical gradients and of bulk changes in water movement. By these criteria, the bile secretory apparatus is not ideal and therefore is not an attractive model for developing concepts of solvent flow and solute transport.

Hepatic bile formation and its disorders can be considered in relation to the major components of bile: water; bile acids; lecithin; cholesterol; bilirubin; and a variety of miscellaneous compounds present in the daily diet or in medications. These compounds include food dyes and preservatives, artificial sweeteners, tranquilizers, diuretics and antibiotics. Complete chemical classification of all the types of solutes dissolved in bile is not possible, but for purposes of this review they can be recognized as organic compounds that may be negatively charged (anions), positively charged (cations) or uncharged (neutral). The problem of cholesterol and lecithin excretion has been reviewed on frequent occasions in the past few years in connection with gallstone formation and will not be further discussed. My focus will be the generation of bile water flow and the transport of bile acids and bilirubin and related organic anions.

FUNCTIONAL ANATOMY

The studies of Rappaport⁵ permit one to delineate a microcirculatory unit that has functional importance within the liver acinus.⁶ The arterial blood entering the acinar lobule supplies first the bile ductules, and then, together with blood from the portal vein, goes to the hepatic parenchymal cells (Fig.1). In this man-

ner, the many compounds entering the mesenteric circulation from the gut are directed to the hepatocytes for processing, before entry into the systemic circulation.

At the sinusoidal level one can start with the knowledge that all blood enters the acinar lobule via the portal venule and hepatic arteriole and leaves via the hepatic venule. The microcirculatory unit that supports the hepatocytes consists of the arteriolar-portal pedicle, a sinusoidal glomus, and at least two terminal hepatic venules. Of functional relevance is the question of whether each hepatocyte is exposed to sinusoidal plasma of identical composition. Logically, it



Figure 1. Microcirculatory Hepatic Unit (Reproduced from Rappaport⁵ with the Permission of the Publisher).

The unit consists of the terminal portal venule (TPV) with the sinusoids branching off it and forming a glomus; the hepatic arteriole (HA) lacing with its branches forms a plexus around the terminal bile ductule (BD). The arterioles empty either directly (1) or via the peribiliary plexus (2) into the TPV and sinusoids. The sinusoids run along the outside of cell plates and cords, inside which are the capillaries of the hepatic secretory and excretory system. The glomus of sinusoids is drained by at least two terminal hepatic venules (THV); LY = lymphatics.

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Supported by a grant (AM-16201) from the National Institute of Arthritis, Metabolism and Digestive Diseases.

is easy to visualize a sinusoidal segment beginning as a continuation of a portal venule and terminating in a hepatic venule with unidirectional plasma flow. From this model it must follow that fluid entering the space of Disse at the portal end with the highest concentration of solutes is directed at adjacent hepatocytes. The amount of solute removed by the hepatocytes will depend on the type of transport system. For compounds that can diffuse through a membrane, the hepatocyte exposed to the highest concentration will contain the greatest amount. Solutes that require a carrier in the membrane for transport will behave differently, depending on the availability of carrier molecules. With a low ratio of carrier to solute molecules, the concentration of solutes will fall very slowly along the sinusoid, and each hepatocyte will be exposed to approximately the same concentration. As the ratio of carrier molecules to solute increases, the concentration gradient will fall more steeply across the sinusoid, and eventually the hepatocytes closest to the hepatic venule may not have any exposure. An autoradiographic study of D-galactose uptake by the hepatocytes illustrates the concept of nonuniform exposure of hepatocytes to solutes.⁷ Assuming uniform capability in galactose removal by membrane carrier, the hepatocytes considered to be closest to the portal venous end of the sinusoid contain more galactose than those closest to the hepatic venous end. A similar apportionment probably applies to many compounds, and can explain the periportal distribution of hepatocyte injury seen with some hepatotoxins.

However, the concept of a gradient along the sinusoid must be tempered with the cinematography of the sinusoidal circulation.⁸ It appears that the course of blood through the sinusoids is not always unidirectional because of an anastomotic network of sinusoids suspended between the portal and hepatic pedicles. Resistances in the sinusoidal network can vary from moment to moment, and therefore the path through the network will change appropriately even though the overall flow remains unidirectional. Branches of the hepatic artery appear to empty intermittently into sinusoids at various angles,⁹ thus rapidly altering the rate and direction of flow. This anatomic arrangement tends to lessen the effect of a gradient in concentration along the sinusoid and provides a mechanism for exposure of the hepatocytes to sinusoidal plasma that in toto is of more uniform composition. The existence of a space of Disse between the hepatocyte and the sinusoidal membrane and pulsatile pressure changes within the sinusoids allow for some mixing of solutes before hepatocyte uptake and function to reduce the steepness of the gradient. Thus, although the basic anatomic arrangement is that of a nonuniform distribution of solutes to hepatocytes, the steep gradient that could occur is modified somewhat by a sinusoidal network that allows for variations in the course of plasma flow and for a stirring of fluid in the space of Disse.

Canalicular bile flow in the lobule is in a direction opposite to sinusoidal plasma flow. The functional im-

plications of this arrangement pertain to the nonuniform distribution of the solute load. Thus, hepatocytes at the portal venous end of the sinusoid can elaborate a canalicular bile containing solutes in higher concentration than those at the hepatic venous end.

The bile, rich in solutes, flows opposite to sinusoidal plasma flow and therefore does not come in contact with hepatocytes that may contain lesser amounts of the same solute. The flow pattern reduces diffusion of solutes from bile into the hepatocyte. Also, for solutes that may enter bile by a carrier-mediated transport mechanism, the high concentration of solute molecules at the canalicular surface would slow the function of the carrier. Thus, the flow of canalicular bile "counter" to the sinusoidal plasma flow favors the formation and maintenance of a bile with a high concentration of organic solutes.

CANALICULAR BILE FLOW

The central problem of bile formation is understanding the mechanisms that generate bulk movement of water into bile canaliculi. The possibilities include all mechanisms postulated for water movement in biologic systems. These are categorized as pinocytosis, active water transport and passive water flow in response to osmotic or hydrostatic gradients. The studies of Brauer¹⁰ using the isolated perfused liver showed that bile flow was largely independent of the sinusoidal perfusion pressure, thus eliminating hydrostatic pressure as an important mechanism for the generation of bile flow. The essential finding was that the hydrostatic pressure used to perfuse the liver and therefore the sinusoidal pressure was less than the hydrostatic pressure produced by elevation of the cannulated bile duct. Under these conditions, bile continued to flow. These data led Sperber¹¹ to suggest that water flow occurred in response to osmotic gradients. Further delineation of osmotic mechanisms has been explored for almost 20 years, and a sizable body of information is available for review. Other possible mechanisms do not appear to be sufficiently attractive to warrant thorough exploration for generation of bile flow, and their exploration may come about only if it is found that osmotic mechanisms cannot fully satisfy the requirements.

QUANTITATIVE ESTIMATION OF CANALICULAR BILE FLOW

The possibility that a reference solute could be used to estimate water flow in the bile canaliculi came about when Schanker and Hogben¹² found that mannitol distributed in liver-cell water. Studies of water flow in the nephron had shown that mannitol accurately reflected glomerular filtration and did not diffuse appreciably from the tubule. Thus, estimation of mannitol output in urine as compared to the plasma concentration indicated total water flow across the glomerular membrane, and the urine-to-plasma concentration ratio indicated the fraction of water reabsorbed distal to the glomerulus. The finding of mannitol in liver-cell water and bile immediately led to the

possibility that it could similarly reflect water movements in the biliary tree. To interpret the excretion rate of mannitol and similar molecules in bile, it is important to review the relation of transmembrane solute and solvent flow in the light of the more recent concept of membrane structure.

The lipid-bilayer concept of the membrane,¹³ together with data indicating that the osmotic permeability of membranes was greater than diffusional permeability,¹⁴ provided evidence of water flow through pores in the membrane. When it was found that mannitol (molecular weight of 182 daltons), which distributed in extracellular volume, entered in the liver cell, it was reasonable to assume that the liver-cell membrane had pores larger than other parenchymal cells. Further evidence for a pore theory could be derived from the finding that slightly larger molecules such as sucrose (molecular weight of 342 daltons) did not gain entry as readily into liver-cell water or bile.

However, polymers such as polyethylene glycol (molecular weight of 4500 daltons) that also distribute in extracellular volume penetrate the hepatocyte and appear rapidly in bile.^{15,16} In common with mannitol, polyethylene glycol 4500 can also be used to estimate glomerular filtration and canalicular bile flow. Yet it seems unlikely that the mechanism by which each compound penetrates the hepatocyte is the same. The hepatocyte was considered exceptional to have pores sufficiently large to accommodate mannitol, and it would be stretching current concepts even further to propose pores large enough to accommodate polyethylene glycol. Thus, it is reasonable to consider other mechanisms by which these molecules may enter the hepatocyte.

More recent studies indicate that cell membranes are mosaics. The lipid bilayer is penetrated by proteins that may be asymmetrically arranged, and lateral movements of both lipids and proteins within the membrane can occur.¹⁷ Of perhaps fundamental importance to water flow were the data indicating that differences in diffusional and osmotic permeabilities are largely artifacts of unstirred layers and that water can diffuse rapidly across lipid bilayers.¹⁸ These more recent findings imply that although mannitol and polyethylene glycol 4500 are present in approximately the same concentration in plasma and liver-cell water, the routes of entry may be different. This alternative view can be supported by recent findings in other biologic systems such as the erythrocyte¹⁹ and toad bladder,²⁰ in which it has been found that solute molecules such as urea do not flow through aqueous pores together with water.

An entirely different conception of solute distribution in vivo has been proposed by Ling, who questions the barrier function of the membrane^{21,22} as the exclusive determinant of intracellular solute distribution. According to Ling, intracellular water is highly oriented because of its hydration or bonding to intracellular proteins and the ionic structure of the cations associated with these proteins. Thus, unlike ex-

tracellular water, which is free to diffuse randomly, intracellular water has fewer degrees of freedom. Solutes therefore do not diffuse intracellularly because energy would be required to reorient the structured intracellular water.

Thus, in addition to the initial interpretation for the presence of mannitol in liver-cell water as entry via pores, more recent studies provide the possibilities of a specialized transport system in the membrane or regulation by cytoplasmic water structure.

The usefulness of mannitol as an index of canalicular water flow is not necessarily dependent on flow through aqueous pores. Rapid equilibration with water entering the canaliculi by any mechanism will suffice, provided the relation remains constant at all flow rates and provided all the mannitol that flows with water into the more distal segments of the biliary tree is recovered in bile. Data derived from the studies of secretin-induced choleresis²³ and taurocholate infusion in dogs,²⁴ taken together with the known behavior of mannitol in the renal tubules, provide strong evidence that diffusion from these distal segments does not occur. Thus, the concentration of mannitol in bile, as compared to the plasma concentration, estimates the fraction of water in bile derived from canalicular segments.

It may be important to specify that because of the rapidity with which mannitol equilibrates at the canalicular level, a flow of water in and out of canaliculi might not be detected. Schanker²⁵ tried to distinguish between direct entry of mannitol into bile via an extracellular route (tight junctions) and entry via the hepatocyte into bile. He found that equilibration of mannitol between plasma water and liver-cell water occurred within 60 seconds. It was not technically possible to ascertain if this rate was appreciably different from equilibration between plasma-cell water and bile water. Although no evidence exists for a canalicular circulation of water, it would be unlikely to be detected by mannitol. Therefore, it is perhaps more accurate to conclude that mannitol estimates net canalicular flow — the volume of water that flows from the canaliculi to the more distal regions of the biliary tree per unit time.

GENERATION OF CANALICULAR BILE FLOW

Since hydrostatic forces are apparently unimportant, only osmotic mechanisms will be discussed. Because of the excellent correlation between bile acid excretion and canalicular bile flow ($r > 0.9$) in all species, including man,²⁶ the term *bile-acid-dependent canalicular bile flow* has been coined to describe this observed relation. Further studies of the phenomenon led to the recognition that bile-acid-dependent flow is demonstrable only at high rates of bile acid output. As the bile acid pool is depleted, canalicular bile flow is relatively well maintained and appears to become independent of bile acid output.

Quantitative estimation of a *bile-acid-independent fraction* cannot be obtained directly. It is derived from

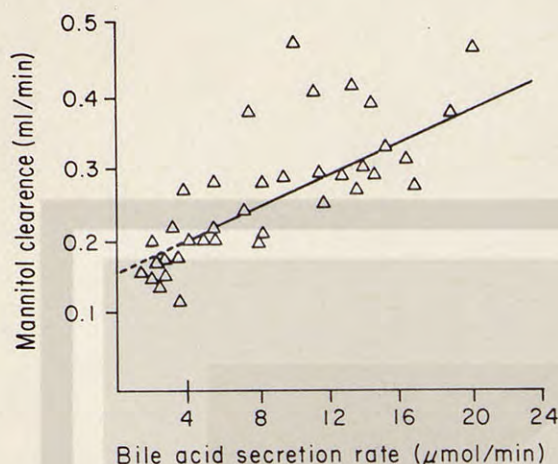


Figure 2. Relation between Bile Acid Excretion and Biliary Mannitol Clearance in 10 Patients Undergoing Total Bile Diversion via a T-tube with an Occludable Distal Limb (Adapted from Scherstén et al.^{26,29}).

Regression equation for mannitol clearance versus bile acid excretion at rates above 5 μ moles per minute, $y = 0.011x + 0.17$. Extrapolation (interrupted line) to zero bile acid excretion gives a positive intercept implying continued bile flow (bile-acid-independent fraction).

studies in which mannitol excretion rates are obtained at several different rates of bile acid excretion and a regression line calculated that is then extrapolated to zero bile acid excretion (Fig. 2). This method was established with use of infusions of sodium taurocholate in the dog²³ but was challenged by observations in the rat,²⁷ guinea pig and rabbit.²⁸ In the latter species it was difficult to establish linearity between canalicular bile flow and bile acid output at high rates over a wide range, particularly when sodium glycodeoxycholate and glycochenodeoxycholate were infused. However, recent findings in man²⁹ clearly confirm the observations in the dog (Fig. 2). Ten subjects, seven to 12 days after uncomplicated operations for cholelithiasis, consented to total biliary diversion via a T-tube with an occludable distal limb. The relation of bile acid excretion to mannitol excretion was determined over a 12-hour period. The bile acid composition of the drained pool consisted of the glycine and taurine conjugates of chenodeoxycholic and cholic acids. Moreover, after depletion of the endogenous pool, infusion of chenodeoxycholate or cholate into the duodenum was followed by rapid excretion in the conjugated forms. The changes in mannitol excretion rates obtained were not different from those occurring during depletion. Thus, a linear relation exists in man at bile acid excretion ratios above 5 μ moles per minute.

BILE-ACID-DEPENDENT BILE FLOW

The physiological basis for the linear relation between canalicular bile flow and bile acid excretion is assumed to be related to the osmotic properties of the bile acids. A physical property of the bile acids is the

formation of polyanionic molecular aggregates or micelles above a certain concentration in aqueous solution. The critical micellar concentration and its molecular size are determined by the species of bile acid and are modified by the ionic concentration of sodium and other salts and also by the cholesterol and lecithin content of bile. With recently available laser light-scattering techniques,³⁰ sodium glycochenodeoxycholate (molecular weight of 472 daltons) was estimated to have a micelle weight of 11,400 in pure solution and 27,000 in a solution in which the molar ratio of bile acid to lecithin was 3:1, a ratio naturally occurring in bile.³¹ No direct estimates are available of the range of molecular weights of the mixed micelles composed of bile acid, lecithin and cholesterol present in bile. Presumably, the linear portion of the relation between bile flow and bile acid excretion occurs because the mixed micelle composition of bile remains constant — only the rate at which these micelles form varies directly with the rate of bile acid secretion. Recently,³² analysis of the relation of canalicular bile flow to bile acid excretion at rates ranging from 2 to 105 μ moles per minute per gram of rat liver showed that a series of regression equations could be derived with varying slopes depending on the bile acid concentration in bile. The slopes of the regression lines showed significant changes ($P < 0.001$) from a value of 0.090 at bile acid concentrations below 10 mM to a slope of 0.012 at concentrations between 30 and 45 mM. Although this very careful analysis confirms the essential role of bile acids in generating canalicular bile flow it implies that micelle size may increase together with bile acid concentration, thereby reducing the osmotic gradient as indicated by the progressive decrease in the slope of the regression equations. The findings also indicate that calculation of the bile-acid-independent fraction by the use of one regression equation is subject to error.

It is not known where molecular aggregation occurs during the process of bile formation. The bulk of the bile acids secreted each day represent the enterohepatic recirculation of the existing bile acid pool. To this pool are added bile acids newly synthesized in the hepatocytes by a process beginning with hydroxylation of cholesterol by microsomal enzymes followed by the conversion of these dihydroxy cholesterol to dihydroxy and trihydroxy coprostanes, and then by degradation of the side chain by mitochondrial enzymes to form C-24 bile acids that are then conjugated to glycine and taurine by enzymes associated with the lysosomal fraction.³³ During the transport process from sinusoidal plasma to hepatic bile, the concentration of bile acids can increase from 0.2 to 60 μ mole per milliliter, a change from molecular to micellar solution. Because the mannitol concentration of hepatic bile is approximately equal to plasma, it is thought that there is little water reabsorption in the biliary tree and that therefore the step-up in bile acid concentration occurs at the level of the biliary canaliculus. The possibilities are that either the bile acids are

prepackaged at some intracellular or membrane site and then secreted in a mixed micelle into bile or there is molecular transport into the canaliculus followed by aggregation into micelles. The presence of cholesterol and phospholipid in the micelle implies a close relation to membrane but does not distinguish between solubilization of these membrane components after micelle formation or formation in association with membrane compounds. The secretion of membrane-associated enzymes, such as 5'-nucleotidase,³⁴ is also correlated with micellar bile acid excretion but also cannot critically distinguish between the two possibilities.

Bile acids that cannot form micelles cause a proportionally greater increase in canalicular bile flow³⁵ than micelle-forming bile acids and do not increase phospholipid excretion. A molecular mechanism for their secretion by some membrane-mediated carrier would be consistent with these findings and by inference also implies that one exists for micelle-forming bile acids. Since hepatic bile has never been demonstrated to be absolutely free of bile acid, and actually prolonged biliary drainage²⁹ has shown continued excretion at rates of approximately 2 μ moles per minute, it seems reasonable to think that a molecular transport system could exist to account for secretion rates below the micellar concentration in bile. Alternatively, however, it could be argued that at low rates of bile acid excretion, there is still accumulation at some prepackaging site before secretion into the canaliculus. After secretion, the micelle could be diluted by the bile-acid-independent fraction of canalicular bile and by the secretion of inorganic salts and water by the bile-duct epithelium, giving a final concentration in hepatic bile below the micellar concentration.

Because canalicular bile flow at low rates of bile acid excretion is greater than would be predicted from extrapolation of the regression line, an independent source for the extra amount of water has been sought. However, by making certain postulates concerning the physiologic properties of the canaliculus, it is possible to argue that the bile acids can account for all canalicular bile flow. The permeability properties of the canalicular membranes and perhaps the proximal smallest ductules determine the effective osmotic pressure generated by the bile acids. The Staverman* reflection coefficient³⁶ (σ) is a quantitative statement of the ratio of solute to solvent molecules that can penetrate a membrane. A value of 1 indicates complete impenetrability of the membrane to solute molecules and therefore the establishment of an osmotic gradient that generates solvent flow towards the impermeant solute. Complete permeability of both solute and solvent ($A_{sf}/A_{wf} = 1$, $\sigma = 0$) implies rapid diffusion of solute across the membrane and no effective osmotic gradient for the generation of water flow. Because the permeability properties of canalicular and

small ductular membranes are not known, no prediction can be made about the osmotic gradients generated by molecular and micellar solutions of bile acids. By postulating a high reflection coefficient for both molecular and micellar species, one would predict that canalicular bile flow would be relatively greater at low concentration before micelle formation begins. As a result of molecular aggregation, the millimolar concentration rises more rapidly than the milliosmolar concentration, causing a different relation to be observed between canalicular bile flow and micellar bile acid excretion. Although hepatic bile is always very close to 300 mOsm per liter, its capacity to generate water flow in vitro can be readily demonstrated if it is placed in a dialysis sac (Visking tubing) against 300 mOsm per liter of sodium chloride. Iso-osmotic saline will rapidly flow into and swell the dialysis sac because the bile acids are relatively impermeable to the cellulose casing, thus generating an osmotic gradient. Until fluid can be collected directly from canaliculi by micropuncture, or more is learned about the permeability properties of canalicular membranes, a complete answer to the role of bile acids in generating canalicular flow seems unlikely.

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*Staverman reflection coefficient, $\sigma = 1 - A_{sf}/A_{wf}$, where A_{sf} = effective pore area available to solute molecules, & A_{wf} = effective pore area available to water (solvent) molecules.

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CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL



Weekly Clinicopathological Exercises

FOUNDED BY RICHARD C. CABOT

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Continued preparation of these Case Records has been made possible by a generous grant from Pfizer Pharmaceuticals to the Massachusetts General Hospital.

CASE 52-1976

PRESENTATION OF CASE

A five-year-old boy was admitted to the hospital because of meningitis.

He was well until 10 days previously, when a sore throat and fever developed. He was seen at another hospital, where erythromycin was prescribed. The sore throat improved, but fever persisted, with malaise, abdominal discomfort and a stiff neck. During the five days before entry his family noticed slight slurring of his speech and a tendency to sway to either side on walking. One day before admission he was seen at a second hospital. The white-cell count was 12,000, with a slight shift to the left. An x-ray film of the chest (Fig. 1) revealed bilateral pleural effu-

sions, questionable bronchopneumonia and cardiac enlargement. A lumbar puncture yielded cerebrospinal fluid that contained 6300 white cells per cubic millimeter, of which 84 per cent were neutrophils and 16 per cent mononuclear cells; the protein was 20 mg, and the glucose 12 mg per 100 ml; a culture yielded *Haemophilus influenzae*, as did a nasopharyngeal swab. Fluids were given intravenously, and ampicillin was administered. On the following day he was transferred to this hospital.

The child was born of a normal, full-term pregnancy and delivery, and his growth and development were normal. There was no history of headache, respirato-

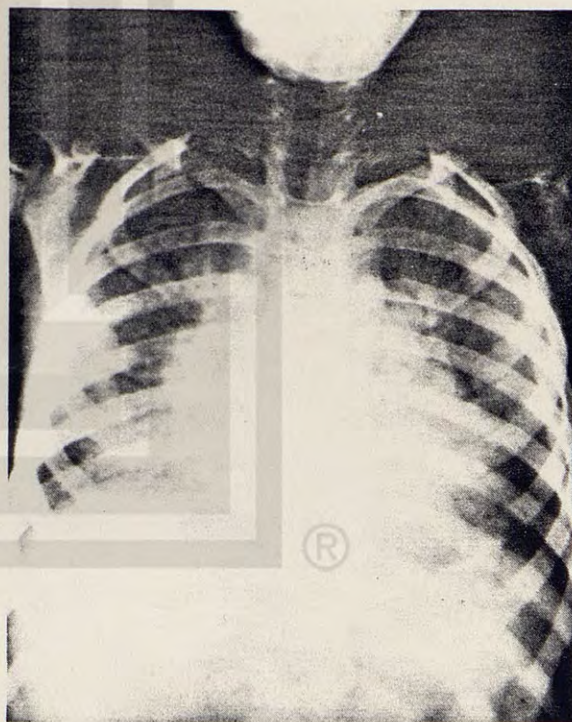


Figure 1. Radiograph of the Chest Obtained One Day before Entry, Showing Cardiac Enlargement, Small Pleural Effusions and Streaky Perihilar Infiltrates Bilaterally.

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MEDICAL PROGRESS

HEPATIC BILE FORMATION

(Second of Two Parts)

NORMAN B. JAVITT, M.D.

BILE-ACID-INDEPENDENT BILE FLOW

Sodium transport is under investigation as a physiologic mechanism for the generation of the bile-acid-independent fraction of canalicular bile flow. The electrolyte content of bile at low bile acid output consists mostly of inorganic sodium salts. Since it is known that sodium transport provides an osmotic gradient for bulk water flow across other membranes, interest has centered on its role in hepatic bile formation.

Studies of the sodium-potassium gradient across erythrocyte membranes have led to the recognition of a membrane carrier.³⁷ A specific macromolecule in the membrane continually transfers sodium outwardly and potassium inwardly. Energy for the "pump" is derived from hydrolysis of adenosine triphosphate by adenosine triphosphatase, which may also function as the carrier molecule. Addition of ouabain or other cardiac glycosides inhibits activity of the system. Because this model results in an exchange of solutes with no net transport, there is no osmotic gradient and no associated water flow.

By interposing two membranes with different biological properties, it is possible to use the sodium pump concept for the generation of water flow. A model was proposed by Curran³⁸ (Fig. 3) in which a sodium pump results in bulk flow of water into the cell, followed by diffusion of electrolyte and water across a second, porous, nonselective membrane. By means of this concept, evidence was developed to demonstrate that the same system exists in the gall-

bladder.³⁹ A general model, the standing gradient hypothesis based also on a strategically located sodium pump in the cell membrane (Fig. 3), was also developed and can account for water flow either into or out from the cell.⁴⁰ The rapid flow of water in response to the localized transport of sodium provides rapid equilibration and an apparent iso-osmotic state.

Because of the general applicability of the sodium-pump model for generation of water flow, it is also considered the leading physiologic mechanism for the generation of *bile-acid-independent canalicular bile flow*. In an attempt to evaluate this mechanism, inhibitors of the sodium pump such as ouabain and ethacrynic acid were given to animals. Initially, a reduction in canalicular bile flow was reported to occur in rabbits,⁴¹ but other studies in rabbits and other species, both in vitro and in vivo, now indicate that these agents increase canalicular bile flow.^{42,43} In retrospect, it may have been naïve to assume that the addition of ethacrynic acid or ouabain to perfused liver systems is analogous to its use in separated erythrocytes or thin epithelial membranes and that any result could be interpreted as evidence for or against the role of a sodium pump. Thus, the concept can still be considered a possibility, but one untested critically.

Other agents, such as theophylline,^{44,45} hydrocortisone⁴⁶ and phenobarbital,⁴⁷ have been found to increase bile-acid-independent canalicular bile flow. Administration of 300 mg of theophylline or 200 mg of hydrocortisone to dogs weighing between 15 and 30 kg causes a prompt 33 to 72 per cent increase in both water and inorganic sodium salt excretion without an increase in bile acid excretion. A dose-response relation could not be demonstrated. With radioactive hydrocortisone, it was possible to show that some of the administered compound was excreted in bile, and its

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Supported by a grant (AM-16201) from the National Institute of Arthritis, Metabolism and Digestive Diseases.

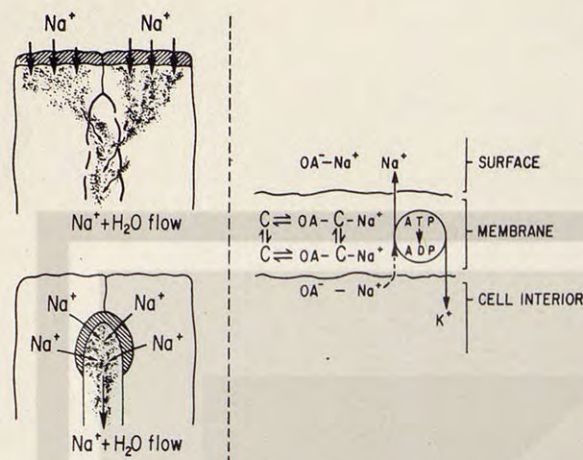


Figure 3. Models of the Sodium Pump for Water Flow and for Organic Anion Transport.

Three figures are shown. The upper and lower left figures show proposed locations of a sodium pump for the generation of water flow. In the upper figure, often referred to as the double-membrane model, the sodium pump is located in the external surface membrane, and an osmotic gradient is generated, causing water flow into the cell, followed by flow of solute and solvent through a nonselective, porous membrane along the lateral surface.

In the lower figure, the "standing-gradient" model is illustrated, with the sodium pump located at a strategic site in the lateral surface membrane, causing a local increase in sodium concentration and a flow of water into the lateral space (adapted from House¹).

The figure on the right (adapted from Schultz and Curran⁵²) illustrates one model of organic anion (OA) transport coupled with sodium. A carrier (C) in the membrane forms a complex with Na and OA at the exterior surface of the membrane, where the extracellular fluid is rich in sodium. The carrier complex then moves across and dissociates at the inner membrane surface, where sodium concentration is low. This action causes uncoupling of the carrier-OA complex and entry of OA into the cell. The transported sodium is then pumped extracellularly through the sodium-potassium exchange mechanism that derives energy from hydrolysis of ATP.

appearance coincided with the onset of cholestasis. With use of less sensitive techniques, theophylline excretion in bile was not established, but could not be excluded completely. The apparently low excretion rate of these agents in bile makes it unlikely that a sufficient osmotic gradient occurred to generate the increase in canalicular bile flow, even though an excretion of 1 μ mole of compound can generate from 8.3 to 23.8 μ liters of bile.⁴⁸

Theophylline is known to increase the intracellular level of cyclic AMP.⁴⁹ It is reasonable, therefore, to attribute its choleretic effect to an increase in hepatocyte cyclic AMP. Further support can be derived from the choleretic effect of glucagon, which is known to increase cyclic-AMP levels in the hepatocyte, and the cholestasis induced by etazolate hydrochloride (SQ20009), a phosphodiesterase inhibitor.⁴⁵ The link between cyclic AMP and water flow is believed to be via sodium transport. In several tissues, including the gallbladder, it has now been shown that elevation in intracellular cyclic AMP inhibits sodium chloride ab-

sorption.⁵⁰ Cholestasis attributable to this mechanism may therefore imply an increase in the loss of sodium chloride and water in bile because of decreased absorption. This interpretation is consonant with the effect of ouabain, a known inhibitor of the sodium pump, that also induces cholestasis. Thus, the findings concerning the mechanism of generation of the bile-acid-independent water flow fit into the framework of knowledge concerning transport in other tissues. However, to establish these reasoned extrapolations as actual mechanisms will require precise data obtained by direct studies of liver tissue.

Phenobarbital will induce an increase in canalicular bile flow in rats beginning six hours after a single intraperitoneal injection. Cholestasis can be blocked by prior administration of either actinomycin D (dactinomycin) or cycloheximide and therefore may be related to protein synthesis in some subcellular component.⁵¹ Further evidence for a relation to protein synthesis can be obtained from the study of Berthelot et al.,⁴⁷ done after four days of phenobarbital administration to 37 rats. Bile flow increased from an average of 9.6 to 14 mg per minute per 100 g of body weight, and liver weight from an average of 9.6 to 13.3 g. Correction for the change in liver weight results in a virtually unchanged bile flow. Thus, the choleretic and other known effects of phenobarbital in rat liver may be mediated by mechanisms different from theophylline and other agents that do not affect liver weight (i.e., protein synthesis).

The sodium pump has also been linked to organic anion transport, and it is fitting that this hypothesis be tested for the movement of bile acids across biologic membranes. Currently proposed sodium-linked transport mechanisms have in common a mobile carrier with high specificity and avidity for the organic anion.⁵² Although a carrier for bile acids has not been isolated from ileal mucosa, the site for active intestinal bile acid transport, a liver membrane fraction has been identified that appears to have specificity toward bile acids.⁵³ The role of sodium in the transport systems has several variations. In one model, the carrier molecule can bind either organic anion or potassium. The carrier thus transports an organic anion from outside to inside, where it exchanges for potassium that is then carried to the outside. The progressive loss of intracellular potassium is prevented by the proximity in the membrane of the sodium-potassium exchange pump that obtains energy from the hydrolysis of ATP. Another version, the ion-gradient hypothesis (Fig. 3), envisions a ternary complex of organic anion, mobile carrier and sodium that occurs in the high sodium environment external to the cell. The carrier complex dissociates at the internal membrane surface, a region of low sodium concentration that is maintained again by the sodium-potassium exchange pump. Other variations of the relation of these variables can be envisioned. The challenge is the designing of experiments that can critically evaluate the many possibilities. The concept of a carrier stereospecific for bile acids but requiring sodium would re-

concile the unique role of the bile acids in generating bile flow with a dependence on sodium for its operation.

The transport of bile acids across ileal mucosa is thought to be sodium dependent,⁵⁴ and substitution of lithium for sodium causes a reduction in bile acid secretion by the liver.⁵⁵ Thus, studies attempting to evaluate sodium dependence in bile acid transport support the general hypothesis.

Models for organic anion transport exist that are unrelated to sodium. By postulating a mobile carrier that undergoes configurational changes at the inner and outer membrane surface with a concomitant change in binding affinity, it is possible to obtain directional solute transport. Control of the configurational state of the carrier could be linked to the activity of other membrane components that may be independent of the sodium-potassium exchange.

CHOLESTATIC LIVER DISEASE

One could consider abandoning the term *cholestasis* because it is not strictly defined and is used in the description of a variety of problems. Nevertheless, it does always connote a disturbance in bile flow. For the purpose of this review it refers to disturbances in the generation of canalicular bile flow that are not associated with gross abnormalities in hepatic or biliary-tract morphology such as hepatitis, cirrhosis or mechanical obstruction. Thus, the focus is on possible disturbances in the generation of bile flow at the molecular level.

Four categories of defects can be envisioned. One group would be genetic or acquired defects in the synthesis of carrier molecules. The second group would relate to energy sources needed to maintain mobile carrier activity such as the sodium-potassium exchange pump requiring ATP and appropriate hydrolyase enzymes. The third category would be changes in the permeability properties of the canaliculi and proximal ductules that normally restrict diffusion of molecules that are actively secreted. Estrogen in high dose is known to reduce canalicular bile flow, and, at least in part, the mechanism appears to be a change in cell permeability as estimated by polyethylene glycol⁵⁶ and by sucrose.⁵⁷

A fourth category that can be associated with cholestasis concerns metabolic alterations in bile acid synthesis affecting the generation of canalicular bile flow. Increased amounts of trihydroxy coprostanic acids have been reported in two families with neonatal cholestasis,^{58,59} but the relation of the metabolic abnormality to the cholestasis has not been defined.

Experimentally, monohydroxy bile acids such as 3 α -hydroxy-5 β cholanoic acid (lithocholic acid) and 3 β -hydroxy-5-cholenoic acid can induce a severe cholestasis. The pathogenesis of the cholestasis is related to the insolubility of the compounds in aqueous solution, and their cholestatic effect is preventable by the simultaneous infusion of chenodeoxycholic or cholic acids.⁶⁰ It has been reported that before cholestasis, lithocholic acid accumulates in bile canalicular mem-

branes and causes a marked increase in membrane cholesterol concentration, with no change in phospholipids.⁶¹ These biochemical alterations are consonant with the distortion of bile canalicular membrane structure that precedes the cholestasis seen by both transmission⁶² (Fig. 4-6) and scanning electron microscopy (Fig. 7 and 8).⁶³ With the onset of cholestasis, evagination of canalicular membrane into the canaliculi and loss of microvilli occur. This sequence of events was not found to occur with either estrogen-induced cholestasis or bile-duct ligation, thus implying a different pathogenesis. Morphologic studies at later times reveal the widely dilated canaliculus devoid of microvilli characteristic of many types of cholestasis.⁶⁴

Since it is now known that 3 β -hydroxy-5-cholenoate is present in the meconium of normal neonates⁶⁵ and in the urine of infants with biliary atresia⁶⁶ it is reasonable to ask if it can be implicated in the pathogenesis of neonatal cholestasis. Favoring cholestasis would be a high ratio of monohydroxy to dihydroxy or trihydroxy bile acids and reduced or absent sulfokinase activity since the ester sulfates of monohydroxy bile acids do not cause cholestasis.

The lack of a cholestatic effect of the ester sulfates and presumably of glucuronides, if found in vivo, is attributable to their enhanced water solubility as com-



Figure 4. Freeze-Fracture Replica of a "Normal" Bile Canaliculus (X 37,400) (Reproduced from Miyai et Al.⁶² with the Permission of the Publisher).

Fractured surfaces of microvilli are studded diffusely with intramembranous granules, which are more numerous on convex surfaces. Inset in right lower corner (X 21,300) is a transmission electron micrograph of a "normal" bile canaliculus.



Figure 5. Freeze-Fracture Replica of Early Bile Canalicular Changes Induced by Lithocholate ($\times 55,200$) (Reproduced from Miyai et Al.⁶² with the Permission of the Publisher).

Some of the microvilli are elongated and flattened. Intramembranous granules are fewer or absent in the microvilli that are disfigured.

pared to the nonesterified compound.⁶⁷ Thus, glucuronidation or sulfation of the single hydroxyl group greatly increases hydrogen bonding to water and diminishes the need for solubilization of monohydroxy bile acids in the micellar structure of dihydroxy and trihydroxy bile acids. The data obtained thus far indicate that the monohydroxy bile acids are present in relatively small amounts and are predominantly ester sulfates, thus minimizing a possible role in cholestasis. However, these findings are in infants with established cholestasis, late in the course of the disease. Until we can identify infants at the inception of the cholestasis, the critical information is unobtainable.

ORGANIC ANION TRANSPORT AND CANALICULAR BILE FLOW

Considerable circumstantial evidence exists that organic anions such as bilirubin, iopanoic acid, sulfobromophthalein and indocyanine green share a common transport system but one that is separate from bile acids. Membrane fractions from hepatocytes have been reported to contain proteins that bind sulfobromophthalein⁶⁸ and bile acids⁵³ with little overlapping specificity. In vivo it has been shown that bolus injection of bile acids does not alter the disappearance rate of sulfobromophthalein from plasma.⁶⁹ Also, infusion of bile acids increases rather than decreases the maximum excretion of the dye.⁷⁰ Abnormalities such as the

Dubin-Johnson syndrome, in which there is a marked decrease in the capacity to excrete sulfobromophthalein and conjugated bilirubin, with normal or near normal serum levels of bile acids,⁷¹ and Byler's syndrome,⁷² with marked elevations in serum bile acids and relatively normal levels of serum bilirubin, also imply independent transport systems.

However, the frequent occurrence of cholestatic jaundice in response to medications suggests that an inter-relation between the two transport systems does exist. One possible inter-relation may be the dependence of organic anion excretion on the generation of canalicular bile flow. It is of interest that the secretion of bilirubin conjugates in bile is not associated with an increase in bile flow even though a 100-fold increase in the concentration of bilirubin conjugates can occur.⁷³ Under certain circumstances, such as the infusion of large amounts of bilirubin into newborn Macaca monkeys, bile flow may almost cease as the concentration of bilirubin rises progressively in bile.⁷⁴ In the rat⁷⁵ and the dog⁷⁶ it was possible to demonstrate a dependence of the maximum excretion rate (T_m) of bilirubin on canalicular bile flow, a finding that supplements the earlier studies of O'Maille, Richards and Short on the enhancement of sulfobromophthalein T_m by bile acid infusion. From this information it seems reasonable to formulate the principle that the transport system specific for bilirubin



Figure 6. Thin Section of the Bile Canalicular Region 30 Minutes after Infusion of Lithocholate ($\times 22,000$) (Reproduced from Miyai et Al.⁶² with the Permission of the Publisher).

A bile canaliculus is dilated, with loss of regularly shaped microvilli. The canalicular membrane is transformed into thin lamellar folds.

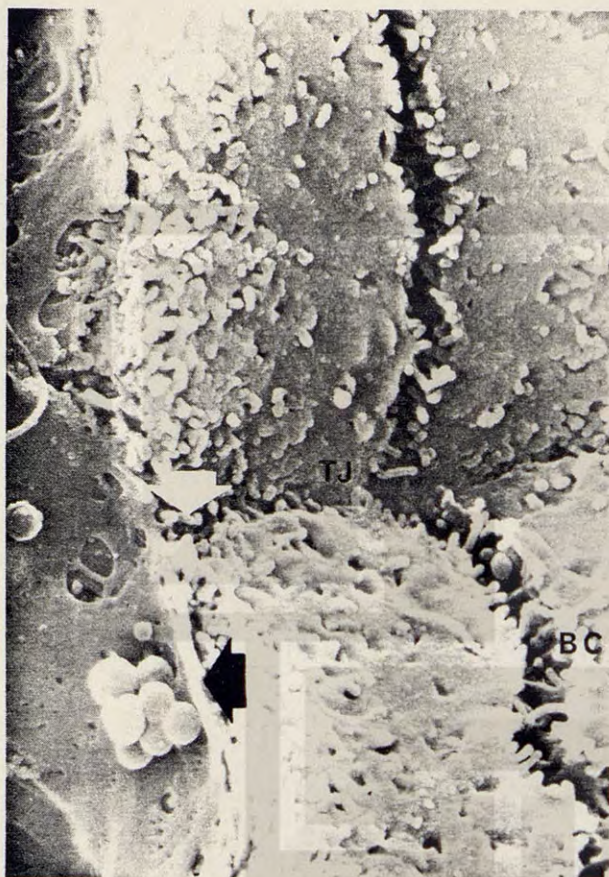


Figure 7. Intercellular Space (White Arrow) between Two Hepatocytes Extending from the Space of Disse (Black Arrow) toward the Area of the Tight Junction (TJ) Adjacent to the Bile Canaliculus (BC) (Scanning Electron Microphotograph, Original Magnification $\times 5000$, Reproduced from Layden et Al.⁶³ with the Permission of the Publisher).

and related organic anions accomplishes the delivery of these compounds into the biliary canaliculi. For further transport through the biliary passages, independent water-flow-generating systems are required. It follows from this relation that any severe disturbance in canalicular bile flow will decrease organic anion excretion. Thus, conjugated hyperbilirubinemia will occur with severe cholestasis even though there may be no primary disturbance in bilirubin transport.

O'Máille, Richards and Short suggested that the increase that occurs in the Tm of organic anion transport in response to augmented flow is attributable to the accelerated rate at which organic anions are swept away from the canalicular membrane, thus enhancing their rate of transfer per unit time. However, it then became apparent that not all agents that increase canalicular bile flow also augment the Tm of sulfobromophthalein. Thus, theophylline, which increases canalicular bile flow, does not increase the Tm of sulfobromophthalein.⁷⁷ At least three possibilities have been proposed to explain the difference between the effect of bile acids and that of theophylline and similar

compounds. Theophylline may be excreted in bile and may inhibit sulfobromophthalein transport, thus lowering concentration of the dye and masking the enhancing effect seen with augmented canalicular flow. Methods used to estimate canalicular bile flow do not distinguish between increased flow per canaliculus and recruitment of additional canaliculi. Thus, theophylline may increase flow per canaliculus but not the number of hepatocytes contributing to bile formation. The bile acids may recruit more hepatocytes and thus raise the number of parenchymal cells contributing to organic anion secretion. Further evidence for a recruitment phenomenon may be derived from the enhanced Tm for sulfobromophthalein attributable to phenobarbital, which increases both liver weight and bile flow. It has also been proposed that the micellar structure of bile acids incorporates sulfobromophthalein molecules, thus lowering the chemical activity of the dye and favoring enhanced membrane transport. However, it has been shown that although phenolphthalein glucuronide, indocyanine green and dibromosulfonphthalein, the dibrom analogue of sulfobromophthalein,⁷⁸ all sediment with the bile acids on ultracentrifugation of bile, only the Tm of dibromosulfonphthalein and indocyanine green is enhanced by administration of taurocholate or taurode-

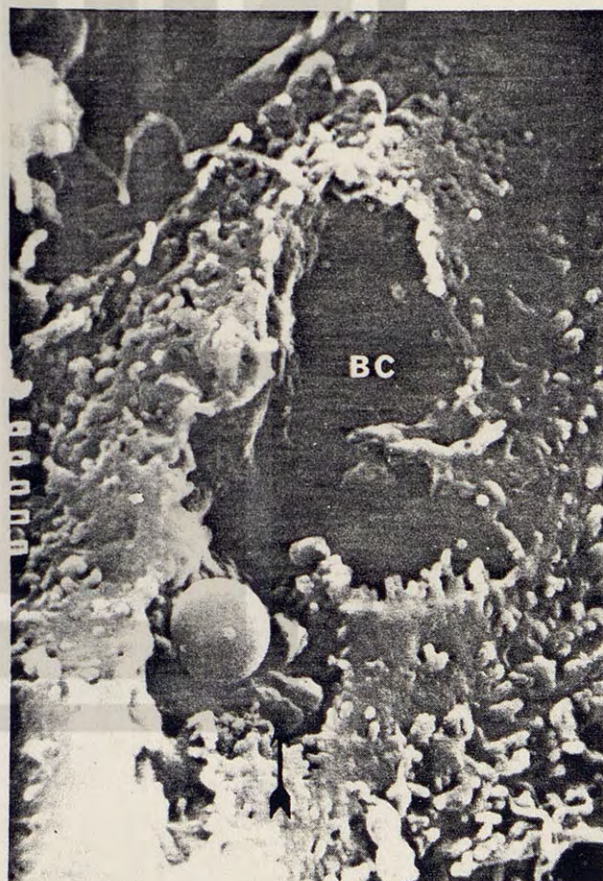


Figure 8. Dilatation of Bile Canaliculus (BC) and Loss of Microvilli with Evaginations of Canalicular Membrane (Arrow) (Original Magnification $\times 5000$) (See Figure 7).

hydrocholate.³⁵ Thus, at least in these instances, interaction with micelles could not be established as the mechanism for enhanced organic anion excretion.

CONCLUSIONS

This review of hepatic bile formation emphasizes the central role of the bile acids in both the generation of canalicular water flow and the excretion of bilirubin and other organic solutes. As a working model one can visualize hepatocytes along a sinusoid successively extracting bile acid until there is virtually none left in plasma. The load of bile acids would determine the number of participating hepatocytes, and the volume of bile generated would determine the excretion rate of other organic solutes that are transported into the canaliculus by independent mechanisms. Failure of bile acid transport shuts that system down. The bile acid transport system can be sodium linked and therefore regulated by agents affecting either sodium or the obligated bile acid anions.

Testing this working model may clarify the issues for cholestasis. Either cholestatic syndromes of the nonmechanical type begin as abnormalities in bile acid transport or the involvement of the bile acid transport system is secondary to undefined mechanisms for the generation of canalicular bile flow.

I am indebted to Drs. Aron M. Rappaport and Richard M. Hays for reading sections of this manuscript.

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RESEARCH PROBLEMS

199-01 Cardiovascular

A. Mechanisms of Cardiovascular Effects

- 1 - Endocrine and Fluid/Electrolyte Factors
- 2 - Cardiac Output, Stroke Volume, Contractility, Circulatory Distribution & Reflex Control
- 3 - Heart Size
- 4 - Venous Compliance
- 5 - Arrhythmias
- 6 - Coronary Effects
- 7 - Myocardial Tissue Effects
- 8 - Vascular Tissue Effects
- 9 - Computer Modeling

B. Amplify Interpretation of Skylab Data

- 1 - Ground Based Correlative Data
- 2 - Correlate LBNP, Tilt Table, and Centrifuge Responses
- 3 - Factors Influencing LBNP Response
- 4 - VCG Changes
- 5 - Improve Ground Based Simulation Techniques

C. Shuttle Reentry G Tolerance

D. Shuttle Scientist Passenger Selection (C-V Factors)

- 1 - Shuttle G Profile/Orthostatic Tolerances of Passenger Types
- 2 - Indices of Susceptibility to Orthostatic Changes

E. Prognostic Indices of C-V Changes In-Flight

(Cont'd)

F. Countermeasures

G. Bioinstrumentation & Methodology Advancements (Plethysmography, Cardiac Output Methodology, Blood Flow, Venous Pressure, Heart Size, Animal Implants, Data Management)

H. Preparation of Flight Experiments

RESEARCH PROBLEMS

199-05 Space Motion Sickness

- A. Etiology and Mechanisms of Space Motion Sickness
 - 1 - Evaluation of Fluid Shift Theory of Etiology
 - 2 - Anatomical and Microelectrical Studies
 - 3 - Functional Studies
 - 4 - Mathematical Modeling
- B. Determination of Individual Predisposition (Selection)
- C. Development of Training Techniques to Diminish Susceptibility
- D. Improved Countermeasures
 - 1 - Drugs
 - 2 - Identification (and Prevention) of Modifying Factors
 - 3 - Other
- E. Bioinstrumentation and Methodology Development
- F. Preparation of Flight Experiments

RESEARCH PROBLEMS

199-07 Bone & Muscle Alterations

A. Mechanisms of Musculoskeletal Changes

1 - Bone

2 - Muscle

B. Amplify Interpretation of Skylab Data (Ground Based Correlative Data)

C. Develop Predictive Indices of Susceptibility to Musculoskeletal Deterioration (Selection)

1 - Bone

2 - Muscle

D. Develop In-Flight Capability for Predicting Skeletal Change Rates

E. Musculoskeletal Countermeasures

1 - Bone

2 - Muscle

F. Bioinstrumentation and Methodology Advancements

1 - Bone

2 - Muscle

G. Preparation of Flight Experiments

RESEARCH PROBLEMS

199-09 Blood Alterations

- A. Mechanisms & Etiology of Red Cell Mass Loss & Blood Volume Decrease
 - 1 - Histological & Cytochemical Studies (E.M., Scanning E.M., Light Microscopy) (Membrane & Intracellular Chemistry)
 - 2 - Physiological & Isotope Studies
 - 3 - Computer Modeling
- B. Amplification of Space Flight Findings (Event and Time Course Data and Significance)
 - 1 - Red Cell Mass & Blood Volume
 - 2 - Immunological Changes
 - 3 - Other
- C. Prediction of Special Predisposition to Blood Changes
 - 1 - Red Cell Mass Loss
 - 2 - Plasma Volume Loss
 - 3 - Other
- D. Countermeasures Against Blood and Blood Volume Changes
- E. Bioinstrumentation and Methodology Development
- F. Preparation of Flight Experiments

RESEARCH PROBLEMS

199-11 Preflight Detection of Disease

(No further breakdown required)

RESEARCH PROBLEMS

199-13 Fluid & Electrolyte Changes

- A. Mechanisms of Space Flight Fluid & Electrolyte Changes
 - 1 - Physiological Studies
 - 2 - Computer Modeling
- B. Amplification of Space Flight Findings (Event & Time Course Data & Significance)
 - 1 - Fluid & Electrolyte and Associated Endocrine Responses
 - 2 - Other Endocrine Responses
 - 3 - Other Metabolic Responses
- C. Prediction of Special Predispositions (Selection)
 - 1 - Fluid & Electrolyte Changes
 - 2 - Other Endocrine
- D. Prognostic Indices of Fluid & Electrolytic Changes in Flight
- E. Countermeasures
- F. Bioinstrumentation & Methodology Development
- G. Preparation of Flight Experiments

RESEARCH PROBLEMS

199-15 Maintenance of Crew Health

(Metabolic Effects)

- A. Mechanisms of Exercise Response Changes
- B. Amplify Interpretation of Skylab Data
- C. Shuttle Scientist Passenger Selection Criteria
(Exercise Response)
- D. Development of Training Techniques (Exercise)
- E. Prognostic Indicators of Status in Flight
- F. Countermeasures
- G. Instrumentation & Technique Development
- H. Preparation of Flight Experiments

RESEARCH PROBLEMS

199-17 Human Behavior & Performance

- A. Develop Behavioral Selection Criteria
 - 1 - Individual
 - 2 - Group
- B. Develop Training Techniques
 - 1 - Individual
 - 2 - Group
- C. Studies of Small Group Dynamics in Social Isolation
- D. Influence of Specific Factors on (Determinates of) Behavior and Performance
 - 1 - Circadian & Work-Rest-Sleep Patterns
 - 2 - Other
- E. Develop Means for Early Detection of Impending Behavioral & Performance Decrement In-Flight (Monitoring Criteria & Techniques)
- F. Countermeasures
 - 1 - Preventive
 - 2 - Corrective
- G. Instrumentation & Methodology Development
- H. Preparation of Flight Experiments

RESEARCH AREAS

199-19 Definition of Physiological Design Requirements (Human Standards and Requirements for Life Support Design)

- A. Gaseous Atmospheres - Pressure & Composition
- B. Food & Nutrition
- C. Thermal Standards & Requirements
- D. Toxicology
- E. Microbial Standards
- F. Other Hazards (Protective Requirements)
- G. Instrumentation & Technique Development
- H. Preparation of Flight Experiments

RESEARCH AREAS

199-27 Clinical Medical Support Systems

- A. Prophylaxis
- B. Diagnosis
- C. Therapy
- D. Medical Data Management
- E. Medical Monitoring
- F. Training Methods & Material
- G. Bioinstrumentation & Equipment Development
- F. Preparation of Flight Experiments

RESEARCH PROBLEMS

199-45 Radiation Effects and Protection

- A. Determination of Acceptable Levels of Radiation Exposure (Responses of Living Systems & Mechanisms)
 - 1 - HZE
 - 2 - Other
- B. Dosimetry of Space Environments for Living Systems
 - 1 - Measurement
 - 2 - Technique & Instrumentation Development
- C. Identification of Human Susceptibility Factors (Selection)
- D. In-Flight (Real Time) Prognostication Techniques
- E. Countermeasures
 - 1 - Preventive
 - 2 - Protective
- F. Preparation of Flight Experiments

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APPLICATION FOR
RESEARCH REVIEW AND/OR SUPPORT

LEAVE BLANK
(For FHPS Headquarters use only)

PROJECT NUMBER

DATE RECEIVED

SECTION I

A. To be completed by Principal Investigator.

1. NAME OR TITLE OF RESEARCH PROPOSAL

Prevention of Disuse Osteoporosis of Bed Rest: Effect of Disodium Dichloromethane Diphosphonate (Cl_2MDP) and Disodium Etidronate (EHDP)

2. TYPE OF APPLICATION

☒ NEW PROJECT

☐ REVISION OF PROJECT NO. _____

☐ CONTINUATION OF
PROJECT NO. _____

☐ SUPPLEMENTAL REQUEST
FOR PROJECT NO. _____

3. NAME AND TITLE OF INVESTIGATOR(S) AND RESPECTIVE INSTITUTIONS

a. Principal Investigator (Last, first, initial)

Schneider, Victor S.

Title

Director, Metabolic Unit

Institution (Name)

U.S. Public Health Service Hospital
San Francisco, California

b. Name (last, first, initial)

McDonald, Janet

Title

Associate Director, Metabolic Unit

Institution (Name)

U.S. Public Health Service Hospital
San Francisco, California

c. Name (last, first, initial)

Title

Institution (Name)

4. TIME REQUIRED TO COMPLETE THIS PROJECT

FROM

February 1978

THROUGH

August 1978

5. TOTAL AMOUNT
ESTIMATED FOR
PERIOD IN ITEM 4

\$

6. AMOUNT REQUESTED OF
FHPS BY THIS APPLICATION
(See Section II)

CCIC

\$ \$15,000

7. PRINCIPAL INVESTIGATOR

Victor S. Schneider MD

Signature

12/6/77

Date

B. To be completed by appropriate station reviewing official(s).

1. CHAIRMAN, STATION RESEARCH COMMITTEE

Thomas R. P. Kemp

Signature

JAN 6 - 1978

Date

2. CHAIRMAN, PHARMACY AND THERAPEUTICS COMMITTEE (if indicated)

Victor S. Schneider MD

Signature

12/20/77

Date

3. DIRECTOR/MEDICAL OFFICER IN CHARGE

Kenneth F. W. Lewis

Signature

1/6/78

Date

SECTION IV - RESEARCH PLAN

NAME OR TITLE OF RESEARCH PROPOSAL Prevention of Disuse Osteoporosis of Bedrest: Effect of Disodium Dichloromethane Diphosphonate (Cl₂MDP) and Disodium Etidronate (EHDP)

☐ NEW PROJECT
OR
PROJECT NO. _____

INTRODUCTION

Objective:

The overall objective of this research is to attempt to modify factors which promote disuse osteoporosis and thereby prevent it from occurring. In addition, an opportunity exists to study bedrest-induced muscular deconditioning, study steady state nutritional requirements, and compare ambulatory and bedrest hormonal responses to a number of stimuli.

Background:

Disuse Osteoporosis: Bone mineral is lost when the mechanical factors present during normal ambulation are removed. Negative calcium balance and/or osteopenia have been observed during several clinical and experimental conditions in man including acute and convalescent phases of paralytic anterior poliomyelitis (1), immobilization due to casting in both fracture patients (2) and normal young men (3) and in space travel (4). The mechanism of bone loss is thought to be a normal adaptive function upon exposure to hypogravic states (5). A reproducible model of disuse osteopenia in studies of healthy young males during prolonged bedrest without immobilization has been developed (6,7). Using bedrest as a model to produce disuse osteoporosis, calcium loss from the whole skeleton has been measured by balance techniques and averages 0.5% total body calcium per month (6). Tenfold greater rates of loss from the central portion of the calcaneus have been observed by gamma ray transmission scanning (6).

Mineral loss during bedrest is probably due to a reduction in the forces which are applied to the skeleton during normal activity (3,6). These one "G" homeostatic forces are absent also in the hypogravic environment of space flight. Loss of bone mineral during space flight is expected on theoretical grounds and has been confirmed in the Skylab experiments (4). This factor will prove hazardous to astronauts on flights of long-term duration, not only because hypercalciuria might lead to the formation of renal calculi during flight, but axial skeletal fractures may occur upon re-entry to earth's gravity.

The need to reduce these hazards has led to a series of experiments. Various therapeutic attempts to prevent the negative calcium balance and loss of calcaneal density during prolonged bedrest have met with only limited success. Other studies designed to apply either compressive and/or tensile forces to the skeleton during bedrest have been performed. Unfortunately, these studies were not able to demonstrate a direct effect of either gravity or exercise in preventing disuse osteoporosis. Mechanical methods tried were: 1) Use of the Exer GenieTM apparatus* (8). The Exer Genie consisted of a rope which was pulled back and forth through a pulley, the friction of which was preset to provide 8 lbs of resistance. An exercise program was performed in the supine

*Purchased from American Fitness of Northern California, 20 Harold Ave., Suite A, San Jose, CA 97117.

SECTION IV - RESEARCH PLAN

NAME OR TITLE OF RESEARCH PROPOSAL Prevention of Disuse Osteoporosis of Bedrest: Effect of Disodium Dichloromethane Diphosphonate (Cl₂MDP) and Disodium Etidronate (EHDP)

☐ NEW PROJECT
OR
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position for 80 min. per day. This program failed to diminish any of the metabolic changes of bedrest and did not prevent mineral loss from the calcaneus. 2) Longitudinal compression was attempted using the gravitational acceleration simulating suit (GASS)*. This was tested in two ways: (a) static compression applying 80% of the body weight from the shoulders to the soles of the feet for 3 hours daily (7,11); and (b) intermittent longitudinal compression at a rate of 45 compressions/min. for 200 min./day (7). This treatment did not prevent either the negative calcium balance nor the usual loss of calcaneal mineral. 3) Impact loading of the axial skeleton to create piezoelectrical forces was tested (9). Thirty-five lbs of impact load to each heel and compression of 80% of body weight to the axial skeleton was performed 6 hours daily. Although calcaneal mineral density remained the same or increased during this therapy, overall calcium loss was equal to that in untreated bedrest. 4) The effect of hydrostatic pressure was studied by quiet standing; i.e., the subject stood without lifting either foot for 3 hrs. each day (7). Neither calcaneal mineral density nor calcium balance was protected by hydrostatic forces on the axial skeleton. 5) Three hours of quiet standing plus 20 min. of bicycle ergometry exercise daily once again did not provide any protection (10). 6) Three hours of normal ambulation plus 21 hours of bedrest started after 6 weeks of total bedrest demonstrated some protection of the usual negative calcium balance (10).

Four biochemical regimens have been studied: 1) synthetic salmon calcitonin (11), a hormone which inhibits bone resorption (100 MRC U), was given daily by injection during 8 weeks of bedrest; 2) phosphate supplements in order to reduce urinary calcium excretion was given in divided doses as a neutral potassium salt (12); 3) oral calcium and phosphate with the hope that the former would increase calcium absorption from the intestine while the latter would decrease calcium excretion in the urine (7,11); and 4) the diphosphonate EHDP either as a 5 mg/kg/day dose or as a 20 mg/kg/day dose (13). Little or no protection was afforded by the first three methods during long-term bedrest. Low dose diphosphonate also showed no beneficial effect.

EHDP was studied in two healthy young men during conditions of strict metabolic balance for 20 weeks of continuous bedrest. The effects of the 20 mg/kg/day dose was assessed by three largely independent techniques: 1) metabolic balance; 2) ¹²⁵I gamma ray transmission scanning of the calcaneus; and 3) ⁴⁷Ca kinetic studies. In weeks 2-12, the usual patterns of hypercalciuria, hyperphosphaturia and negative calcium and phosphorus balances were seen. During the last 8 weeks of bedrest, a shift towards positive calcium and phosphorus balance occurred in both subjects. Urinary hydroxyproline excretion decreased during bedrest in distinct contrast to the rise which is usually seen. The serum phosphorus level rose promptly, persisted at levels of about 3 mg/dl above baseline during EHDP therapy and fell to normal levels promptly upon discontinuation of the drug. Serum alkaline phosphatase values tended to decline throughout the treatment period. Serum total and ionized calcium concentrations remained unchanged during the 20 weeks of bedrest. Both

*This apparatus was designed and maintained by James Gatts, M.D.

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subjects lost significant amounts of mineral from the calcaneus during the first 17 weeks of bedrest. During the last 3 weeks of bedrest, the usual progression of calcaneal mineral loss was no longer observed. Calcium kinetic studies revealed that bone accretion and resorption rates fell progressively and in parallel fashion to levels 50% below baseline by the end of bedrest.

Thus, re-examination of the diphosphonate as a therapeutic modality in the prevention of disuse osteoporosis appears warranted.

Diphosphonates: A central role for inorganic pyrophosphate in the stabilization of hydroxyapatite crystals has recently been postulated by Fleisch and others (14-16). Pyrophosphate has been isolated from bone and teeth (17), urine (18,19) and serum (16) and has been shown to inhibit both precipitation and dissolution of hydroxyapatite crystals in vitro in concentrations similar to those in normal urine (18,19). Because of its hydrolytic instability enhanced by high temperature, low pH and certain enzymes including alkaline phosphatase, pyrophosphate seems to have limited application for experimental or therapeutic use in clinical situations (20,21).

Diphosphonates are a class of synthetic organic compounds which are structurally related to inorganic pyrophosphate. Unlike pyrophosphate, which is characterized by a P-O-P bond, the diphosphonates which have exhibited interesting physiological properties contain a P-C-P bond. Such compounds are known as geminal diphosphonates, i.e., they contain both phosphorus groups on one carbon atom. It is the presence of this P-C-P configuration that is thought to be responsible for the resistance of such compounds to chemical and enzymatic hydrolysis; this permits them, in vivo, to reach the sites of their physiological action intact, despite the presence of phosphatases in animal tissues (20-23).

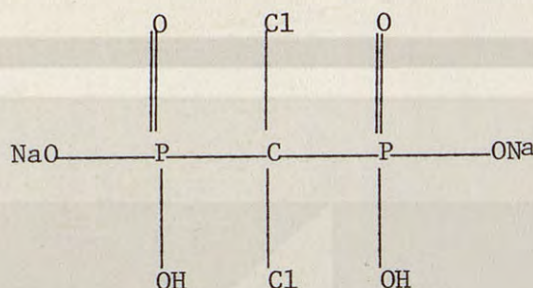
The first diphosphonate which has been investigated extensively in animals and man is disodium etidronate (disodium ethane-1-hydroxy-1, 1-diphosphonate, abbreviated EHDP). It has been shown to have profound effects on the rates of bone resorption and mineral accretion as well as on soft tissue calcification. Clinical trials have shown EHDP to be an effective therapeutic agent in the treatment of Paget's disease of bone and in diseases characterized by heterotrophic ossification. This drug is currently in the final phases of FDA approval for clinical use (13,24-27).

The second compound in this group which may be of potential therapeutic interest is disodium clodronate (disodium dichloromethane diphosphonate, abbreviated Cl₂MDP) (28). The compound has the following structural formula:

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The compound is a white powder with a molecular weight of 289; it is very soluble in water and insoluble in most other solvents. The molecule itself is very stable; three-year storage tests have shown that the only significant change which occurs is a slight increase in the moisture content of the powder.

Rationale:

Diphosphonates such as EHDP and Cl₂MDP have been shown to inhibit both soft-tissue calcification (22) and bone resorption in a variety of in vitro (23) and in vivo (25) systems.

Although EHDP and Cl₂MDP have similar chemical structures, these compounds appear to affect processes in model systems to different degrees. At low levels, Cl₂MDP appears to be more effective in inhibiting pathologic resorption. EHDP has been shown in animal models to inhibit normal skeletal mineralization at lower levels than does Cl₂MDP, and has been associated with an accumulation of osteoid tissue both in animals and man when given for extended periods (29,30).

In animals, Cl₂MDP has been shown to be more effective than EHDP (24, 29,31-36). In one study, the Cl₂MDP produced denser but not osteopetrotic bone. It is postulated that Cl₂MDP acts primarily by reducing the rate of bone resorption while stopping bone formation. Thus, prevention of loss of mature bone which possesses a higher calcium and phosphate content than does newly mineralized bone is seen (32). EHDP, on the other hand, appears to reduce the rate of bone resorption and to stimulate bone formation.

This effect of EHDP was confirmed in this laboratory when EHDP (20 mg/kg/day) was started with the onset of prolonged bedrest in humans. However, negative calcium balance developed during the first 12 weeks of bedrest in spite of an immediate decrease in alkaline phosphatase activity and urinary hydroxyproline (13). This suggests that preloading with diphosphonate prior to bedrest may condition the bone so that the effects of bedrest may be modified earlier.

In addition, a bone scanning computerized axial tomography unit will be used to try to develop a new technique for measuring bone loss during prolonged bedrest or space flight.

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An opportunity also exists to determine hormonal responses (1) to glucose intake during ambulation and recumbency and (2) to measure the diurnal variation of parathormone (PTH), calcitonin (CT), 1,25-dihydroxycholecalciferol (1,25-DHCC), adrenocorticotropin (ACTH) and cortisol.

Carbohydrate ingestion can change calcium homeostasis during normal ambulation and recumbency, and carbohydrate tolerance changes during recumbency (37).

Recently, ACTH and cortisol secretion has been shown to change during bedrest (55) and, therefore, may be a factor in the development of disuse osteoporosis.

SPECIFIC AIMS

1. To determine whether oral medication with the diphosphonates EHDP or Cl₂MDP will modify or prevent 17 weeks of bedrest-induced disuse osteoporosis as assessed by standard metabolic balance, gamma ray transmission scanning of the calcaneus and ⁴⁷Ca kinetic techniques.
2. To determine the diurnal variation of PTH, CT, and 1,25-DHCC in controls and treated subjects (EHDP or Cl₂MDP) during normal ambulation and prolonged bedrest.*
3. To determine the longitudinal effects of 17 weeks of bedrest on PTH, CT and 1,25-DHCC on controls and treated subjects.*
4. To determine the diurnal variation of ACTH and cortisol during prolonged bedrest.
5. To evaluate pancreatic endocrine hormones before and during bedrest by using a standard oral glucose tolerance test.
6. To determine the efficacy of measuring bone mineral content during prolonged bedrest with a computerized axial tomography bone scanner.

METHODS OF PROCEDURE

Subjects will be studied during 1 week of equilibration, 9 weeks of control ambulation, 17 weeks of bedrest and 3 weeks of reambulation. Progress will be monitored by: (a) calcium, phosphorus, and nitrogen balance studies and urinary hydroxyproline and creatinine; (b) gamma ray transmission scanning of the calcaneus and long bones; (c) ⁴⁷Ca kinetic studies; (d) hormonal responses to oral glucose ingestion.

*From the laboratory of Claude Arnaud, M.D., Veterans Administration Hospital, 4150 Clement Street, San Francisco, CA 94121

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Research Subjects:

Eighteen subjects will be studied during 17 weeks of continuous bedrest; 8 will receive Cl₂MDP (at 2 dosage levels); 4 will receive EHDP and 6 will serve as untreated controls. The allocations of drugs and placebo will be random. The study will be blind as follows: the volunteers receiving medication and the individuals making densitometric and chemical determinations in the laboratory will not know who is receiving drug and who is receiving placebo. The principal investigator and research associates will have access to drug code sheets and data with regard to urinary and fecal excretions for the purpose of on-site monitoring during the study.

The subjects will be Caucasian males between the ages of 19-48, who respond to a request for research subjects from the general population. The volunteers will be screened by members of the metabolic staff. A psychological test will be administered as an adjunct in screening, as needed, and routine laboratory screening will be done prior to acceptance, as deemed necessary.

Each subject participating in the study must provide the investigator with signed consent, which gives the investigator permission to study the volunteer. The consent form to be signed will indicate that the volunteer has been informed about the objectives of the study, procedures to be used and of all known possible problems the volunteer may experience as a study participant.

Subjects will be housed on the Metabolic Ward of the U.S. Public Health Service Hospital in San Francisco, in semi-private rooms. During the equilibration week, all respective research subjects will undergo a prestudy examination as outlined below. All tests will be carried out according to standard techniques in the clinical departments of the Public Health Service Hospital. These examinations will provide baseline observations and, in addition, will serve to detect and eliminate volunteers who do not fulfill patient selection criteria.

Characteristics which will exclude a volunteer from the study include:

- a. Acute disease other than acute upper respiratory infections and minor skin diseases, e.g., dermatophytosis.
- b. Chronic diseases other than minor skin diseases, e.g., acne.
- c. Medications which by their action interfere with the interpretation of results, e.g. fluoride, estrogen.
- d. Recent sub-standard nutritional status which may have altered the metabolic milieu.

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Medical History and Physical Examination:

A medical history will be taken and physical examination performed and recorded. In addition to the routine examination, additional tests will include:

- | | |
|--|--------------------------------------|
| 1. Neurologic evaluation | 14. Serum total bilirubin |
| 2. Dental, ear, nose & throat examination | 15. Serum SGOT |
| 3. Eye examination including slit lamp examination and tonometry | 16. Serum creatinine |
| 4. Standard 12 lead electrocardiogram | 17. Serum blood urea nitrogen |
| 5. Chest x-ray | 18. Serum alkaline phosphatase |
| 6. Abdominal (flat plate) x-ray | 19. Serum sodium |
| 7. Urinalysis | 20. Serum potassium |
| 8. Stool for occult blood | 21. Serum chloride |
| 9. Complete blood count | 22. Serum carbon dioxide |
| 10. Fasting blood glucose | 23. Serum calcium |
| 11. Serologic test for syphilis | 24. Serum phosphorus |
| 12. Serum total protein/albumin & globulin | 25. Serum Australian antigen |
| 13. Serum uric acid | 26. A screen for addicting drugs |
| | 27. Serum cholesterol & triglyceride |

Study Design:

The 30 week study will be divided into three parts and will involve a total of 18 normal test subjects. Each part of the study will be divided into five phases:

1. Phase I (week 1) will consist of orientation. The subjects will start the metabolic diet. The subjects also will receive polyethylene glycol 4000, undergo metabolic equilibration, and receive a complete physical examination.
2. Phase II (week 2-4) will be the ambulatory control period during which the subjects will continue on the metabolic diet. Baseline measurements of calcium, phosphorus and nitrogen balance, blood PTH, CT, 1,25-DHCC, urine hydroxyproline and densitometric scans of the calcaneus and long bones will be obtained.
3. Phase III (weeks 5-10) will be the ambulatory load period during which the subjects will receive EHDP, high dose Cl₂MDP, low dose Cl₂MDP, or placebo, the details of which will be described below. Baseline metabolic balance and hormonal and densitometric measurements will be continued during this period. ⁴⁷Ca kinetics with diurnal hormonal studies and glucose tolerance will be determined.

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4. Phase IV (weeks 11-27) will be the bedrest period, during which the subjects will be at strict bedrest and will receive EHDP, high dose Cl₂MDP, low dose Cl₂MDP, or placebo. Metabolic balance will continue and hormonal and densitometric measurements will be obtained. ⁴⁷Ca kinetics with diurnal hormonal studies and glucose tolerance will be determined. The diphosphonates will be discontinued at the end of bedrest.
5. Phase V. (weeks 28-30) will be the reambulation period. Balance and densitometry will continue during the reambulation program.

<u>Study A</u>	<u>No. of Subj.</u>	<u>Load Period (wks. 5-10)</u>	<u>Bedrest (wks. 11-27)</u>
A	2	1600 mg/d Cl ₂ MDP	1600 mg/d Cl ₂ MDP
B	2	20 mg/kg/d EHDP	20 mg/kg/d EHDP
C	2	Placebo	Placebo
<u>Study B</u>			
A	2	800 mg Cl ₂ MDP	800 mg Cl ₂ MDP
B	2	1600 mg/d Cl ₂ MDP	1600 mg/d Cl ₂ MDP
C	2	Placebo	Placebo
<u>Study C</u>			
A	2	Cl ₂ MDP	Placebo
B	2	EHDP	Placebo
B	2	Placebo	Placebo

This study will start with testing ambulatory load therapy with either high dose Cl₂MDP or EHDP and the therapy will continue throughout the bedrest phase. If the Cl₂MDP is effective, a smaller dose will be compared with the larger dose. Assuming both the Cl₂MDP and EHDP are effective, Study C will look at treatment during the ambulatory load period only. An alternate approach will be to repeat the most successful therapy during Study C.

The studies will be done on an ongoing basis over the course of several years. Two or more subjects will be allowed to begin a study as soon as they are identified and additional subjects will be added as they are identified.

Medications Studied:

EHDP and Cl₂MDP Administration. The investigational new drugs (IND) will be supplied to the investigator as 200 mg tablets or capsules which will be packaged in bottles of 500. The placebo will be supplied as tablets or capsules which are in appearance, method of packaging and firmly attached labeling, indistinguishable from the active drug preparations. The medication will be labeled with a "tear off" label. The "tear off" portion will bear that

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information necessary for the interstate shipment of an investigational new drug and will be removed prior to dispensing the drug by the principal investigator (who will be aware of the nature of the medication). The firmly attached portion of the label will bear a space for the investigator to fill in the volunteer's name and an instruction section which will read as follows:

"Take _____ tablets with a glass of fruit juice at least one hour before lunch."

The IND sponsor will provide the necessary forms which will enable the investigator to keep accurate written records of all the investigational new drugs received from the IND sponsor, dispensed to the volunteer, and unused drugs returned by the investigator to the IND sponsor.

The amount of medication for the EHDP will be based upon the initial body weight and modified monthly according to the following table:

Body Weight (Kg)	# Tabs/day (200 mg)	Mg EHDP /day p.o.
35-45	4	800
45-55	5	1000
55-65	6	1200
65-75	7	1400
75-85	8	1600
85-95	9	1800

This schedule will provide an EHDP dose level of approximately 20 mg/kg/day. The Cl₂MDP will be given at a fixed dose of either 800 mg/day (low dose) or 1600 mg/day (high dose). Allocation of the subjects to drug and placebo groups will be random.

Other Medications:

All subjects will receive a Hexavitamin tablet once daily which, with the diet, will provide approximately 14,000 I.U. of vitamin A, 630 I.U. of vitamin D, 3 mg thiamin, 5 mg riboflavin, 42 mg niacin, and 192 mg ascorbic acid. Dioctyl sodium sulfosuccinate (Colace[®]) will be given orally in a dose of 100 mg twice daily to prevent constipation. Polyethylene glycol 4000 will be used as a fecal marker and will be given in an oral dose of 500 mg three times daily.

The use of other medications will be kept to the minimum consistent with good medical practice and the best interest of the volunteers. Any such use of medication will be completely documented as to type of medication, the amount used, the time administered and the justification for its use.

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Test Procedures:

Bone Densitometry. Mass of the os calcis will be measured biweekly throughout the study by gamma ray transmission scanning. The gamma ray transmission scans will be performed by Dr. John Vogel or the investigators, according to the technique reported in NASA Contract T-58941-G and summarized as follows (38):

Instrumentation. The scanning system consists of a custom-designed rectilinear scanner interfaced with control and data acquisition electronics. The basic elements of the scanner consist of (A) scanning head with source and detector, (B) scanner drive mechanism, and (C) control electronics.

The source and detector are spaced 6 in. apart and move synchronously. The object to be scanned is placed on a fixed platform between these units, thereby allowing the radiation beam to pass through to the detector.

Whereas both ^{241}Am (59.4 keV) and ^{125}I (27.5 keV) sources have been used, ^{125}I was chosen as the preferred source since the available ^{241}Am source had a limited photon yield. Iodine-125 from 100 to 400 mCi activity can easily be stored and shielded and has an excellent photon yield. Initially, sources were made by absorbing carrier-free ^{125}I onto Dowex 1 X 8 resin beads. Recent sources were obtained from Atomic Energy of Canada, Ltd. They are prepared by electroplating ^{125}I onto charcoal beads, sealing in a metal capsule, and placing in a brass holder. This source is held in an aluminum housing which has a 3/32 in. straightbore collimator 1/2 in. in length mounted in direct apposition to the detector collimator and housing. A 2-mil tin filter is used to filter out the small number of 31- and 35-keV photons with a resulting beam energy of 27.5 keV.

To minimize background and to accommodate a low-energy gamma output, a Harshaw detector with a 1.5-cm diameter X 3-mm thick (NaI (Tl) crystal covered with a thin beryllium window is employed. The detector housing is fitted with a 3/32-in. collimator consisting of two lead disks 1/4 in. thick and spaced 1 in. apart.

Data obtained from the detector are fed through a single-channel analyzer adjusted to accept the photopeak pulses from the source within a 16-keV window and are subsequently stored in a Nuclear Data Model 3300 multichannel-analyzer system operating in a multiparameter mode. Count data are stored in the 4,096-channel analyzer memory in a 256 X 16 matrix. Storage address of the data is determined by the voltage obtained from a position-indicating potentiometer on the x-axis screw.

Accumulated data are transferred to digital magnetic tape for storage. Readout on punched paper tape and a high-speed parallel printer is used for data calculation. Two display formats are available. The contour display is a planar representation of the 16 X 256 data matrix. Intensity modulation governed by count data provides a display analogous to a radiograph. This visual display is used to verify position reproducibility of a scan, each row line representing a 1/8-in. interval. The isometric display mode provides a

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spatial representation of the scan, giving a three-dimensional effect to the viewer. Analog counting-rate information is apparent in the display.

Data calculation utilizes the formula illustrated in Fig 5. Each row is calculated separately. The average counting rate at each side of the bone (through tissue and water only) represents 100% transmission value (I^*). The counting rates represented by I and I_0^* are accumulated during each 1/64 movement of the scanner since data addressing relates to the position of the potentiometer on the x-axis screw. Changes in speed alter sampling time but not the distance scanned. During a scan the speed is maintained constant by the use of precision stepping motors, thereby assuring a constant sampling time. The speed chosen is that which gives an I_0^* counting rate greater than 500. The transmission value through bone for each data point, I , is ratioed to I_0^* . The natural logarithm of these ratios is determined and summed for the row. The sum is then expressed in computer units as a positive value and is related to the absorption due to the bone interposed in that slice, increased computer unit values representing increased bone mineral.

Radiation dose to the heel is less than 3 mR/scan at these speeds and counting rate.

Data are normally calculated using a time-share computer terminal, with data entered automatically from the punched tape. Manual calculations also may be done using a programmed Monroe 325 computing calculator.

Systems check. Prior to patient measurements, a daily systems check is performed. A spectrum analysis of the source is taken to identify the location of the photopeak. Coincidence circuitry can be used to identify the channels in which the photopeak resides, and the gain and window settings of the single-channel analyzer can be adjusted until a 16-keV window is centered at 27.5 keV.

Scanning of a plastic-embedded bone provides a day-to-day standard for overall systems operation. Analysis of these check values yielded a 1.8% variation (95% confidence) over a 2-month period.

Weekly calibration checks of the system are performed by scanning a step wedge containing varying concentrations of calcium hydroxyapatite uniformly suspended in a dental material. The wedge has three different concentration zones, each containing six steps giving a total of 18 data points. Plotting the known hydroxyapatite in mg/cm^2 against the computer-derived absorption values gives a linear relationship of $y = 0.00240 + 0.05x$ where y is the computer unit absorption value per data location and x is the hydroxyapatite content expressed as mg/cm^2 .

Patient positioning. A fitted plexiglass foot rest is placed in a plexiglass box on a platform between the source and detector. The patient's foot is then positioned in the foot holder and water is added. The addition of water compensates for variations in tissue thickness and thus provides a uniform tissue-equivalent thickness. Prior to the initial scan, x-rays are taken of the subject's foot in the mold. The x-ray, with scan lines superimposed, is

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used for future reference. Scanning is begun 2 in. from the bottom of the os calcis.

Any study involving multiple scans over a prolonged period of time demands accuracy of repositioning which is dependent upon the adequacy of the mold. Reproducibility scans have been determined for test subjects. Repetitive single scans were performed on an arbitrary line. After each phase, the subject removed his foot from the mold, walked for 2 min., and then replaced his foot in the mold. Five passes were made in that manner. The 2 SD from the mean was 1.3%. Reproducibility of scans was found to be within 3.5% of 95% confidence level when an ambulatory subject was repetitively scanned over a 6-month period.

In addition, the lower tibia will be scanned every 2 to 3 weeks using the Densitom II bone densitometry system, a computerized tomography analysis of the quantification of different parameters of bone mineralization by means of visualization of cross sections of long bones.

Calcium Kinetics:

Calcium kinetics will be measured by following the disappearance of ⁴⁷Ca from the blood after intravenous injection of 10 μ c of ⁴⁷CaCl₂ according to the method of Neer et al (30). Blood samples will be drawn following injection at appropriate intervals as described in the method. Urine will be collected and measured as individual voidings for the first 44 hours and as 24 hour pools for the next 19 days. Fecal specimens will be collected throughout the 21 day period, homogenized and pooled at variable intervals. All specimens will be measured for radioactivity and total calcium. Data will be analyzed according to a four-compartment model for the miscible calcium pool to determine bone accretion rates. Kinetics data will be obtained during baseline (week 8), in the 5th week of bedrest (week 15), the period when maximal urinary calcium loss and maximal bone turnover is anticipated, and in the 17th week of bedrest (week 26).

Oral Glucose Tolerance Test:

The standard oral glucose tolerance test will be performed twice during the study: the first test will be done during Phase III (week 6); the second during Phase IV (week 16), i.e., the 6th week of bedrest. The test will be performed as follows: A 19 gauge butterfly needle will be placed in an antecubital fossa vein and kept open with a flow drip of normal saline after an overnight fast. Blood specimens will be taken 15 min. before, just before, and at one-half hour intervals for 3 hours after 75 g of a glucose solution is consumed over a 3-5 min. period. The glucose preparation will be that which is available from the Clinical Laboratory for the standard oral glucose tolerance test. Samples will be taken for glucose, potassium, insulin, glucagon, growth hormone and calcium. Urinary calcium will be measured for the 2-hour period prior to the glucose tolerance test and the 3 hours immediately after ingesting the glucose. Twenty cc of blood will be taken at each time period.

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Diurnal Hormonal Studies:

The diurnal hormonal studies will evaluate variations in the hormones PTH, CT, 1,25-DHCC, ACTH and cortisol. Blood samples will be drawn every 4 hours on the calcium kinetic study days. Thus, for a 24-hour period, the research subject will have a 19 gauge butterfly needle in an antecubital fossa vein kept open with a flow drip of normal saline. Twenty-five cc of blood will be withdrawn for these studies at each time interval.

Safety:

1. Bedrest. In previous studies, in which longer bedrest periods were used than those contemplated for the present study, no adverse effects were recorded. As measured by gamma ray transmission scanning, mineral content in the os calcis had returned to baseline after an ambulatory period of time which was less than the period spent in bed. None of our subjects has developed hypercalcemia, renal stones, renal damage, decubitus ulcers, thrombophlebitis, syncope, fractures, or psychiatric or orthopedic problems.
2. Radiation hazard:
 - (a) ⁴⁷Ca - the dose of ⁴⁷Ca contemplated for this study, 10 microcuries administered three times, will deliver approximately 0.24 REM to the whole body and 2.40 REM to the bones within a period of 6 months.
 - (b) ¹²⁵I - radiation dose to the bone is less than 3 millirem per scan at the speeds and counting rate used. Thirty such determinations will be performed.
 - (c) X-Ray Radiation - chest and KUB x-ray will be done on the subjects during the control ambulation period and as a follow-up at the end of the study. Each x-ray will give a dose of 3 REM. Anticipated total x-ray dose therefore will be 12 REM.

The maximum radiation dose received during the 30 week experiment will be 14.49 REM. This quantity is within the acceptable limits outlined for people who work with radiation.

3. Polyethylene glycol 4000. This substance is a wax-like material which is colorless and tasteless and has been

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found to have low toxicity. It has been studied extensively in animal and human subjects and has been available for many years as a solublizer for use in lotions, creams and suppositories (56,57,58). In addition, it is used by humans internally as a food additive, pill excipient and as a solublizer for toothpaste. In recent years it has become the standard fecal marker for metabolic balance studies. An IND #7781 was obtained in 1971 informationally for the FDA, since an approved drug was being used for a non-approved reason. We have administered this drug to 25 subjects who have participated in our studies over the last six years without any observed side-effects. In addition, this substance is being used without an IND in most other centers.

4. EHDP. Disodium etidronate inhibits resorption but may allow formation of hydroxyapatite crystals. At high doses it prevents normal calcification of bones in growing rats and dogs (30,40). Studies of rats receiving various doses of EHDP have shown a dose response relationship; increased osteoid seam width has been observed at higher doses of this drug (41). Osteoporotic patients receiving up to 20 mg/kg/day of the drug for a period of three months had increased osteoid width on iliac crest biopsies (42). All follow-up studies indicate the complete reversibility of this effect. At doses above 5 mg/kg/day there is usually a rise in serum phosphorus; the maximum level observed to date has been 7 mg% (13, 42,43). This hyperphosphatemia stabilizes despite continuation of the drug, is completely reversible following discontinuation and has not been associated with any untoward clinical effects. EHDP has been found to have beneficial effects on patients with a variety of bone diseases including myositis ossificans progressiva and Paget's disease (26,44). Over 175 persons have received this investigational new drug. Isolated, unexpected abnormalities have developed in persons in these studies both on placebo and on disodium etidronate therapy: 1) In one patient the clotting time was at times prolonged and leukopenia followed. These blood changes were not correlated with the dosage of medication. 2) Another patient developed an abnormal gait thought to be due to emotional problems. 3) An adult patient was found to have neuritis, an elevated sedimentation rate and a positive antinuclear antibody titer with negative LE cell preparations. Her symptoms probably antedated medication with EHDP and assessment of the role of this medication in her total

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clinical picture is difficult. In summary, EHDP at the doses proposed for this study has not been shown to have definite, serious, or permanent toxic effects.

5. Cl₂MDP. Disodium dichloromethane diphosphonate inhibits both resorption and formation of hydroxyapatite crystals. Cl₂MDP has been shown to prevent disuse osteoporosis of immobilization and to prevent the effect of corticosteroid-induced osteoporosis in animals (45). Human toxicity studies have revealed that 1-3% of Cl₂MDP is absorbed from the GI tract. Fifty per cent is absorbed by bone and 50% is excreted in the urine. Maximum dosage given to humans has been 3200 mg/day for 30 days. During that period of time, no untoward side-effects were reported (46).

In animals, a common response to the administration of Cl₂MDP, orally or parenterally, is gastrointestinal distress characterized by irritation of the gastrointestinal tract, emesis and diarrhea. It was anticipated that the projected dose of 3200 mg/day would produce mild nausea, vomiting or diarrhea in sensitive subjects. This did not occur. Because the condition, once produced, is rapidly reversible upon withdrawal of the Cl₂MDP, the risk to subjects is slight and the symptoms may provide a good marker for the upper limit of tolerance for the drug.

In one study in rats, a slight drug-related anemia demonstrated by low hemoglobin and hematocrit was seen (47). A reproducible drug-related phenomenon observed in rats is the elevation of serum levels of enzymes SGPT and SGOT. This was without apparent toxic significance upon histological examination of the liver and heart tissue and was reversible upon withdrawal of the drug. The rat is the only species so far examined which has demonstrated this response to Cl₂MDP (47).

Lower nephron nephrosis is seen in both the young and adult rabbit (47) on massive doses of Cl₂MDP which would not be applicable to the current study.

Dilated and degenerating seminiferous testicular tubules were seen in the young adult and the adult rat in one of several studies (47). Once again, the dose given was much greater than that given in the present study.

A decrease in serum phosphorus has been noted in rats (47), a decrease in serum magnesium in adult dogs (47), and an increase in serum copper in rats (47).

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In summary, Cl₂MDP at the doses proposed for the study has not been shown to have serious or permanent toxic effects.

6. Oral glucose tolerance tests. No side-effects are anticipated. Some subjects may complain of nausea, and vomiting does rarely occur. Minor bruising and/or hematoma at the site of the scalp vein insertion may occur. The possibility of reactive hypoglycemia is slight inasmuch as the research subjects will be eating immediately after the glucose tolerance test is completed.
7. Diet. A solid food diet which is nutritionally adequate will be given. No adverse effects are expected from the diet.
8. Blood withdrawal. Using accepted aseptic techniques, blood samples will be taken. There is the possibility of bruising at the site of needle stick. There is also a possibility of thrombophlebitis. Over the 30 weeks of the study, a total of 1750 cc will be withdrawn; 750 cc for routine laboratory determinations, 500 cc for the 3 diurnal hormonal variation studies and ⁴⁷Ca kinetic studies, and 500 cc for the 2 oral glucose tolerance tests. The iron loss will be 880mg over the 30 weeks. Iron intake is 15 mg/day, 50% higher than the recommended dietary allowance (54), and will cover the iron loss. The average blood loss will be 60 cc/week. In previous studies at this facility, anemia has not developed.

Side-Effects and Adverse Reactions:

Unexpected reactions, illnesses or behavior during the period of the study, whether interpreted as medication-related or not, will be recorded for each volunteer (including those on placebo) on the special report form "Side Effects and Adverse Reactions," a copy of which is attached. When the reaction is severe or highly unusual, it will be reported by a phone call or telegram to the drug supplier. Severe occurrences will be described in a more extensive follow-up letter reporting the reaction. An attempt to determine the cause and/or mechanism of reactions will be made and reported on the report form.

Termination of the Study:

All studies made during "Pre-Study Examination of Volunteers" will be repeated at the termination of the study, and the results recorded. In addition, the "Final Report Form" for the volunteers will be completed.

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Documentation of the Study:

- A. Record keeping. The results of all histories, physical and other examinations and laboratory measurements or determinations including radiographic studies, etc. will be recorded on appropriate report forms.
- B. Disposition of case reports and other documentation. 1) Data will be forwarded to the IND sponsor as it becomes available. 2) Copies of the data and other study documentation will be retained in the investigator's file for a period of at least two years after the IND sponsor notifies the investigator that an NDA (New Drug Application) has been filed with the Food & Drug Administration or the IND sponsor notifies the investigator of the new drug that they have withdrawn their IND (Investigational New Drug Application) filed with the Food & Drug Administration.

Data Analysis:

In general, all data from the twelve individuals receiving EHDP or Cl₂MDP will be compared with those from the six subjects at bedrest without treatment. The efficacy of the three treatment regimens will be judged by analysis of the differences between these three groups in (a) overall mineral loss, estimated by calcium and phosphorus balance, (b) mineral loss from the calcaneus and tibia, judged by gamma ray transmission scanning, (c) bone resorption, judged by calcium kinetics and urinary hydroxyproline excretion, and (d) bone formation, judged by calcium kinetics and serum alkaline phosphatase activity. The effect of dose on response will be evaluated by comparing the results obtained from individuals receiving 0 and 20 mg EHDP/kg of body weight/day, 0 and 1600 mg/day Cl₂MDP or on the basis of absorbed doses calculated from urinary excretion data.

Correlation with our previous bedrest studies will be assessed by comparing data of the untreated control subjects with those of other untreated control subjects from earlier experiments.

Activity:

During the baseline period, the subjects will be allowed to engage in activity ad lib, but will be accompanied by a member of the metabolic staff when not on the ward. During bedrest the subjects will be under close supervision in bed or on a wheeled stretcher; random supervision will be accomplished by frequent observation by the metabolic nursing staff. Freedom of horizontal movement in bed will be permitted, and the subjects will be allowed to raise themselves on one elbow for eating and reading. They will not be allowed to sit up or dangle their legs over the bed, and defecation and micturition will be performed while supine. During reambulation the subjects will be allowed to resume activities ad lib, but they will be cautioned to avoid excessive walking during the first two weeks, since this previously has

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been shown to lead to marked tenderness of the soles of the feet.

Balance Diet:

The diet will be prepared in the same fashion as in previous studies (6-13). The solid food diet will be composed of seven daily menus, each consisting of three meals and an evening snack. All foods except some staples, soft drinks and meats will be purchased in common lots prior to the study to assure maximal constancy. Fresh fruits will be avoided and canned whole milk and frozen homogenized eggs will be used. Distilled water will be used for food preparation, drinking, and utensil cleansing. The subjects will be required to eat all food, lick their plates and drink a distilled water rinse of their glassware. On the average, the diet will contain 1 g of calcium and 1.7 g of phosphorus. To determine the exact mineral and nitrogen content of the diet, each month duplicates of the menus for one week will be weighed and homogenized separately, pooled, and an aliquot stored frozen for subsequent digestion and analysis.

Serum, Stool, and Urine Collections:

Approximately 35 ml of blood will be drawn every two weeks for complete blood count, ionized calcium, total serum calcium, phosphorus, creatinine and alkaline phosphatase determinations. If possible, parathyroid hormone*, calcitonin* and 1,25-DHCC* will be assessed. An additional 30 ml of blood will be drawn every four weeks for a partial thromboplastin time, erythrocyte sedimentation rate, lupus cell preparation, serum sodium, potassium, chloride and carbon dioxide, total serum protein and albumin, serum bilirubin, SGOT and BUN. Urinary protein, sugar, and sediment will be checked weekly by the research technician. On three occasions through the study (during baseline and during the 5th and 17th weeks of bedrest), 35 ml blood samples will be drawn at four-hour intervals for 24 hours to evaluate diurnal variations of ionized calcium, total serum calcium, phosphorus, protein, alkaline phosphatase, cortisol, ACTH, CT*, PTH*, 1,25-DHCC.*

Urine and stools will be collected between weeks two and thirty, inclusive. Twenty-four hour urines will be collected daily, acidified with 1 ml of 12 normal HCl/100 ml urine, and stored at 4° C. At the end of each seven day period, total creatinine content of the daily urines will be determined. For each subject the seven creatinine values will be averaged and any value which is more than 20% from the mean will be discarded and a new mean obtained; any value which is more than 10% from the new mean will be assumed to represent a collection error and discarded. For each subject, the remaining urines will be combined into a pool representing the seven day period, and an aliquot will be stored at -22° C for subsequent analysis of calcium, phosphorus, nitrogen and hydroxyproline. A 1000 ml aliquot of urine with a label indicating the subject's number, dates of collection and total volume of the seven-day sample may be shipped to Procter & Gamble for total EHDP and Cl₂MDP determination. Seven-day collections of stools will be obtained beginning and ending 24 hours after the seven-day urines to provide a partial correction for the intestinal transit time. Stools will be collected in bedpans lined

*From the laboratory of Claude Arnaud, M.D., Veterans Administration Hospital, 4150 Clement Street, San Francisco, CA 94121

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with Mylar film and will be subsequently transferred with distilled water rinsing into epoxy-lined 1 gallon canisters and refrigerated. Upon completion of the seven-day collections, the stools will be further diluted with distilled water to a final weight approximately three times the initial weight. They will be homogenized for 30 min. on a paint shaker and an aliquot stored at -22° C for subsequent analysis of calcium, phosphorus, and nitrogen. A 100 ml sample of this homogenized sample, labeled as described above for the urine samples, will be frozen and may be shipped to Procter & Gamble for total EHDP and Cl₂MDP determination. Initially the urine sample collected during weeks 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 & 26 from volunteers receiving EHDP or Cl₂MDP will be analyzed for total EHDP or Cl₂MDP. Fecal samples collected during weeks 8, 10, 16, 24 & 26 from volunteers receiving EHDP or Cl₂MDP will also be analyzed for total EHDP or Cl₂MDP. If necessary, to establish an adequate picture of the excretory pattern, additional samples will be analyzed. Total weights and volumes for each collection will accompany all samples.

Laboratory Determinations:

Stools and diet specimens will be digested in nitric acid for calcium determinations and in sulfuric acid for phosphorus and nitrogen determinations as previously described (6,10). Calcium will be determined by atomic absorption spectrophotometry on an automated Perkin-Elmer Model 303 (48). Phosphorus will be analyzed by the Technicon autoanalyzer adaptation of the Fiske and SubbaRow method, using standards adjusted to the pH of the samples (49). Creatinine will be determined using the Technicon autoanalyzer adaptation of the Folin Wu method (50). Nitrogen will be determined by the standard Technicon autoanalyzer method (51). Hydroxyproline will be determined by the method of Kivirikko and Prockop (52), and alkaline phosphatase will be determined by the automated modification of the Bessey-Lowry-Brock method (53). All methods have been validated initially by recovery studies which are repeated periodically, and quality control is maintained by including standard sera in all runs. All assays are carried out in duplicate and results accepted only when the disparity between the two determinations is less than 5%. Calcitonin (59), PTH (60) and 1,25-DHCC (61) are to be done in Dr. Claude Arnaud's laboratory.

SIGNIFICANCE OF THE RESEARCH

The diphosphonates have received attention because of their potential therapeutic usefulness in a wide variety of skeletal disorders for which no satisfactory treatment is yet known (26,32,44). As stable analogues of naturally occurring pyrophosphates, they have been shown to retard both the formation and dissolution of hydroxyapatite crystals in vitro (22). Inhibition of bone formation and resorption has also been demonstrated in tissue culture and in several species of intact animals. EHDP has been shown to effectively retard bone resorption in humans (13) and, in fact, evidence suggests that a paradoxical enhancement of bone formation may occur at an optimal dosage level. EHDP is in its final phase before being given FDA marketing approval and will be used in the treatment and prevention of metastatic calcification and in Paget's disease.

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Cl₂MDP in animal studies (32,45) has been shown to be more effective in the prevention of bone mass loss in immobilization osteoporosis and corticosteroid-induced osteoporosis. Thus, it is important to be able to develop a method of prevention of disuse osteoporosis so that space travelers have no fear of fracture upon re-entry into one "G" conditions. In addition, other practical aspects may include the ability to prevent disuse osteoporosis associated with aging and to help those individuals who are prone to pathological fractures secondary to the osteoporosis of aging.

Diurnal studies of PTH and calcium have been done in this laboratory on several previous occasions; however, the data were inconclusive. The lack of PTH effect with bedrest may be due in part to the PTH assays performed. CT and 1,25-DHCC have not been determined in bedrest studies. Therefore, with the new techniques available to measure these hormones more accurately, it is important to find out whether these factors play a major role in disuse osteoporosis.

A recent communication indicates that there is a marked change in the diurnal variation of ACTH associated with bedrest in women (55). At the end of 30 days of bedrest, ACTH was found to be highest at 4:00 p.m. in all subjects although significant blood cortisol changes were not seen. The subjects had kept normal daytime activities and nighttime sleep patterns. If the normal pituitary-adrenal axis control mechanisms are altered with bedrest, it will be important to elucidate these changes to determine (1) whether a change in the cortisol excretion pattern causes disuse osteoporosis; and (2) to determine if the pituitary-adrenal sensitivity changes during bedrest in man.

FACILITIES AVAILABLE

All studies will be conducted on the Metabolic Unit of the U.S. Public Health Service Hospital, San Francisco, California.

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The Joint US-USSR Biological Satellite Program

Kenneth A. Souza

Cooperation between the USA and USSR in space biology and medicine began in 1971 with the signing of the US-USSR Science and Applications Agreement. A Joint Working Group for Space Biology and Medicine was established, which continues to meet periodically to exchange information obtained during spaceflight and to discuss problems and areas of mutual scientific interest.

A major accomplishment of this working group was to establish a Joint US-USSR Biological Satellite Program. Under the aegis of this program, a variety of U.S. biological and radiation physics experiments were flown on two unmanned Soviet satellites, Cosmos 782 and Cosmos 936. Cosmos 782, launched on 25 November 1975 and recovered 19.5 days later, carried 11 U.S. experiments. Seven U.S. experiments were aboard Cosmos 936, which was in orbit 18.5 days from 3 to 22 August 1977.

The experiments flown on Cosmos 782 and Cosmos 936 were designed to determine the effects of spaceflight on a variety of biological species, including animals, plants, fish, and insects. Although unmanned, many of the experiments aboard both flights focused on problems common to man and animals during prolonged spaceflight. For example, rats were used to investigate alterations in bone and muscle, and in general physiology resulting from spaceflight—alterations that have been observed in astronauts and cosmonauts during and following space travel. Rats were also used with a variety of other biological and nonbiological material to measure cosmic radiation and to assess the haz-

ard it poses to man during prolonged spaceflights.

In addition to the United States and the Soviet Union, Czechoslovakia, Hungary, Poland, Rumania, and France participated in the scientific investigations of the Cosmos 782 mission; together with these nations, Bulgaria and East Germany participated in the Cosmos 936 mission. Results of all experiments on both spacecraft are scheduled to be published soon by the Soviets. The purpose of this article is to (a) familiarize the U.S. scientific community with U.S. involvement in the two US-USSR missions, (b) describe the spacecraft and some of the methods and flight hardware used to support the biological experiments, and (c) highlight some of the data U.S. investigators obtained.

THE SPACECRAFT

The Cosmos 782 and Cosmos 936 were modified versions of the Vostok spacecraft used in early Soviet manned spaceflights. The Vostok was a spherical craft approximately 2.4 m in diameter, which carried a 900-kg payload and had a gross weight of about 2300 kg (Fig. 1). During flight, batteries supplied electric power. The atmosphere within the craft was maintained at approximate sea-level conditions, and the total pressure ranged from 745 to 815 mm Hg, with a pO_2 of 145–210 mm Hg and a pCO_2 of 2–4 mm Hg. Gaseous impurities generated within the cabin during flight, such as ammonia and methane, were kept at low levels by circulating the air in the cabin through canisters containing absorbent materials.

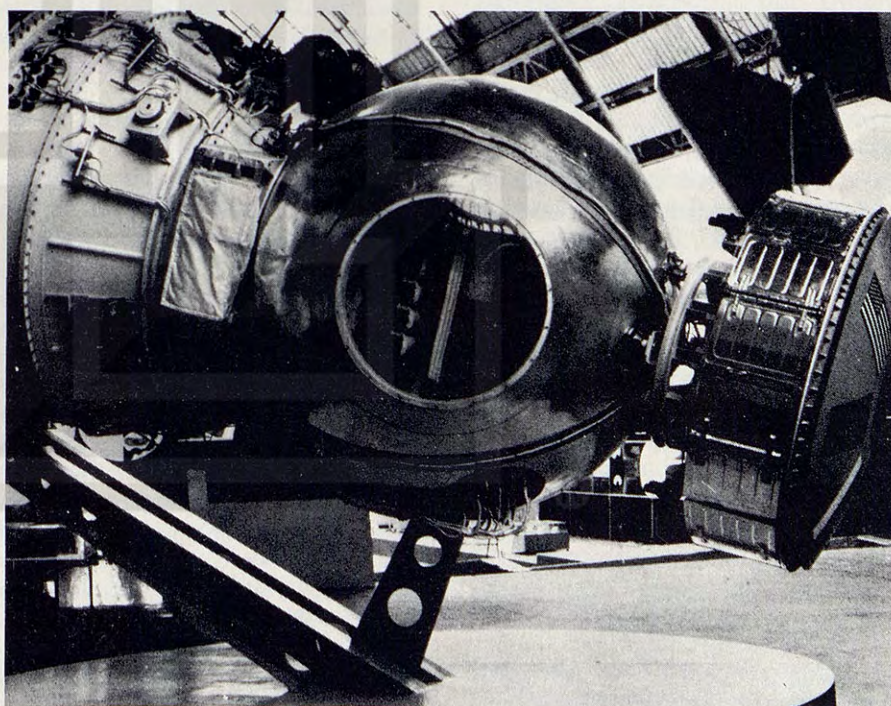


Fig. 1. Cosmos 782 on display in the Soviet Space Museum, Astakeno, USSR. A circular viewport was installed in the spherical portion of the craft for display purposes.

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Within the spacecraft, biological specimens and experiments were contained in a variety of hardware. Of primary interest to U.S. investigators were the rat-holding units (Fig. 2). Rats were contained throughout the flight in individual cylindrical cages, each with its own light, food, water, and air-circulation and waste-management systems. Each cage was approximately 20.5 cm long and 9.4 cm in diameter. Light at a 2-lux intensity was regulated to a 12-hr-light, 12-hr-dark cycle within each cage. Ten grams of a special paste diet was provided to the animals four times per day at 6-hr intervals throughout the flight. This same diet was provided both to flight and to ground-control animals. All animals began the special diet approximately 2 weeks before launch and remained on the diet throughout the flight and throughout the 25-day recovery phases of the mission. Water was provided *ad libitum* at all times.

Cabin air was drawn through each cage from the rear and was dispersed from the cage ceiling through a series of holes in a plastic cage liner. The airflow passed downward over the animal, forcing animal wastes and debris into a waste-collection trap, which rotated to present a clean trap to the animal at 2-day intervals. Air passing through the waste trap was then circulated through activated charcoal filters and returned to the cabin. Surrounding the plastic cage liner was an electric wire coil, which was used to measure changes in conductivity, reflecting gross body movements of the animal.

The rat cages were arranged in groups of five, with each group sharing a common food and water reservoir. Only the quantity of food and water delivered to each group of five cages was measured, and this only once, at the end of the experiment. Cosmos 782 contained five such groups (25 cages) and Cosmos 936 contained four (20 cages); in both cases, the rats housed within these cages constituted the group of animals exposed to weightlessness.¹

In addition to the 20 cages described above, Cosmos 936 contained 10 other cages, divided into two groups of 5 cages each; these latter two groups were placed on two on-board centrifuges. The centrifuges were designed to provide an artificial-gravity environment by rotating

¹Although the term *weightlessness* is used, complete weightlessness (absence of all accelerations) was not achieved on either flight. The spacecraft tumbled in orbit, imparting accelerations of 1.7×10^{-7} to 1.5×10^{-4} G to experiments at the edge of the spacecraft.

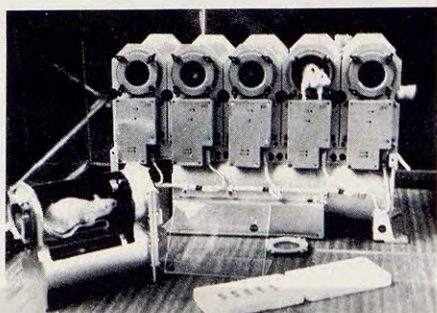


Fig. 2. Rat-holding unit and individual rat cage. Four and five such units were included in the Cosmos 936 and Cosmos 782, respectively.

continuously during the flight. Each centrifuge had a radius of 32 cm, measured from the centrifuge center to the longitudinal axis of the animals during rotation. Centrifuges were switched on when the spacecraft reached its orbit and were switched off approximately 5 hr before landing. The rotation rate was 53.5 ± 3 rpm, which provided an acceleration of 1 G to the animals. The five cages on each centrifuge were similar to the cages used for the animals exposed to weightlessness; the only difference was that airflow through the cages, instead of being from top to bottom, was through holes in the cage doors, and the centrifuge rotation forced the air through the cages.

Cosmos 782 contained no centrifuges for rats, but it did contain a single centrifuge on which a variety of biological specimens, including fruit flies, carrot tissue, and fish embryos, were subjected to artificial gravity during spaceflight.

MISSION OPERATIONS

In support of the experiments aboard Cosmos 782 and 936, two ground control groups, designated the "synchronous" and "vivarium" controls, were used. The synchronous control was designed to provide an environment as similar as possible to that experienced by the biological specimens during spaceflight. A spacecraft mockup was loaded with all of the experiments and specimens contained in the actual spacecraft (Fig. 3). Control specimens were housed in the same kind of hardware as the flight specimens, and, in the case of rats, food, water, lighting, and airflow were similar for both control and flight groups. Centrifuges were also included in the mockup and rotated at the same rate as the centrifuges within the orbiting spacecraft. However, because of terrestrial gravity, the resultant acceleration applied to the

control specimens was about 1.5 G as opposed to the 1 G to which the flight animals were exposed.

Just prior to the initiation of the synchronous control experiment, specimens were subjected to launch stresses similar to those experienced during the actual launch. The specimens were then loaded into the mockup spacecraft and exposed to an environment regulated to simulate that of the orbiting spacecraft. Upon completion of the control experiment, reentry stresses were applied to the specimens, after which they and the flight group were treated identically.

A vivarium control was also conducted during the mission to provide a group of minimally stressed rats for comparison with the flight and other control groups. Animals were housed in standard polyvinyl cages ($450 \times 310 \times 160$ mm) with five animals per cage and maintained in that arrangement throughout the flight and postflight periods. A paste diet identical to that fed to the flight and synchronous-control animals was provided once per day in 40-g/animal quantities during the preflight and on-orbit phases of the mission. However, no attempt was made to ensure that each animal in a cage received the full portion of food allocated for each animal; thus, animals within a cage undoubtedly received different quantities of food.

A third ground control, designated "Omega Bios," was conducted during the Cosmos 936 mission. For that study, the Omega Bios rats experienced centrifugation on the ground at the same rotation rate as those centrifuged in space, but differed from the animals centrifuged in the synchronous control in that the radius of the centrifuge used for Omega Bios was 9.2 rather than 32 cm (Fig. 4). The resultant acceleration imparted to the animals during centrifuge rotation was 1.05 G. The cages on both centrifuges were identical.

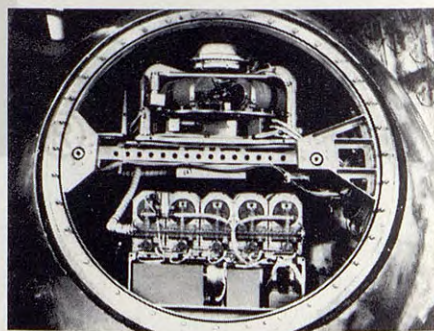


Fig. 3. Spacecraft mockup used for synchronous-control experiment during the Cosmos 936 mission.

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On 25 November 1975, Cosmos 782 was launched into an elliptical orbit with an apogee of 405 km, a perigee of 226 km, an orbital inclination of 62.8°, and a period of revolution of 90.5 min. Cosmos 936 was launched on 3 August 1977, into a similar orbit. At the time of each launch, the vivarium experiments commenced at the Institute of Biomedical Problems in Moscow. The synchronous-control experiment was started 5 and 4 days after the launch of Cosmos 782 and Cosmos 936, respectively, in order to permit spacecraft environmental data to be received and used to program the environment within the spacecraft mockup. The Omega Bios experiment (Cosmos 936) was started 7 days after the launch.

During the on-orbit phase of the missions, total gross body movement of the flight and synchronous-control animals was monitored during 2-hr blocks of time on the odd-numbered days of flight. Body temperature was also measured during flight, using deep-body temperature transmitters implanted in the peritoneal cavities of 10 rats approximately 3 weeks before flight.

The flights of Cosmos 782 and Cosmos 936 lasted 19.5 and 18.5 days, respectively. In both cases, a recovery team of Soviet scientists and engineers, flown to the landing sites (Siberia for Cosmos 782, Central Asia for Cosmos 936), assembled a field laboratory and soon began animal autopsies and experimental analyses. For example, Cosmos 936 landed at 6:27 A.M. (Moscow time) on 22 August 1977, near the Central Asian city of Kustanay; within 45 min a recovery team arrived at the scene. A field laboratory was fully deployed and experiments removed from the spacecraft within 3 hr; autopsies began 1 hr later. Autopsies, which took about 30 min each, were performed on about half the rats at the recovery site; the rest of the rats were returned to Moscow.

For 25 days following their return to Earth, rats from both missions were allowed to readapt to terrestrial gravity. Throughout this period, animals from the flight, synchronous-control, and Omega Bios control groups were individually held in the standard vivarium cages and were given one feeding each morning of 45 g of the paste diet. The vivarium-control animals also had their food quantity increased from 40- to 45-g/animal/day during the recovery period.

Following completion of control experiments, autopsies and analyses were performed by the same recovery team

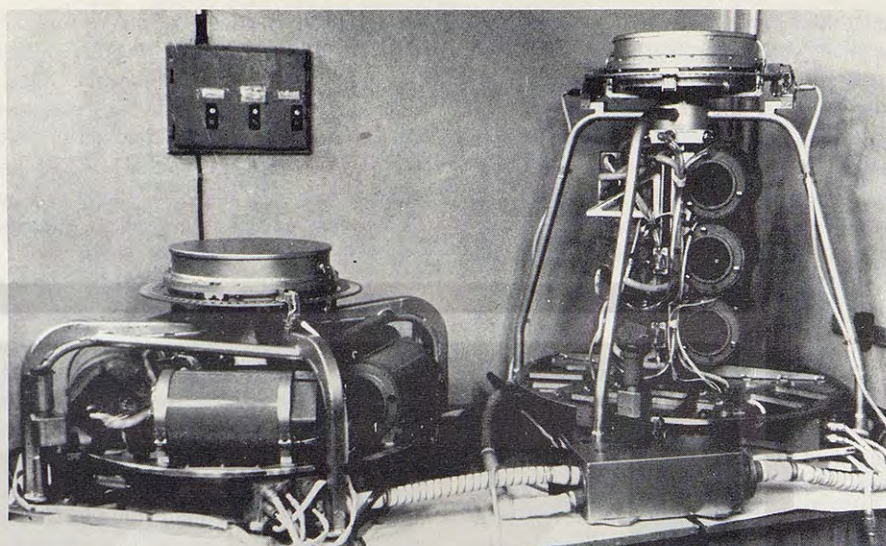


Fig. 4. Rat centrifuges used during the Cosmos 936 mission. The short-radius centrifuge (on right) was used for the Omega Bios control experiment. The standard centrifuge (on left) was used to provide a 1-G acceleration to rats during spaceflight.

members who performed similar functions during the flight experiment. In addition, ground-control experimental procedures were initiated at the same time of day and in the same sequence as those performed during the flight experiment. Once preliminary analyses and experimental procedures were completed, all specimens were frozen, preserved, or packed as live specimens and shipped to U.S. laboratories, where they arrived in excellent condition.

U.S. EXPERIMENTS ABOARD COSMOS 782 & COSMOS 936

There were 11 U.S. experiments aboard Cosmos 782 (Table 1); 10 were biological experiments with the common objective of determining the effects of spaceflight on the morphology, physiology, and development of plants, animals, fish, and insects. The one experiment not sharing this objective was a radiation dosimetry study, which sought to measure the radiation environment inside the spacecraft to assess the hazard to man and animals from cosmic radiation, particularly HZE particles. The results of this latter experiment have been published elsewhere and will not be discussed here (Benton et al. 1978).

The Cosmos 936 mission included seven U.S. experiments (Table 2). Five were designed to supplement and expand experiments flown on the previous joint mission. The two new experiments involved a study of the effects of spaceflight on hepatic enzymes involved in carbohydrate and lipid metabolism in

rats and a study of the changes in rat muscle fibers induced by spaceflight. The significant results of all U.S. biological experiments are discussed below, beginning with the experiments with rats.

Experiments with Rats

Wistar strain, specific-pathogen-free male rats were used for both the Cosmos 782 and Cosmos 936 missions and were supplied by the Institute of Endocrinology, Slovakian Academy of Sciences, Bratislava, Czechoslovakia. At the launch of both flights, animals were about 63 days old and weighed approximately 215 g. Upon recovery, the following general observations of the flight animals were made: (a) the animals exposed to weightlessness had a soiled and disheveled appearance, suggesting that they did not groom themselves during flight; (b) animals exposed to weightlessness exhibited coordination and balance problems not seen in either ground-control specimens or those exposed to artificial gravity in orbit; and (c) five of the animals in the Cosmos 936 weightlessness group suffered broken leg bones, probably from an unusually hard landing.

The rats aboard both spacecraft were used for a wide variety of studies, most of which focused on problems observed in man during spaceflight. For example, in 1973 the United States launched the first manned Skylab mission, which housed a three-man crew for 28 days. In later flights, a second three-man crew occupied Skylab for 59 days, and another

three-man crew flew in Skylab for 84 days. During these flights, the level of calcium in the urine and plasma of the astronauts increased (Leach and Rambaut 1977). In addition, densitometry studies of the heel bone indicated that bone density decreased during the flights, suggesting that elevated levels of calcium in the urine and plasma reflected a loss of calcium from the bones, a potentially serious problem for men during prolonged space travel.

To investigate this problem, various parameters of bone formation, mineralization, and resorption were studied in the rats flown on Cosmos 782. The most striking effects of spaceflight were observed in bone formation (Morey and Baylink 1978). Postflight studies indicated that, during flight, rats formed significantly less periosteal bone than did rats in either ground-control experiment. An arrest line at both the periosteum and

the endosteum of the tibiae of animals subjected to spaceflight suggested that bone growth ceased during the flight. However, when a group of flight rats was allowed to readapt to terrestrial gravity for 25 days, bone formation not only resumed, but took place at a greater rate during that period, compared with control animals.

Rats exposed to weightlessness during the 18.5-day flight of Cosmos 936 also exhibited a decreased rate, and perhaps complete cessation, of bone formation. Surprisingly, the bone-formation rates of rats subjected to artificial gravity during this flight also decreased, although the bone growth rate of this group of rats returned to normal much sooner in the postflight period than did that of members from the weightlessness group. Arrest lines, indicative of a cessation of bone growth, were also much less pronounced in the centrifuged rats than in

those exposed to weightlessness. These results suggested that factors other than, or in addition to, weightlessness (e.g., confinement and launch stresses) may affect bone growth during spaceflight. However, centrifugation did prevent a decrease in femur-breaking strength during flight, suggesting that weightlessness may have been primarily responsible for this effect (Holton et al. 1978).

The Cosmos 782 red blood cell experiment of Leon et al. (1978) also stems from observations made on the crews of Skylab: A significant decrease in total body red-cell mass occurred during the Skylab flights (Kimzey 1977). This result could have been due to a suppressed erythropoiesis, increased hemolysis, or a combination of the two. The Cosmos 782 experiment was designed to study the effects of spaceflight on red-blood-cell hemolysis by using rats as surrogates for humans. Rats were injected with $2\text{-}^{14}\text{C}$ glycine 15.5 days before launch, which labeled the hemoglobin molecules in newly formed red blood cells.

When these red blood cells die and hemolyze, the radioactive carbon is released as carbon monoxide. Red-blood-cell hemolysis appears to be the sole source of endogenously produced carbon monoxide in the rat; measuring ^{14}CO in the breath of rats that received a prior injection of $2\text{-}^{14}\text{C}$ glycine measures red-blood-cell hemolysis. As the red blood cells approach the end of their lifespan, labeled carbon monoxide output increases dramatically, reflecting the death of red cells that were labeled by the $2\text{-}^{14}\text{C}$ glycine injection. Prior to age-related death, the rate at which labeled carbon monoxide is released is proportional to the hemolysis of cells dying from a variety of random events.

Following the flight of Cosmos 782 and throughout the readaptation period, breath samples were collected from the flight and control animals. Analysis of the data indicated that random hemolysis of red blood cells was approximately three times greater in flight than in control rats. In addition, the mean red-cell lifespan of the flight rats decreased by about 4 days, that is, from 63 to 59 days (Leon et al. 1978). The spaceflight-associated factors responsible for these results are not known, and additional studies are planned to pursue these interesting observations, especially because accelerated hemolysis may have been responsible for, or contributed to, the decrease in red-cell mass observed in astronauts during Skylab flights.

As was the case of the rats aboard

TABLE 1. U.S. experiments flown on Cosmos 782.

Study	Investigators	Affiliation
PLANTS		
1. Responses of carrot crown gall tumors to spaceflight	R. Baker et al.	Colorado State University
2. The morphogenetic responses of cultured totipotent cells of the carrot (<i>Daucus carota</i>) at zero gravity	F. C. Steward, A. D. Krikorian	State University of New York at Stony Brook
FRUIT FLY		
3. Effects of weightlessness on the embryonic development and aging of <i>Drosophila melanogaster</i>	J. Miquel, D. E. Philpott	Ames Research Center
FISH		
4. <i>Fundulus heteroclitus</i> embryogenesis	H. W. Scheld et al.	Lyndon B. Johnson Space Center
RAT		
5a. Bone-quantitative analysis of selected bone parameters	E. Holton, D. J. Baylink	Ames Research Center, Veterans Administration Hospital (Seattle, WA)
b. Histological studies on tibial bones, jaws, teeth	C. W. Asling, I. Asling	University of California at San Francisco
6a. Immune system: effect of spaceflight on cell-mediated immunity	A. D. Mandel, E. Balish	Ames Research Center, University of Wisconsin
b. Histological examination of inguinal lymph nodes	L. Kraft	Ames Research Center
7. Effect of spaceflight on gastric ulceration	J. Vernikos-Danellis, P. A. Brown	Ames Research Center
8. Effect of spaceflight on random hemolysis and mean lifespan of red blood cells	H. A. Leon, S. A. Landaw	Ames Research Center, Veterans Administration Hospital (Syracuse, NY)
9. Effects of spaceflight on the hormonal content of the pituitary gland	S. Ellis, R. E. Grindeland, L. Keil	Ames Research Center
10. Histological and ultrastructural examination of cosmos and control eyes	D. E. Philpott, G. Harrison	Ames Research Center
RADIATION DOSIMETRY		
11. HZE particle dosimetry	E. V. Benton, D. D. Peterson, M. Tran	University of San Francisco

Cosmos 782, rats flown on Cosmos 936 experienced a threefold increase in random hemolysis of red cells. Rats exposed to artificial gravity during flight exhibited a less dramatic increase in hemolysis, suggesting that weightlessness was the probable cause for the increased hemolysis (Leon and Landaw 1978). If one extrapolates these results from rats to man, the decreased red-cell mass in crews of Skylab could be attributed to an increase in random hemolysis, although one cannot rule out the possibility that red-cell production may be depressed during flight and thus may contribute to the overall decrease in red-cell mass.

To determine if stress associated with spaceflight caused gastric ulceration or erosion of the gastric mucosa, the stomachs from the flight and control rats were examined macroscopically and histologically. Neither ulcers nor gastric erosion was observed among rats in either of the groups (Brown and Vernikos-Danellis 1978). These observations were somewhat surprising because we knew before the flights that the animals would be subjected to a wide variety of stresses, including the short-term stresses of launch and reentry and the long-term stresses of confinement and weightlessness.

The cell-mediated immune response was also studied in the flight and ground-control rats. Five days prior to flight, groups of rats were immunized with 10^6 formalin-killed *Listeria monocytogenes* cells suspended in Freund's complete adjuvant. Following recovery, lymphocyte cultures were prepared from spleens of rats in the presence of *Listeria* antigens, phytohemagglutinin, Concanavalin A, and purified protein derivative (PPD), and measured for their uptake of ^3H -thymidine. The lymphocytes of all rats gave a blastogenic response to phytohemagglutinin and Concanavalin A. Although individual rats varied considerably, all flight and immunized control rats gave a blastogenic response to *Listeria* antigens and PPD. These results did not indicate that spaceflight had a detrimental effect on cell-mediated immunity (Mandel and Balish 1977); there was, however, a striking increase in the number of necrotic cells with evidence of phagocytosis of cellular debris in the inguinal lymph nodes of flight rats. These changes may have been caused by exposure to cosmic radiation or weightlessness.

Spaceflight-associated stresses were also linked to some postflight changes in rat plasma and pituitary hormone levels.

TABLE 2. U.S. experiments flown on Cosmos 936.

Study	Investigators	Affiliation
FRUIT FLY		
1. Effects of weightlessness on the development and aging of <i>Drosophila melanogaster</i> *	J. Miquel, D. E. Philpott	Ames Research Center
RAT		
2. Effects of weightlessness and centrifugation ($1 \times G$) on erythrocyte survival in rats subjected to prolonged spaceflight*	H. A. Leon, S. A. Landaw	Ames Research Center, Veterans Administration Hospital (Syracuse, NY)
3. Effects of spaceflight on some liver enzymes concerned with carbohydrate and lipid metabolism in the rat	S. Abraham, H. P. Klein	Bruce Lyon Memorial Research Laboratory (Children's Hospital Oakland, CA), Ames Research Center
4. Quantitative analysis of selected bone parameters*	E. Holton, D. J. Baylink	Ames Research Center, Veterans Administration Hospital (American Lake, WA)
5. Cosmic ray effects on the eyes of stationary and centrifuged rats flown on Cosmos 936*	D. E. Philpott, G. Harrison	Ames Research Center
6. Spaceflight effects on muscle fibers	K. R. Castleman, L. A. Chui, J. P. Van Der Meulen	Jet Propulsion Laboratory, University of Southern California School of Medicine
RADIATION DOSIMETRY		
7. Space radiation dosimetry on board Cosmos 936*	E. V. Benton et al.	University of San Francisco

*Similar experiment flown on Cosmos 782.

For example, following the flight, the plasma growth-hormone level was lower in flight rats; however, no significant differences were observed in plasma concentrations of adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and melanocyte-stimulating hormone (MSH) among flight and control rats. Pituitary concentrations of the hormones measured were not significantly different between flight and control animals. Hematocrits were similar for all groups, and anterior pituitary and testicular weight taken from rats following flight did not differ significantly from control animals (Grindeland et al. 1978).

Light flashes, observed by astronauts and cosmonauts when their eyes were closed during spaceflight, have been related to the impact of cosmic ray HZE particles on the eyes, which may pose a hazard to man, particularly during long stays in space (Pinsky et al. 1974). Similar flashes were seen by persons whose retinas were exposed to cyclotron HZE beams in terrestrial accelerators (Demircioglyan 1974). Ultrastructural alterations were observed in the outer segments and cells of the pigment epithelium in the retinas of pocket mice exposed to accelerator HZE beams

(Philpott et al. 1972). Because cellular damage to the retina could affect man's vision during spaceflight, the retinas of rats flown on Cosmos 782 were examined. Most of the eye tissues of the rats flown in space were normal; however, some necrotic cells were found in tissue of flight rats that were not observed in control rats. Furthermore, tissue necrosis was similar to that in the retinal tissue of rats exposed to accelerator HZE beams (Philpott et al. 1978b).

During the Cosmos 936 mission, rat retinas were again examined for evidence of tissue damage attributable to cosmic radiation. The results obtained were similar to those of the Cosmos 782 mission: Necrotic cells and a variety of other pathological conditions were observed in flight rats but not in ground-control rats. No differences were noted between the weightlessness group of animals and those provided with artificial gravity during flight, a finding that tends to eliminate weightlessness as a cause of the observed abnormalities (Philpott et al. 1978a).

On the Soviet biological satellites, Cosmos 605, Cosmos 690, and Cosmos 782, significant changes were noted in lipid concentrations in the liver and other tissues of rats subjected to spaceflight (Belitskaya 1977, Yakoleva 1977). De-

spite overall losses in total body fat in rats subjected to weightlessness, plasma triglycerides, cholesterol, and phospholipids increased. Decreased phospholipid content of the microsomal fractions from muscle tissue of flight rats was also found by Soviet scientists, suggesting that the membranes of these tissues may be affected by spaceflight (Tigranyan 1976). Data from the Skylab missions also indicated that spaceflight affected carbohydrate and fat use by the astronauts (Berry 1973).

Because of these observations, a comprehensive survey of key hepatic enzymes of carbohydrate and lipid metabolism in the rats flown on Cosmos 936 was undertaken. This survey showed that significant changes in the activities of at least five enzymes involved in lipid and carbohydrate metabolism occurred in rats following 18.5 days of near weightless spaceflight. The livers of those rats contained approximately twice the glycogen content of control rats when examined postflight. None of these changes was observed in rats centrifuged at 1 G during flight, and all of the changes had completely disappeared within 25 days postflight (Abraham et al. 1978). Both of these findings lend credence to the alterations noted in the group of rats exposed to weightlessness.

A second U.S. experiment with rats, which, like the liver enzyme study, flew for the first time on Cosmos 936, focused on changes in skeletal muscle-fiber type and structure following spaceflight. Skeletal muscle contains two types of muscle fibers in proportions characteristic of the particular muscle, the so-called "type I" and "type II" fibers. Type I fibers contract by an oxidative kind of energy metabolism and thus require an oxygen supply. The "type II" fibers contract by a glycolytic-anaerobic energy metabolism. Type II fibers contract rapidly but are readily fatigued; type I fibers contract more slowly but are fatigue-resistant. The slow fibers are particularly important in maintaining posture against gravity, and the fast fibers are required for quick, forceful movements. Moreover, muscle-fiber size usually increases as a result of exercise, electrical stimulation, and hypergravity (Martin and Romond 1975) and decreases in response to inactivity (Dubowitz and Brooke 1973). Fiber atrophy has also been observed in animals and humans as a result of spaceflight (Ilyina-Kakueva et al. 1976).

The complete extensor digitorum longus (edl) muscle was excised from flight-weightlessness and ground-control rats.

No centrifuged rats were available for this study. The edl muscle was serially sectioned and stained for alkaline and acid ATPase, NADH, and PAS reactions. Sections were analyzed using microscopic- and computer-assisted image enhancement techniques of the Medical Image Analysis Facility at the Jet Propulsion Laboratory. Of the three experimental groups examined (flight plus synchronous and vivarium controls), the average muscle-fiber diameter was greatest in the vivarium controls and smallest in the flight specimens. The intermediate size of the synchronous-control muscles was probably the result of hypokinesia from cage confinement.

No significant differences were found in the ratio of type II to type I fibers between the flight and control groups. However, the edl muscle contains a preponderance of type II fibers. Additional studies are required using muscles with predominantly slow, and an approximately equal mix of, fiber types (i.e., soleus and gastrocnemius, respectively) before conclusions can be reached regarding the effects of spaceflight on the conversion of muscle-fiber types (Castleman et al. 1978).

Experiments with Fish Embryos

Embryos of the minnow, *Fundulus heteroclitus*, were flown to study the effects of spaceflight on their development, particularly the development of their vestibular systems. At launch, embryos ranged in age from 32 to 128 hr, which covered the developmental stages from late gastrula to first appearance of statoliths. After the completion of the Cosmos 782 flight, some fish were subjected to behavioral tests, and others were fixed, sectioned, stained, and examined.

Similar experiments were flown aboard Skylab 3 and in the Apollo-Soyuz Test Project (Scheld et al. 1975). The results from the three flight experiments did not identify any significant effects on normal development attributable to weightlessness (Scheld 1978).

Experiments with Plants

Two U.S. experiments with plants were flown on the Cosmos 782 satellite. In one, carrot tumor growth during spaceflight was studied as an indicator of metabolic rate; in another, a tissue culture of undifferentiated totipotent carrot cells was used to determine if weightlessness altered carrot cell growth and development. In the first study, the tumor

masses produced by plants exposed to weightlessness were significantly smaller than those plants centrifuged at 1 G during flight. This was the opposite reaction predicted from previous Earth-based, gravity-compensated experiments. Why tumors were smaller than expected in the weightless group remains to be determined (Baker et al. 1978).

Analyses of the carrot tissue culture experiment revealed that the totipotent somatic cells used in the experiment underwent normal growth and morphogenesis to produce viable and fully competent embryos at 0 G. Also, following the flight, normal plants were grown to maturity from the tissues subjected to weightlessness (Krikorian and Steward 1978).

Experiments with Insects

The development and aging of *Drosophila melanogaster* were studied to determine if they were altered by spaceflight. Following the Cosmos 782 flight, investigators examined flies using morphological, biochemical, and behavioral techniques. Scanning and transmission electron microscopy revealed that the external morphology and internal fine structure of the flies were not appreciably affected by the lack of normal gravity. The lifespan of flies subjected to spaceflight was also apparently unaltered. There was, however, a decrease in activity and mating competence of the flies exposed to weightlessness. These changes were thought to be a result of wing damage incurred during flight by flies striking the container walls. The absence of similar damage in ground-control flies suggested that, in orbit, flies may be more active or disoriented, or both, in their flying patterns (Miquel et al. 1978).

The principal differences between the *Drosophila* experiment on Cosmos 782 and that on Cosmos 936 were that (a) the Cosmos 782 flight used 4-day-old flies at launch, whereas the Cosmos 936 flight used flies approximately 3, 7, and 26 days old at launch, and that (b) a Soviet strain of *D. melanogaster*, Domodo-devo-32, was used for the Cosmos 782 experiment, and an American strain, Oregon R, was used for the experiment on Cosmos 936. As in the flight of Cosmos 782, a detailed examination of the flight flies, by scanning and transmission electron microscopy, failed to reveal any abnormalities. However, young (7 days old) and middle-age (26 days old) flies exhibited reduced vitality, as measured by flying and mating behavior. As in the

previous mission, these effects were attributed to increased activity levels and O₂ consumption during spaceflight and also to wing damage, which probably resulted from disoriented flying patterns leading to impact with the container walls. Although a decreased lifespan of young flies was observed, it too was attributed to increased activity levels (Miquel and Philpott 1978).

CONCLUDING REMARKS

The Joint US-USSR Biological Satellite Program has provided U.S. scientists with an opportunity to conduct a variety of biological experiments in space. Without such an opportunity, experiments would have had to wait until the mid-1980s, when the U.S. Space Shuttle will have an on-orbit capability beyond 14 days. The program has also provided a forum for an exchange of topics and data of mutual scientific interest.

Although both Cosmos missions in which the USA participated were limited in the complexity of experiments that could be performed, that is, those that required virtually no on-orbit manipulations, data recording, or telemetry, the information derived from postflight analyses was extremely useful. We now know that spaceflight factors, principally weightlessness, have a measurable effect on the erythropoietic and musculo-skeletal systems and on the carbohydrate and lipid metabolism of rats. We learned that artificial gravity produced by centrifugation has the potential for counteracting some of the detrimental effects of spaceflight. Observations of rat retinal tissue during both flights suggested that cosmic radiation may be a hazard to man during prolonged stays in space. We also learned that spaceflight had little (if any) effect on the growth and development of a variety of biological tissues, including the carrot (*Daucus carota*), the minnow (*Fundulus heteroclitus*), and the fruit fly (*Drosophila melanogaster*), although additional studies of the activity and behavior of adult *Drosophila* during spaceflight are needed.

ACKNOWLEDGMENTS

On behalf of all members of NASA and the U.S. scientific community who participated in the Cosmos 782 and 936 missions, I would like to extend our sincere thanks to the Soviet government for making our participation possible and to our Soviet colleagues for their superb assistance in the execution of our experiments.

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Effects of Neutral Amino Acids on the Antihypertensive
Action of Methyldopa in Spontaneously
Hypertensive Rats

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Short title: Neutral Amino Acid Effects on Methyldopa Action

The mechanism by which methyldopa (alpha-methyl-3,4-dihydroxyphenylalanine) lowers blood pressure probably involves its uptake into the central nervous system (CNS) and its decarboxylation to form an amine (Henning & Van Zweiten, 1968; Chalmers & Wurtman, 1971; Heise & Kroneberg, 1973; Van Zweiten, 1973; Maitre, Hedwall & Waldmeier, 1974; Chalmers, 1975; Scriabine, Clineschmidt & Sweet, 1976; Scriabine, Ludden & others, 1977). Inhibition of the enzyme aromatic amino acid decarboxylase (AAAD) within the CNS thus abolishes methyldopa's antihypertensive effect, while inhibition of AAAD only in peripheral organs not only fails to block the fall in blood pressure, but actually potentiates it (Henning & Van Zwieten, 1968; Scriabine, Ludden and others, 1977). That a CNS locus mediates methyldopa's antihypertensive action is also suggested by the observation that application of the methyldopa metabolite alpha-methylnoradrenaline to various brainstem and hypothalamic loci also lowers blood pressure (DeJong, Nijkamp & Bohus, 1975; Struyker-Boudier, Smeets & others, 1975; DeJong & Nijkamp, 1976).

Since methyldopa is a large neutral amino acid (LNAA) similar in structure to the naturally occurring amino acids phenylalanine and tyrosine, one might expect that its transport from the blood to the brain would be mediated by the same specific uptake system as that transporting the LNAAs normally present in blood (Pardridge & Oldendorf, 1975;

1977; Wurtman & Fernstrom, 1976). Indeed, competition among methyl dopa and other amino acids for transport in the kidney and intestine is well documented (Young & Edwards, 1964; 1966), and recent studies from our institution have shown that a concurrent injection of LNAAs can decrease the proportion of a methyl dopa dose that is subsequently detected in the brain of the normal rat (Markovitz & Fernstrom, 1977). The following observations provide evidence that plasma concentrations of LNAAs can also determine the magnitude of methyl dopa's effects on blood pressure.

Male Spontaneously Hypertensive Rats of the Okamoto strain (Charles River Laboratories, Wilmington, Mass.), weighing 230-320 g and having systolic blood pressures of 180-220 mm Hg, were exposed to light (Vita-Lite, Duro-Test Corp., North Bergen, N. J.) from 0700 to 1300 h daily and given ad libitum access to water and to food (Charles River Rat-Mouse-Hamster formula, 24% protein). Food was removed at noon on the day before an experiment, and all experiments were begun soon after the onset of the daily light period (0700 h). Systolic blood pressure was measured by the tail cuff method (see Udenfriend, 1976) using a Narco Biosystems Pneumatic Pulse Transducer. Rats were warmed at 38°C for 15 min prior to each reading, and at least six readings were obtained and averaged for each time point. Rats were pre-conditioned by measuring blood pressure on at least

four occasions on as many days. At least six days were allowed to pass between successive experiments using a given group of rats.

In each experiment, blood pressure was measured immediately before an injection and again 3 h later (the interval after which, in preliminary studies, LNAAs maximally inhibited methyldopa's actions). Rats were maintained at ambient temperatures of 28°C and given free access to water during the 3-h interval. Methyldopa (3, 10, 25, 50, 100, or 250 mg/kg), alone or in combination with LNAAs (the mixture or phenylalanine alone), was suspended in 0.05 N HCl and injected intraperitoneally with a 20-gauge needle. In one series of experiments, a LNAA mixture was used (25 mg/kg each of phenylalanine, tyrosine, leucine, isoleucine, and valine, making 125 mg/kg total, or 0.88 mmoles/kg). In the second series, phenylalanine was administered in a concentration equimolar to the above mixture (146 mg/kg). Preliminary experiments showed that the amino acid mixture had no effect on blood pressure.

In one experiment, rats were given methyldopa, 100 mg/kg, alone or with the LNAA mixture, and were decapitated immediately after the second determination of blood pressure. Methyldopa levels in the whole spinal cord were measured by a fluorometric method (Dominic & Moore, 1971).

Nine or 10 animals were used in each experimental group. Results were expressed as change in blood pressure,

in mm Hg, for each rat (in relation to his own control values); data from each group were averaged, and statistical significance between groups was assessed by Student's t-test.

The concurrent administration of the LNAA mixture or of phenylalanine produced a highly significant attenuation of methyldopa's effect on blood pressure in the middle dose range (25-100 mg/kg) (Figs. 1 and 2). The effect of the LNAAs on blood pressure among rats receiving 100 mg/kg of methyldopa (i.e., to reduce it to 37.7 ± 3.7 mm Hg; as opposed to 58.0 ± 7.2 mm Hg in animals receiving only the methyldopa; $P < 0.002$) paralleled the decrease in spinal cord methyldopa levels (6.5 ± 0.4 μ g/g in rats receiving LNAAs plus methyldopa vs. 10.5 ± 0.06 μ g/g in animals receiving only methyldopa, $P < 0.001$).

The effect of methyldopa was dose-related up to the point of maximum response (i.e., about 100 mg/kg): the highest dose used (250 mg/kg) failed to decrease blood pressure any further, but did override the suppressive effect of the LNAA mixture and of phenylalanine (Figs. 1 and 2).

These observations suggest that, by competing with methyldopa for uptake into the CNS, LNAAs decrease the proportion of each dose reaching the proposed site of action where it can be converted to biologically active metabolites that act on monoaminergic receptors and thereby attenuate the resulting fall in blood pressure.

Phenylalanine was tested alone because it has the lowest K_m for the brain LNAA transport system (Pardridge and Oldendorf, 1975). It was thus expected to bind more tightly to the LNAA carrier (at the blood-brain barrier) than the other LNAAs and, hopefully, to cause a greater inhibition of methyldopa uptake than the LNAA mixture. This was not observed (Figs. 1 and 2), possibly because phenylalanine was more rapidly metabolized than some of the other LNAAs. Hence, the levels of LNAAs acting at the blood-brain barrier might not have been comparable. No other single amino acid has been tested.

The exact loci at which methyldopa metabolites act to lower blood pressure are currently unknown. They might involve the brainstem, the synapses made by descending bulbospinal neurons (Chalmers & Wurtman, 1971; Scriabine, Clineschmidt & Sweet, 1976) or other sites in the CNS. LNAAs could be expected to reduce methyldopa uptake at any of these loci. None of the LNAAs that we administered is thought to have any significant direct effect (at the doses used) on the enzymes involved in methyldopa's biotransformation once the drug is taken up into the CNS. It seems likely that the amino acid pattern of the plasma will be found to be a determinant of the effectiveness not only of methyldopa, but also of any CNS-active drug that happens to be a neutral amino acid (Ordonez, Ambrus & others, 1974).

Acknowledgements

These studies were supported in part by grants from the National Institutes of Health (AM-14228) and the National Aeronautics and Space Administration (NGR-22-009-627). Dr. Zavisca is a Research Fellow in the Department of Anesthesiology of the Massachusetts General Hospital, Boston, Mass., and is supported by a Public Health Services Training Grant (GM-01273) to that department.

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FIGURE LEGENDS

FIG. 1. Relation between dose of methyldopa, with or without concurrent administration of LNAAs, and fall in systolic blood pressure among Spontaneously Hypertensive Rats.

Each point represents the mean $\pm$ SEM of the fall in blood pressure (in mm Hg) 3 h after rats received methyldopa alone (X) or methyldopa plus large neutral amino acids (O) intraperitoneally. Each point represents the mean obtained from 9 or 10 rats.

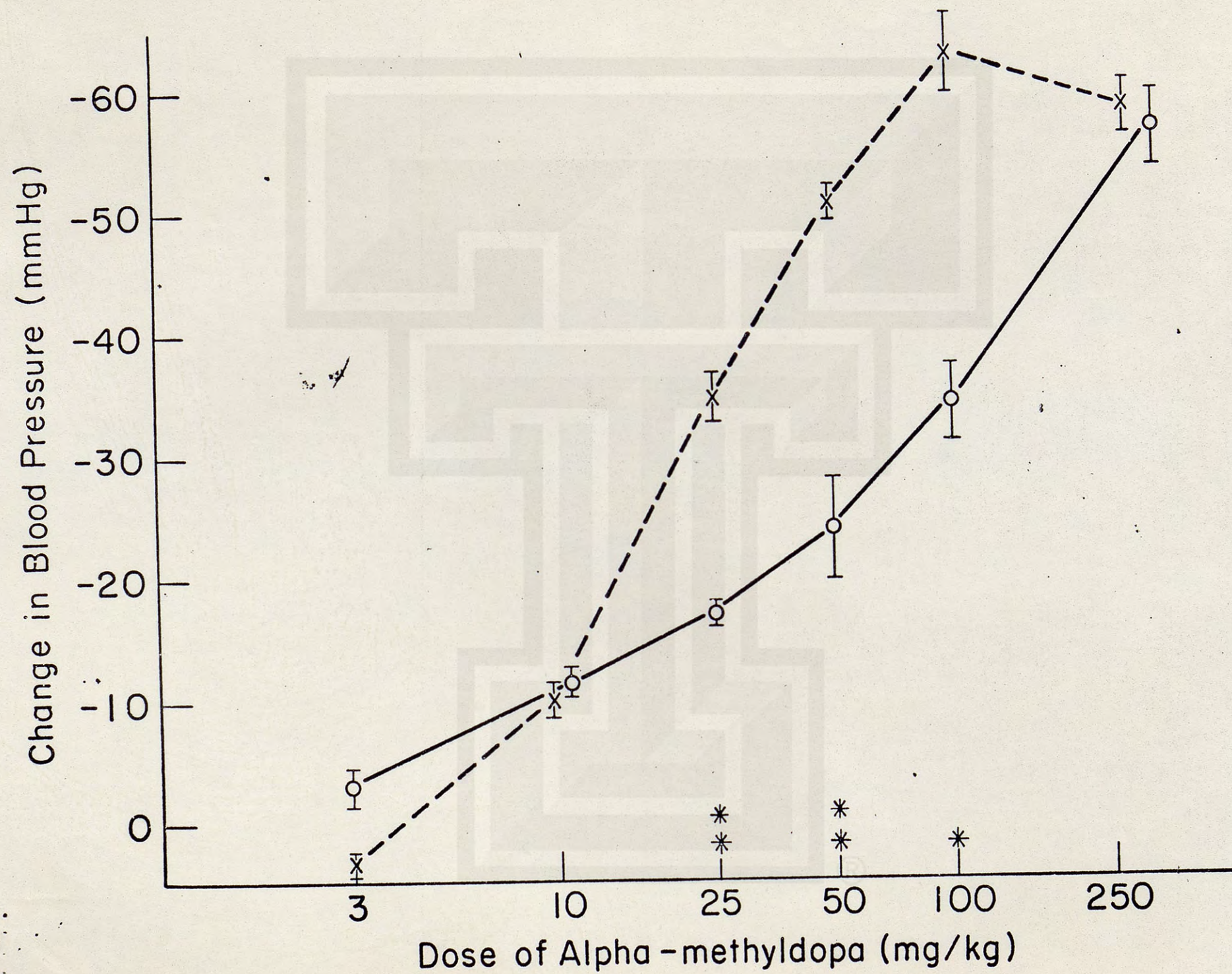
*P < 0.01 differs from rats receiving methyldopa alone.

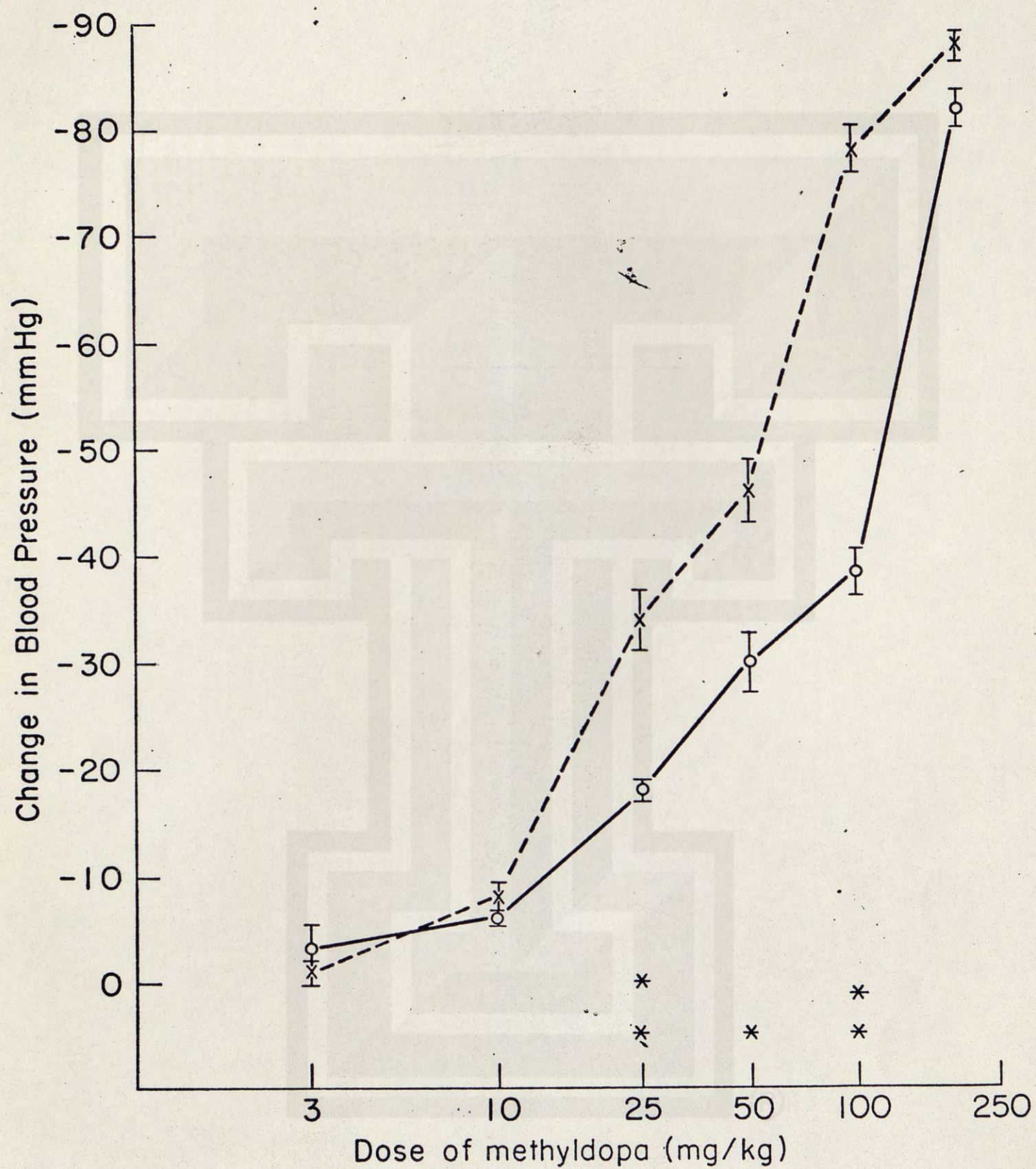
**P < 0.001 differs from rats receiving methyldopa alone.

FIG. 2. Relation between methyldopa dose, with or without concurrent administration of phenylalanine, and fall in systolic blood pressure among Spontaneously Hypertensive Rats. Each point represents the mean $\pm$ SEM of the fall in blood pressure (in mm Hg) 3 h after rats received methyldopa alone (X) or methyldopa plus phenylalanine (O) intraperitoneally. Each point represents the mean obtained from 9 or 10 rats.

*P < 0.01 differs from rats receiving methyldopa alone.

**P < 0.001 differs from rats receiving methyldopa alone.





SALICYLATE REDUCES SERUM INSULIN
CONCENTRATIONS IN THE RAT¹

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(Received in final form July 19, 1976)

Summary

Administration of sodium salicylate (50-500 mg/kg, i.p.) reduced serum insulin concentrations in nonfasted rats. This treatment also suppressed the rise in serum insulin that followed oral administration of glucose (by stomach tube) to fasted rats; this effect is only partly attributable to the blunted increase in serum glucose concentrations.

Salicylates are widely used as analgesics, antipyretics, and anti-inflammatory agents. Their administration has also been found to modify blood sugar levels; however, previous descriptions of these modifications have been inconsistent. For example, salicylate administration caused hyperglycemia in dogs (1), rats (2), and humans (3,4), but produced hypoglycemia in normal and mildly diabetic humans (5,6), and in adrenalectomized rats (2). The effects of salicylates on blood glucose have variously been attributed to epinephrine release (2), alterations in hepatic glycogen metabolism (7), and changes in peripheral glucose utilization (8,9).

Another potential locus at which salicylates could act to modify blood sugar levels is the beta-cell of the pancreas. Here again, available data have failed to delineate a clear pattern of stimulation or inhibition of insulin secretion. Some investigators found that salicylates either do not modify (10,11) or mildly stimulate (4,6) insulin secretion in human subjects. In contrast, Coore and Randle (12) observed decreased insulin secretion in fragments of rabbit pancreas incubated *in vitro* with glucose when salicylate was included in the medium. The following results support this latter observation, by showing *in vivo* that salicylates profoundly suppress insulin secretion in the rat: serum insulin

¹ Acknowledgments: These studies were supported in part by grants from the National Science Foundation (BMS 74-12298) and the National Aeronautics and Space Administration (NGR-22-009-627). M.A.A. holds a predoctoral fellowship from the NIMH (1 F31 MH05066-01); J.D.F. is a Sloan Foundation Fellow in Neurochemistry.

levels attained at particular serum glucose concentrations in salicylate-injected rats are much lower than those in control animals.

Materials and Methods

Male Sprague-Dawley rats weighing 190-200 g (Charles River Breeding Lab., Wilmington, MA) were kept at 20-22°C in our animal quarters for one week prior to experimentation. During this time, they had *ad libitum* access to food (Charles River Rat and Mouse Formula, 22% protein) and water, and were exposed to light (300 $\mu\text{W}/\text{cm}^2$; Vita-Lite, Dura-Test Corp., North Bergen, NJ) between 7 AM and 7 PM daily. In some experiments, nonfasted rats¹ were injected intraperitoneally between 10 and 11 AM with saline (0.9% NaCl, 0.5 cc) or sodium salicylate (50, 125, 250, or 500 mg/kg in a saline solution adjusted to pH 7.4 with 0.1 M NaOH) and were killed 30 or 60 min later. In other experiments, fasted rats (food removed at 7 PM on the preceding evening) received saline or sodium salicylate (125 mg/kg, i.p.) between 10 and 11 AM. Thirty min later they received glucose in solution (0, 0.5, or 1.0 M solution; 1 ml/100 g body weight) by stomach tube and were killed after an additional 30-min period. Blood samples were collected from the cervical wound, centrifuged, and the sera frozen until assay. Serum insulin concentrations were measured by radioimmunoassay (13); in our assay system, purified rat insulin (a mixture of I and II, kindly provided by Dr. R.E. Chance, Eli Lilly Lab., Indianapolis, IN) serves as the standard. Assays were performed to determine whether the presence of salicylate in the assay mixture altered the binding of labeled insulin to the antisera. At salicylate concentrations as high as 60 mM (the highest concentration tested), no effects on binding were observed. This concentration is greater than that seen in human serum following oral consumption of a 400 mg/kg dose (14). Serum glucose concentrations were estimated by the o-toluidine method (15,16). Data were analyzed by analysis of variance, regression analysis, and the Student's *t*-test (17).

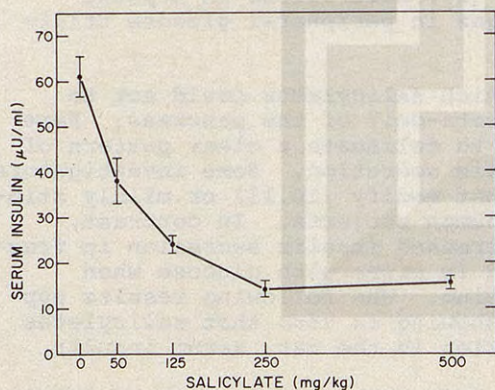


FIG. 1

Effect of various salicylate doses on serum insulin concentrations in nonfasted rats. Groups of animals were killed one hr after drug injection. Vertical bars represent standard errors of the mean. The reduction in serum insulin level was significant for each dose when compared to the 0 mg/kg dose (saline) (F-Test; $P < 0.01$ for each dose).

¹ Food removed 2 hr before drug treatment to minimize potential effects of uncontrolled consumption just prior to drug injection.

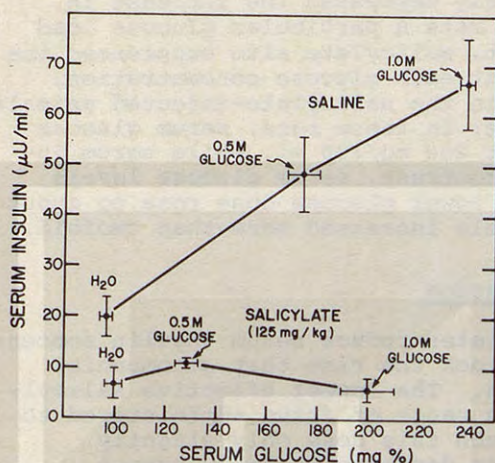


FIG. 2

Relationship between serum glucose levels and insulin concentrations in fasted rats receiving saline or salicylate (125 mg/kg, i.p.) 30 min prior to glucose intubation (0, 0.5, or 1.0 M, 1.0 ml/100 g). Animals were killed 30 min after glucose intubation. Vertical bars represent standard errors of the mean for serum insulin; horizontal bars indicate standard errors for serum glucose. Data were analyzed by regression analysis (17). The slope of the regression line for salicylate-injected rats is significantly smaller than

that for saline-injected animals, suggesting that the drug reduces the ability of glucose to stimulate insulin secretion (Student's *t*-test, $P < 0.01$).

Results

Initially, groups of nonfasted rats were injected with a high dose of salicylate (500 mg/kg) or its vehicle. Thirty min later serum insulin levels had fallen from 42.4 ± 5.3 to 19.1 ± 2.4 microunits/ml ($P < 0.001$); by 60 min, serum insulin levels were still depressed in salicylate-injected animals (19.1 ± 2.2 , compared with 40.6 ± 2.2 microunits/ml in saline-injected rats, $P < 0.001$). Similar reductions were noted in three subsequent experiments.

We next characterized the dose-response relationship for the suppression of serum insulin by salicylates. Groups of nonfasting rats received one of several salicylate doses (0, 50, 125, 250, or 500 mg/kg, i.p.) and were killed one hr later. Each dose tested caused a substantial reduction in serum insulin (Fig. 1). In this and subsequent experiments, 50 mg/kg proved to be the minimal effective dose; doses of 250 or 500 mg/kg produced maximal lowering of serum insulin. Serum glucose concentrations were similarly reduced (by 22-25%, $P < 0.01$) in rats receiving 125 or 250 mg/kg of salicylate, but not in those receiving the lowest or highest dose.

To determine whether salicylates could reduce serum insulin concentrations without lowering the concentration of serum glucose (which, by itself, would be expected to suppress insulin secretion), we gave fasting rats an oral glucose load to yield blood sugar values ranging between 90 and 240 mg/100 ml. We then determined whether pretreatment with salicylate would alter the serum insulin levels attained at any particular glucose concentration (Fig. 2). Groups of rats, fasted overnight, received salicylate (125 mg/kg, i.p.) or saline between 10 and 11 the next morning, followed 30 min later by glucose, *per os*, at one of two doses. They were killed 30 min after glucose intubation, a time previously observed to correspond to the maximal elevation in serum insulin

concentrations. Salicylate clearly depressed the increase in serum glucose produced by giving rats a particular glucose load (Fig. 2). Apart from this effect, salicylate also suppressed the rise in serum insulin occurring at each glucose concentration. This effect was most noticeable in the salicylate-injected animals receiving the higher glucose dose; in these rats, serum glucose concentrations increased to about 200 mg/100 ml, while serum insulin levels actually fell. In contrast, serum glucose levels in rats receiving saline and the lower glucose dose rose to about 175 mg/100 ml, while insulin levels increased more than twofold.

Discussion

These data show that salicylates reduce serum insulin concentrations in nonfasted rats and block the rise that accompanies glucose intubation in fasted rats. The lowest effective salicylate dose, 50 mg/kg, is within the range of doses administered to human subjects (*e.g.*, 6,18). Since this dose only slightly lowered serum glucose levels, this decrease probably cannot account for the apparent action of salicylate on insulin secretion. The 125 and 250 mg/kg salicylate doses substantially reduced both serum glucose and insulin, and therefore, at these dosages, at least part of the drug's action on insulin may be mediated by the decline in serum glucose. Paradoxically, the highest salicylate dose (500 mg/kg) did not lower serum glucose concentrations. The actions of salicylates on glucose distribution and utilization appear to be complex and are poorly understood. However, one mechanism that could explain why serum glucose levels did not fall after the highest salicylate dose might involve epinephrine release. Salicylates may stimulate epinephrine release *in vivo* (2). If so, the highest salicylate dose could both increase circulating epinephrine levels (and thus, hepatic glycogenolysis) and suppress insulin release (12,19). Indeed, epinephrine secretion could mediate all or part of the suppression of insulin release by salicylates (20). At lower doses, salicylates may also induce epinephrine release, but perhaps in amounts sufficient only to inhibit insulin release (20) without stimulating glycogen breakdown to the extent necessary to compensate for the drug-induced fall in blood sugar levels. We are currently investigating these possibilities.

The experiments in which glucose was given by stomach tube show that, despite the suppression by salicylates of serum glucose levels, these levels do rise to values sufficient to stimulate insulin secretion in untreated animals (*e.g.*, after the 1.0 M glucose dose). The failure of insulin levels to rise may reflect a direct action of salicylates on the pancreas; of course, other actions could have an identical--but indirect--effect, such as an inhibition of insulin release by epinephrine after its secretion in response to salicylate.

At present, we are unable to account for the apparent differences in the effect of salicylate on insulin levels in rats and human beings. In humans, the drug has been reported to elevate slightly (4,6) or not to modify (10,11) serum insulin concentrations. As far as we know, no depressing action *in vivo* has yet been reported. We hope our data will renew interest in the pharmacology of salicylates, especially their effects on insulin re-

lease and overall carbohydrate metabolism. A common underlying mechanism may yet emerge, that ultimately can account for these apparent species differences.

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ORAL CHOLINE ADMINISTRATION TO PATIENTS WITH TARDIVE DYSKINESIA

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Abstract We gave pharmacologic doses of choline to patients with tardive dyskinesia in an attempt to suppress involuntary facial movements. Choline is the physiologic precursor of acetylcholine, and its administration elevates brain acetylcholine levels in laboratory animals and, possibly, in human beings. Hence, we thought that its use could benefit patients with diseases like tardive dyskinesia, which is believed to result from deficient central cholinergic tone. Twenty patients with stable buccal-lingual-masticato-

ry movements took oral doses of choline for two weeks according to a double-blind crossover protocol. Plasma choline levels rose from 12.4 ± 1.0 to 33.5 ± 2.5 nmol per milliliter (mean $\pm$ S.E.M.; $P < 0.001$) during this period. Choreic movements decreased in nine patients, worsened in one and were unchanged in 10. Thus, oral doses of choline can be useful in neurologic diseases in which an increase in acetylcholine release is desired. (N Engl J Med 297:524-527, 1977)

TARDIVE dyskinesia is a choreic movement disorder characterized by involuntary twitches in the tongue, lips, jaw and extremities.¹ It typically occurs in susceptible persons after chronic ingestion of neuroleptic drugs and may involve an imbalance in the postulated reciprocal relation between dopaminergic and cholinergic neurons in the basal ganglia.²⁻⁴ Thus, drugs that either block catecholamine synthesis (alpha-methyl-p-tyrosine),^{3,5} deplete the brain of monoamines (reserpine, tetrabenazine)⁶ or antagonize dopamine's actions on synaptic receptors (phenothiazines, haloperidol)⁷ often suppress tardive dyskinesia, whereas drugs that indirectly stimulate dopamine receptors (amphetamine, levodopa) often exacerbate the abnormal movements.^{3,8} Drugs assumed to increase the amount of acetylcholine within brain synapses (physostigmine, deanol) also tend to suppress the chorea of tardive dyskinesia, whereas anticholinergics (scopolamine) make it worse.^{3,9}

Cohen and Wurtman showed that choline administered by injection¹⁰ or dietary supplementation¹¹ increases blood and brain choline and brain acetylcholine levels in rats. These increases are present within the terminals of cholinergic neurons¹² and are associated with postsynaptic effects compatible with enhanced release of the transmitter.^{13,14} In human beings, oral doses of choline caused both a dose-related rise in blood choline levels and a significant increase in the choline concentration of cerebrospinal fluid.¹⁵ These observations suggest that choline administration might be useful in treating patients with diseases that, like tardive dyskinesia, presumably arise from deficient central cholinergic tone. Early reports have tended to support this inference.^{16,17} We have examined the possible utility of choline therapy in a dou-

ble-blind crossover study of 20 patients with tardive dyskinesia.

MATERIALS AND METHODS

We selected 20 subjects at random from a large group of inpatients with stable chronic buccal-lingual-masticatory dyskinesia at Medfield State Hospital, Medfield, Massachusetts. Each patient had received phenothiazines or haloperidol in the past, and 13 were still taking such drugs when the study began (Table 1). Anticholinergic medications (benztropine or trihexyphenidyl) were discontinued during the study, but the doses of all other medications, including neuroleptics, were unchanged.

Each patient and his or her family gave informed consent for participation in the study according to the provisions of a protocol (on choline administration to patients with tardive dyskinesia) that had been approved by both the Medfield State Hospital Committee for Research on Human Subjects and the Committee on the Use of Humans as Experimental Subjects at the Massachusetts Institute of Technology.

We evaluated the severity of the chorea by counting the number of eye blinks, tongue protrusions, slow tongue movements inside the mouth, jaw closures, or lip movements visible during a 30-second interval. Each patient sat in a quiet private room with the ward nurse and two investigators, one of whom (J.H.G.) was present during all examinations. The investigators counted the movements independently on two separate days before the study began and made subsequent counts every three days thereafter. We tabulated the mean counts during the control periods, the second week of choline and the second week of placebo ingestion and scored the percentage change as follows: -25 to +25 per cent, no change (i.e., falling within the anticipated day-to-day variation); 25 to 50 per cent, moderately changed; and >50 per cent, greatly changed. We took movies of the facial movements before the drug trial and again during the final weeks of choline and placebo administration.

Choline chloride (150 mg per kilogram per day during the first week and 200 mg per kilogram per day during the second week) was mixed in a sweet commercial beverage (Kool-Aid) and dispensed in three daily divided doses. The placebo, sucrose octa acetate, was also dissolved in Kool-Aid (10 mg per liter) and administered in the same manner and volume as the choline. Both solutions tasted bitter, but the placebo did not impart the "fishy" odor sometimes noted in patients who chronically ingest choline.^{18,19}

Half the patients received choline, and the other half placebo, for two weeks; these schedules were reversed after a 10-day interval during which neither choline nor placebo was dispensed. The patients, nursing staff and examiners were all unaware of whether a particular dose of Kool-Aid did or did not contain choline.

Blood samples for choline measurements were collected from every patient before the drug trial began and on three subsequent occasions: during the second week of therapy, on the ninth day of the drug-free interval and during the second week of the crossover period (the final two weeks of therapy). All blood samples were collected before breakfast. During the treatment periods, they were obtained one hour after the subjects ingested the Kool-Aid. Serum

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Supported in part by grants from ADAMHA (MH-28783), the National Aeronautics and Space Administration (NGR-22-009-627) and the John A. Hartford Foundation (Dr. Growdon is the John R. Whittier Fellow of the Committee to Combat Huntington's Disease, and Ms. Hirsch is the recipient of a predoctoral traineeship [MH 05479-01] from the U.S. Public Health Service).

Table 1. Clinical Characteristics of 20 Patients with Tardive Dyskinesia.

CASE No.	AGE	SEX	PRIMARY DIAGNOSIS	SEVERITY OF TARDIVE DYSKINESIA	CURRENT MEDICATION	DOSAGE
	yr					mg/day
1	36	F	Schizophrenia	Moderate	Thiothixene	100
2	55	F	Schizophrenia	Moderate	Chlorpromazine Trifluoperazine	300 40
3	38	F	Schizophrenia	Moderate	Haloperidol Phenytoin Phenobarbital	15 300 100
4	75	F	Senile dementia	Severe	Thioridazine	75
5	63	M	Schizophrenia	Severe	Phenytoin	300
6	85	F	Senile dementia	Severe	Diazepam Phenytoin	8 300
7	79	F	Schizophrenia	Moderate	None	
8	66	F	Mental retardation with psychosis	Severe	Phenytoin	300
9	73	F	Schizophrenia	Severe	None	
10	48	F	Schizophrenia	Severe	Haloperidol Phenobarbital	5 120
11	72	F	Schizophrenia	Moderate	Chlorprothixene	150
12	80	F	Schizophrenia	Severe	Thioridazine Diphenhydramine	50 100
13	63	F	Schizophrenia	Mild	Thioridazine	300
14	52	F	Schizophrenia	Mild	Chlorpromazine Phenytoin Phenobarbital	100 300 100
15	62	F	Schizophrenia	Moderate	None	
16	37	M	Schizophrenia	Mild	Fluphenazine	25
17	76	M	Senile dementia	Severe	Diphenhydramine	50
18	32	M	Mental retardation with psychosis	Moderate	Haloperidol Phenytoin	40 300
19	37	F	Mental retardation with psychosis	Severe	Phenytoin Phenobarbital	300 160
20	66	M	Schizophrenia	Mild	Thioridazine	400

samples were separated, frozen and assayed for choline content by a radioenzymatic method.²⁰

RESULTS

Before treatment, plasma choline levels ranged between 8.6 and 20.5 nmol per milliliter (12.4 ± 1.0 , mean $\pm$ S.E.M.). During the second week of choline ingestion (200 mg per kilogram per day), plasma choline levels in blood obtained one hour after a choline dose increased in all patients and ranged between 18.2 and 60.1 nmol per milliliter (33.5 ± 2.5 , mean $\pm$ S.E.M., a 170 per cent increase; $P < 0.001$ by Student's *t*-test). Plasma choline levels measured during placebo administration and at the end of the 10-day "washout" period did not differ significantly from control levels.

Buccal-lingual-masticatory movements lessened in nine patients during the period of choline administration; five patients improved greatly, and four improved moderately (Table 2). Case 1 had rapid, tremulous tongue movements, which virtually ceased during choline therapy. Cases 2 and 3 had slower, rolling

tongue movements within the mouth; these movements, too, were greatly suppressed during choline treatment, but not during placebo administration.

Tongue movements also decreased markedly, but not completely, in two patients with more severe dyskinesia. Case 4 protruded her tongue ("serpent's tongue") 20 times per 30 seconds during the initial observation period. In the final week of choline thera-

Table 2. Clinical Effect of Choline Administration on the Buccal-Lingual-Masticatory Movements in 20 Patients with Tardive Dyskinesia.

CLASSIFICATION	NO. OF PATIENTS	MEAN NO. OF MOVEMENTS/30 SEC		% CHANGE*
		BEFORE CHOLINE	DURING CHOLINE	
Greatly improved	5	12.6	4.2	+74 - +84
Moderately improved	4	21.2	11.7	+41 - +55
Unchanged	10	13.4	13.6	+18 - -21
Worsened	1	4.5	27.5	-511

*+ indicates improvement, & - worsening of the chorea.

py, the rate decreased to five times per 30 seconds, although the tongue continued to roll inside her mouth. (Placebo ingestion had no effect on the rate of tongue protrusions.) Two weeks after she stopped taking choline, her tongue protrusions returned to their pre-treatment rate of 20 per 30 seconds. Within a week of the beginning of a second course of choline treatment (200 mg per kilogram per day), the rate of tongue protrusions again decreased to six times per 30 seconds.

Case 5 protruded his tongue 20 to 30 times per 30 seconds during initial observations but did not protrude it at all during the second week of choline ingestion. It continued to move inside his mouth, but the movement frequency decreased by 49 per cent.

Buccal-lingual-masticatory movements decreased moderately (25 to 50 per cent) in another four subjects. Cases 6 and 7 had fewer jaw movements during choline ingestion, but their tongue motions did not change. The number of jaw movements also diminished during choline therapy in Case 8, although the frequency of her eye blinks did not change. Tongue and lip movements decreased during choline ingestion in Case 9, but jaw movements were unaffected.

The frequency of tongue movements increased markedly in Case 20 (from four to 27 times per 30 seconds) during the period of choline ingestion, but returned to control counts when the choline was discontinued. Neither choline nor placebo altered buccal-lingual-masticatory movements in the remaining 10 patients.

Another patient with severe akathisia was included in the study; she did not exhibit facial chorea, however, and is not listed in the tables. She was unable to sit still and moved her feet 30 times every 30 seconds. These movements were not altered during placebo ingestion but nearly ceased during choline administration.

No serious side effects were encountered in any subject during the course of the study. Cases 2 and 3 were more withdrawn than usual and possibly depressed during choline treatment. Three patients (Cases 4, 7 and 15) experienced symptoms of mild cholinergic toxicity, including lacrimation, blurred vision, anorexia and diarrhea, while taking 200 mg of choline per kilogram per day. All the effects were dose related and subsided when the dosage was reduced.

DISCUSSION

The 20 patients who participated in our study all exhibited "permanent" buccal-lingual-masticatory characteristic of tardive dyskinesia,^{1,21} and all had taken neuroleptics in the past (although these drugs had been discontinued in seven patients before the study began). Most subjects were elderly women who had taken neuroleptics for many years; the drugs and doses listed in Table 1 are minimal estimates and do not include medications received before admission to

Medfield State Hospital. Since the onset of tardive dyskinesia was documented only in Case 13, it is possible that some patients in the series had senile chorea, or the mannerisms of mentally retarded or psychotic patients, and not true drug-induced tardive dyskinesia. The variety of their responses to choline (nine better, one worse and 10 unchanged) suggests that the patient sample was indeed heterogeneous, at least in the involvement of cholinergic mechanisms. This confusion about causes will remain a problem until an accurate diagnostic test for tardive dyskinesia is found.

Most attempts to treat tardive dyskinesia are based on the theory that neuroleptic drugs, by blocking intrasynaptic dopamine receptors, cause a reflex overactivity of dopaminergic neurons, which may be due to increased dopamine turnover²² or to "denervation" supersensitivity.²³ Either action would excessively suppress striatal cholinergic neurons (which receive inhibitory impulses from the dopaminergic nigrostriatal pathway²⁴) at times of day when the blockade of dopamine receptors was incomplete. Although no therapy is completely satisfactory, numerous efforts to treat stable tardive dyskinesia have employed drugs thought to decrease the amount of dopamine released into central synapses; such approaches were the subject of a recent review.²⁵ Other therapeutic strategies designed to increase cholinergic tone at the next synapse distal to that employing dopamine have had only limited success. The choline precursor deanol apparently does produce some benefit in a few specific cases²⁶⁻²⁸ but not in most others,^{29,30} and its efficacy has not been established in a double-blind study. Intravenously administered physostigmine, a centrally active anticholinergic drug, reportedly decreased buccal-lingual-masticatory movements in some patients with tardive dyskinesia^{17,31}; its route of administration, however, precludes its use in chronic therapy.

The demonstration that exogenous choline elevates brain choline and acetylcholine levels in rats^{10,11} suggests that choline administration might also increase brain acetylcholine levels in human beings, and thus provide a practical way to restore deficient cholinergic tone. Shortly after the results of animal studies were published, Davis et al. described a patient in whom the administration of choline (16 g per day) decreased the choreiform movements of tardive dyskinesia.¹⁶ The therapeutic utility of choline presumably arises from the ability of orally administered choline to elevate plasma choline levels significantly in human beings,^{15,32} a finding confirmed in the present study.

Buccal-lingual-masticatory movements decreased in nine patients during the period of choline consumption but were unaffected by the placebo. The occurrence of cholinergic side effects in three of our subjects provides additional indirect evidence that exogenous choline enhances neuronal acetylcholine syn-

thesis and release in human beings as it does in rats¹³ and probably accounts for the suppression of chorea in the nine patients who improved during choline therapy. The buccal-lingual-masticatory movements of these patients were not sufficiently different from those displayed by the group as a whole to permit identification of particular movements that might be especially responsive to choline treatment. The mean blood choline levels, both before and during treatment, in the patients who responded to choline did not differ significantly from the mean of the group as a whole — in nine responders, 13.1 ± 1.4 nmol per milliliter before and 31.5 ± 2.5 during treatment, and in the entire group, 12.4 ± 1.0 nmol per milliliter before and 33.5 ± 2.5 during treatment — nor did their age (63.3 years vs. 59.8 years), sex (predominantly women), primary diagnosis (predominantly schizophrenia), or concurrent neuroleptic medication (Table 1). Intravenous physostigmine was not administered in the current study, but a choreic patient's response to this drug might help to predict his or her ability to benefit from choline, as another study showed.¹⁷

Oral doses of lecithin, the major source of dietary choline, may be an alternate way to treat patients with tardive dyskinesia. We observe that lecithin administration, like that of free choline, elevates blood choline levels in human subjects³³; however, its ability to suppress buccal-lingual-masticatory movements in patients with tardive dyskinesia remains to be tested.

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NASA PROGRESS REPORT AND PLANNED STUDIES: Sept. 15, 1977

1. Effect of salicylates on serum insulin: (reprint; ongoing studies: amino acids; hope to do human studies).
2. Diet, neurotransmitters, and control of pituitary function:
 rat prolactin
 rat growth hormone
 effect of choline on human plasma cortisol, etc.
3. Diet, neurotransmitters, and sleep: Await funding.
4. Relationship between dietary protein content and plasma amino acids in normal and cirrhotic subjects: Many data.
5. Effect of tyrosine availability on brain catecholamine synthesis.
6. Effect of choline availability on acetylcholine synthesis and cholinergic function: rat studies
 clinical studies
7. Effect of diet composition on the effectiveness of antihypertensive drugs that are also amino acids (e.g., aldomet).

PHYSIOLOGICAL CONTROL OF BRAIN CATECHOL SYNTHESIS BY BRAIN TYROSINE CONCENTRATION

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(Received 15 July 1976; accepted 15 October 1976)

Abstract—Treatments that raise or lower brain tyrosine concentrations in rats cause parallel changes in the rates at which their brains accumulate DOPA (after aromatic L-amino acid decarboxylase is inhibited by RO4-4602). When the neutral amino acid drug *p*-chlorophenylalanine (PCPA) was injected i.p. into rats (100 mg/kg), it decreased brain tyrosine concentrations over a wide range, partly by competing with tyrosine for uptake into the brain. Brain DOPA accumulation after RO4-4602 decreased in parallel. At this dosage, PCPA did not inhibit tyrosine hydroxylase activity *in vivo*, and, at very high concentrations (10^{-3} M) *in vitro*, it inhibited the enzyme only slightly. Hence, the decrease in DOPA accumulation probably derived from a decrease in the availability of tyrosine (the substrate for tyrosine hydroxylase) rather than from a change in enzyme activity. Similarly, the neutral amino acids, valine and isoleucine (which have been used previously to modify brain tyrosine concentrations and, hence, catechol synthesis), had no effect on tyrosine hydroxylase activity *in vitro* in concentrations up to 10^{-3} M. At high concentrations (10^{-3} M), leucine slightly inhibited the enzyme. When rats ingested a single meal containing 40 per cent casein, their brain evidenced increases in both tyrosine levels and catechol synthesis. Thus, the availability of tyrosine to the brain may be one of the factors normally controlling brain catecholamine synthesis.

Mammalian brains synthesize the catecholamines dopamine and norepinephrine and the indoleamine serotonin from the amino acid precursors L-tyrosine and L-tryptophan. The first (and probably the rate-limiting) step in the synthesis of each of these monoamine neurotransmitters involves the addition of a hydroxyl group to either the aromatic ring of tyrosine or the indole nucleus of tryptophan [1]; the enzymes tyrosine hydroxylase [2] and tryptophan hydroxylase [3] catalyze these reactions. DOPA and 5-hydroxytryptophan, the hydroxylated products, are then decarboxylated (by the enzyme aromatic L-amino acid decarboxylase [4]) to form dopamine and serotonin.

The rate of serotonin synthesis in rat brains can be accelerated by physiologic manipulations that raise brain tryptophan concentrations (e.g. feeding rats a single protein-free meal [5]). This finding is compatible with the view that the tryptophan hydroxylase enzyme is not saturated with its amino acid substrate *in vivo* [6]. Brain tryptophan concentration depends, in turn, upon the pattern of amino acids in the plasma: it varies directly with plasma tryptophan concentration and indirectly with the plasma concentrations of other neutral amino acids that compete with tryptophan for transport into the brain [7, 8]. We recently described preliminary evidence that brain tyrosine concentrations might also influence the rates at which catecholamine neurons synthesize their neurotransmitters [9]. In that study the injection of amino acids that did or did not modify brain tyrosine levels caused parallel changes in the rates at which rat brains accumulated DOPA after decarboxylase inhibition.

We have now examined further the relationship between brain tyrosine level and catechol synthesis, which we found to be linear over a wide range. We

also found that the activity of the rate-limiting enzyme tyrosine hydroxylase was not affected by the experimental conditions used, and that a physiologic treatment that raised brain tyrosine also accelerated catechol synthesis.

MATERIALS AND METHODS

Groups of 6-8 male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), each weighing 150 g (except where noted), were housed in hanging cages, given access to water and a 26 per cent protein diet (Charles River Rat and Mouse Formula), and maintained under light (300 microwatts/cm²; Vita-Lite, Duro-Test Co., North Bergen, NJ) between 8 a.m. and 8 p.m. daily. Rats used for diet experiments were fasted overnight immediately prior to the study and then allowed to consume the experimental diet starting at 10 a.m. Diets were prepared in agar gel (35 g/1000 ml of water) with the compositions outlined in Table 1. All injections were intraperitoneal.

We estimated catechol synthesis in the rats' brains by measuring the accumulation of DOPA 30 or 60 min after the administration (800 mg/kg) of the DOPA decarboxylase inhibitor, RO4-4602 (generously provided by the Hoffman-LaRoche Co., Nutley, NJ) [6, 9]. The accumulation of DOPA was linear for at least 60 min after decarboxylase inhibition; brain dopamine and norepinephrine levels were not significantly depressed during this interval. In some experiments, the methyl ester of *p*-chlorophenylalanine (PCPA) (Regis Chemical Co., Chicago, IL)—a synthetic amino acid that, among other actions, competes with tyrosine for uptake into the brain [9, 10]—was administered (100 mg/kg) at various

Table 1. Composition of protein diets

Dietary constituent	Casein diet* (g/1000 ml water)					
	0%	8%	18%	24%	40%	75%
Casein†	0	80	180	240	400	750
Destrose	270	241	207	184	133	3
Sucrose	221	198	167	167	151	4
Dextrine	270	241	207	184	133	3
Salt mix‡	40	40	40	40	40	40
Vitamin mix§	22	22	22	22	22	22

* Each diet also contained 150 ml of Mazola Oil (Best Foods, CPC International Inc., Englewood Cliffs, NJ).

† From General Biochemicals, Chagrin Falls, OH.

‡ Roger's-Harper's, Teklad Test Diets, Madison, WI.

§ From Nutritional Biochemicals, Cleveland, OH.

times before injection of RO4-4602. In the diet experiments, rats fasted overnight were allowed access to one of the test diets for 1 hr, injected with RO4-4602, permitted to eat for 30 min, and then killed. All amino acids were administered in doses of 100 mg/kg; in the subsequent discussion, doses are given as $\mu\text{M/kg}$.

Tyrosine hydroxylase activity was assayed by measuring the evolution of $^{14}\text{CO}_2$ produced by the enzymatic decarboxylation of carboxyl-labeled DOPA formed from carboxyl-labeled L-tyrosine [11]. Whole brains and corpora striata were homogenized in 5 and 10 vol. respectively, of 0.05 M Tris-acetate buffer, pH 6.0, containing 0.2 per cent Triton X-100 (Harleco, Philadelphia, PA). The homogenates were centrifuged at 10,000 g for 10 min, and the supernatant fluid was decanted for assay [2]. The assay medium contained (in a total vol. of 110 μl): 50 μl of brain homogenate; 65 nmoles of DMPH₄ (2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride [synthetic cofactor obtained from Calbiochem, San Diego, CA]); 29 nmoles of pyridoxal phosphate; 4 nmoles of 2-mercaptoethanol; 240 units of catalase; 0.01 m-moles of phosphate buffer; and 10 μl of aromatic L-amino acid decarboxylase prepared from hog

kidneys [11]. Samples were preincubated at 37° for 2 min.

The reaction was started by adding 10 μl of L-[1- ^{14}C]tyrosine (sp. act., 54.6 mCi/m-mole [from New England Nuclear Corp., Boston, MA], diluted with 1 mM tyrosine to a final concentration in the assay medium of 0.1 mM) to the sample and incubating it at 37° for 30 min. The assay was stopped by injection of 0.5 ml of 10 per cent trichloroacetic acid through the rubber stopper. The acidified medium was shaken at 37° for an additional 2 hr to recover $^{14}\text{CO}_2$, which was trapped by folded filter paper strips in 0.2 ml of NCS tissue solubilizer (Amersham/Searle, Arlington Heights, IL). The strips were then placed in scintillation vials containing 10 ml of Aquasol (New England Nuclear), and their radioactivity was counted.

Within an enzyme range of 20–50 μl , the assay was linear with time for 45 min. Samples of boiled enzyme or complete assay mixtures containing 0.2 mM moniodotyrosine were used as blanks.

Brains used for amino acid analysis were homogenized in 5 vol. of 6 per cent trichloroacetic acid; the acid was then removed from the supernatant fraction by 3 or 4 washes with equal vol. of absolute ether. Samples were lyophilized overnight, made up to 1 ml with 0.2 N sodium citrate buffer, pH 2.2, and filtered through a Millipore filter (Millipore Corp., Bedford, MA) before being placed on the Beckman 119 or 121 C amino acid analyzer (Beckman Instruments, Inc., Fullerton, CA) [12].

Previously described methods were used to assay brains for tyrosine [13] and DOPA [14, 15].

RESULTS

Brain catechol synthesis at various intervals after PCPA. We have previously observed that PCPA administration produces parallel decreases in brain tyrosine concentration and catechol synthesis [9]. In our present study, we used PCPA given at various inter-

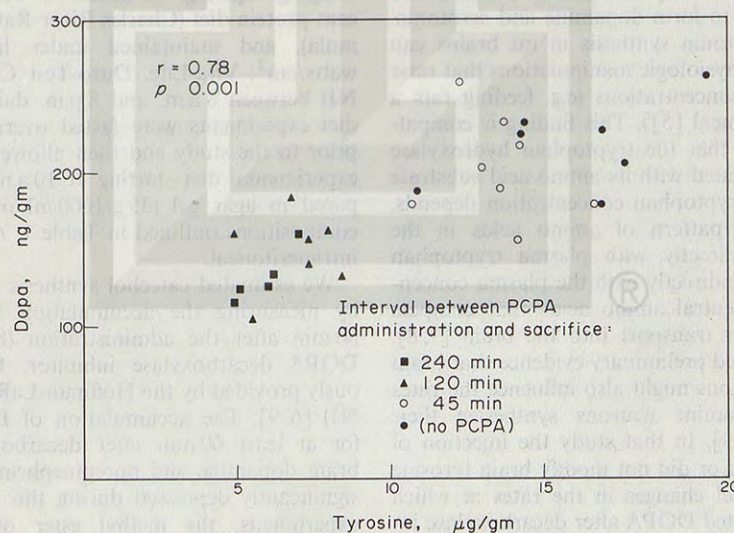


Fig. 1. Relationship between brain DOPA concentration and brain tyrosine level after PCPA administration. The animals were given injections of PCPA (100 mg/kg, i.p.) at various times and were killed 1 hr after a second injection of RO4-4602 (800 mg/kg). Each point represents DOPA and tyrosine values obtained from a single brain sample.

Table 2. Tyrosine hydroxylase activity of brain homogenates from animals given PCPA or valine

Treatment	Number of animals	Per cent of control activity	Statistical significance*
1 hr after valine injection	4	110.5 $\pm$ 3.2	N.S.
1 hr after PCPA injection	4	92.6 $\pm$ 23.3	N.S.
2 hr after PCPA injection	5	112.2 $\pm$ 6.3	N.S.

Male Sprague-Dawley rats weighing 200 g were injected (100 mg/kg, i.p.) with valine, PCPA, or their vehicle (water) and killed 1 or 2 hr later. A 50- μ l aliquot of whole brains homogenized in Tris-acetate buffer (pH 6.0) was used for assay of tyrosine hydroxylase activity. Tyrosine hydroxylase in control samples (9 animals) was 0.201 nM 14 CO₂/mg protein/hr.

* N.S. = not significantly different from control animals.

vals before RO4-4602 to produce a range of decreases in brain tyrosine; we then determined the extent to which the rate of DOPA accumulation paralleled the tyrosine concentration in each rat's brain. Rats received PCPA either 15 min after RO4-4602 administration or 60 or 180 min before the decarboxylase inhibitor. All rats were killed 1 hr after the injection of RO4-4602.

The decreases in brain tyrosine concentrations after PCPA administration varied over a wide range, from 15.95 μ g/g (control) to 7.63 μ g/g (animals killed 4 hr after receiving the drug; $P < 0.001$) (Fig. 1). Brain DOPA accumulation showed a parallel decrease from 217 ng/g (control) to 149 ng/g ($P < 0.001$). Brain DOPA and tyrosine concentrations correlated significantly ($r = 0.78$; $P < 0.001$) over this range (Fig. 1).

Tyrosine hydroxylase activity after PCPA or neutral amino acid administration. PCPA inhibits the biosynthetic enzyme tryptophan hydroxylase [16] and has been widely used in the hope that it would specifically deplete brain serotonin. PCPA also inhibits phenyl-

alanine hydroxylase activity in the liver but has little or no effect on tyrosine hydroxylase activity. We have used PCPA to modify brain tyrosine levels (Fig. 1). In an earlier study, we showed similar effects with several of the branched-chain amino acids (leucine, valine, and isoleucine) which are in the same brain transport group as tyrosine [9], i.e. the large neutral amino acids [17]. In all these experiments, a parallel reduction in DOPA accumulation accompanied the decrease in brain tyrosine level (Fig. 1) [9].

To determine whether the fall in DOPA accumulation was related to a drug-induced decrease in tyrosine hydroxylase activity rather than to altered substrate (tyrosine) availability, we measured tyrosine hydroxylase activities in the brains of animals receiving either PCPA or one of the other neutral amino acids. Animals were injected with PCPA (37 μ M/kg) or with the neutral amino acid valine (85 μ M/kg) (which lowered brain tyrosine to 67 per cent of control and brain DOPA accumulation to 69 per cent of control); they were killed 1 or 2 hr later. Whole brains were removed, homogenized in 0.05 M Tris-acetate buffer, and assayed for tyrosine hydroxylase activity. Neither valine nor PCPA, administered *in vivo*, significantly changed tyrosine hydroxylase activity, as assayed *in vitro* (Table 2).

In other studies, PCPA or one of the other neutral amino acids was added to the assay mixture in various concentrations, *in vitro*, and tyrosine hydroxylase activity was determined with homogenates of whole brains or striata as the enzyme source. The striata showed considerably greater activity. The branched-chain amino acids valine and isoleucine had no effect on the tyrosine hydroxylase activity of whole brain or caudate homogenates, even when they were present in concentrations as high as 1 mM. Leucine at this concentration did somewhat inhibit tyrosine hydroxylase activity in homogenates of whole brains. PCPA had no effect on tyrosine hydroxylase activity in concentrations of 1 μ M to 0.1 mM, but, at a concentration of 1 mM, it did suppress enzyme activity by 40 per cent (Table 3). In contrast, phenylalanine concentrations as low as 0.1 mM significantly inhib-

Table 3. Effects of neutral amino acids, added *in vitro*, on tyrosine hydroxylase activities of homogenates of whole brains or caudate nuclei

Amino acid added to the medium	Control value	Tyrosine hydroxylase activity at various amino acid concentrations (nmoles ¹⁴ CO ₂ /hr/mg protein)			
		10 ⁻⁶ M	10 ⁻⁵ M	10 ⁻⁴ M	10 ⁻³ M
A. Caudate nuclei					
PCPA	2.70	2.31	2.60	2.40	1.56
Valine	2.70	2.28	2.68	2.69	2.50
B. Whole brains					
PCPA	0.145	0.154	0.140	0.140	0.089
Isoleucine	0.145	0.136	0.156	0.154	0.155
Leucine	0.169	—	0.155	0.149	0.113
Phenylalanine	0.150	0.154	0.172	0.095	0.023

PCPA or one of the neutral amino acids (in a vol. of 10 μ l) was added to the assay mixture, containing 50 μ l of homogenate prepared from whole brains or caudate nuclei from untreated animals, and the evolution of 14 CO₂ was measured.

Data are given as averages of duplicate determinations.

ited the conversion of labeled tyrosine to DOPA, and hence to $^{14}\text{CO}_2$ (Table 3). This concentration of phenylalanine is close to that normally found in the brain.

To determine whether the concentration of branched-chain amino acids examined *in vitro* for possible inhibition of tyrosine hydroxylase were as high as the brain levels attained *in vivo* after administering the described doses, we gave rats valine, isoleucine or leucine and then measured their brain concentrations of these substances after 1 hr. The valine concentration was 217 μM in valine-treated rats (as compared to 71 μM in control animals), the isoleucine concentration rose to 66 μM (as compared to 41 μM in control animals), and the leucine concentration was 68 μM in leucine-treated rats (as compared to 42 μM in control rats). Hence, the *in vitro* concentrations of these compounds needed to inhibit tyrosine hydroxylase (i.e. 1 mM for leucine and greater than 1 mM for valine and isoleucine) (Table 3) were considerably greater than those occurring *in vivo*. (Although we did not determine PCPA levels in brain, they were probably less than 0.1 mM: Gal *et al.* found a maximum PCPA concentration of 0.72 $\mu\text{M/g}$ 6 hr after injecting rats with 405-mg/kg doses of the ethyl ester of PCPA [19].)

Using the coupled decarboxylation system and the synthetic cofactor DMPH₄, we determined an *in vitro* K_m for tyrosine of approximately 0.10 mM in whole brain homogenates. From the DOPA accumulation data obtained with PCPA, we obtained a double reciprocal plot of substrate concentration versus velocity, as described by Carlsson *et al.* [6]. Using weighted least-squares analysis [20], we estimated the *in vivo* K_m of tyrosine hydroxylase for tyrosine to be 0.043 mM. The concentration of tyrosine in brains of normal rats (fed *ad lib.* with rat chow) was 0.088 mM, a value which is very close to the measured K_m ; hence, tyrosine hydroxylase is probably not saturated with its amino acid substrate *in vivo*. Treatment with PCPA (or other neutral amino acids) lowered the brain tyrosine concentration to 0.036 mM, which is well below its K_m (for tyrosine hydroxylase) and thus could account for the reduced rate of tyrosine hydroxylation.

Brain catechol synthesis after ingestion of a protein meal. To determine whether the brain tyrosine level normally varies and, if so, whether such variations are sufficient to modify catechol synthesis, we measured catechol synthesis (by decarboxylase inhibition) in animals consuming a single high-protein meal which would be expected to elevate brain tyrosine. Groups of rats fasted overnight were given access to either a carbohydrate (protein-free) or 40 per cent casein meal for 1 hr; they were then injected with RO4-4602 and killed 30 min later. In all of twelve similar experiments, the ingestion of the casein meal caused 60–70 per cent increases in both brain tyrosine concentration ($P < 0.001$) and brain DOPA accumulation ($P < 0.001$) (Table 4A). The ingestion of a carbohydrate meal generally increased brain tyrosine level and brain DOPA accumulation (i.e. compared to these levels in animals that continued to be fasted). Occasionally, the consumption of the carbohydrate meal did not elevate brain tyrosine (Table 4B)—a finding that was especially common in studies on

Table 4. Effect of single meals containing 0 or 40 per cent protein on the synthesis of DOPA in rat brains

Treatment	Brain tyrosine level ($\mu\text{g/g}$)	Brain DOPA level (ng/g)
A. 200-g rats		
Fasted	12.9 $\pm$ 0.6	66.5 $\pm$ 4.2
Protein-free diet	18.8 $\pm$ 0.6*	116.5 $\pm$ 6.0†
40% protein diet	26.9 $\pm$ 0.7*	108.0 $\pm$ 5.3†
B. 100-g rats		
Fasted	14.6 $\pm$ 0.5	53.0 $\pm$ 4.2
Protein-free diet	15.0 $\pm$ 0.5	53.1 $\pm$ 3.6
40% protein diet	25.3 $\pm$ 0.9*	82.3 $\pm$ 7.0†

Male Sprague-Dawley rats weighing 200 g (A) or 100 g (B) were fasted overnight and given access to food at 10 a.m. At 11 a.m., they received RO4-4602 (800 mg/kg, i.p.). They were killed at 11:30 a.m. Animals on the protein-free and 40 per cent protein diets consumed 6.6 and 9.2 g, respectively, in group A, and 8.2 and 9.7 g, respectively, in group B.

* $P < 0.001$ differs from fasted animals.

† $P < 0.01$ differs from fasted animals.

younger animals (weight, 100 g). In all these cases, brain DOPA accumulation also failed to increase. The rate of DOPA accumulation tended to be somewhat lower in the younger rats (Table 4).

Effect of varying the protein content of the diet on catechol synthesis. In order to vary the rats' brain tyrosine concentrations physiologically, we fed fasted rats single meals in which the protein content ranged from 8 to 75 per cent; brain tyrosine level and DOPA accumulation were measured after decarboxylase inhibition, as above.

With increasing protein content in the meal, brain tyrosine level also increased; however, at very high concentrations of protein (75 per cent casein), the food-induced increase in brain tyrosine was somewhat suppressed (Table 5), possibly because the very large increases in plasma neutral amino acid concentrations suppressed tyrosine uptake into the brain. Among animals consuming meals of different composition, the rates at which the brain accumulated DOPA (after RO4-4602 administration) roughly paralleled the food-induced increases in brain tyrosine concentration (Table 5). DOPA accumulation was less rapid in animals consuming the 75 per cent protein meal than in those eating 8 per cent casein, even though the brain tyrosine levels were not significantly different (Table 5). This disparity could reflect a greater inhibition by phenylalanine of tyrosine hydroxylase activity after the high-protein meal (Tables 3 and 6).

DISCUSSION

Our observations affirm that the concentration of tyrosine in rat brain influences the rate at which the rat brain synthesizes catecholamines. This influence is possible because the tyrosine hydroxylase enzyme is not saturated with its amino acid substrate (tyrosine) *in vivo* and becomes less so after short-term fasting or the administration of another neutral amino acid. Our data further demonstrate that the relationship between brain tyrosine concentration and cat-

Table 5. Effect of varying the protein content of the diet on catechol synthesis

Treatment	Brain tyrosine level ($\mu\text{g/g}$)	Brain DOPA level (ng/g)	Serum tyrosine level ($\mu\text{g/ml}$)
Fasted	12.25 $\pm$ 0.45	66 $\pm$ 7	20.69 $\pm$ 1.08
8% casein diet	19.84 $\pm$ 0.81*	123 $\pm$ 11†	23.31 $\pm$ 1.21
18% casein diet	20.75 $\pm$ 1.32*	91 $\pm$ 4‡	30.20 $\pm$ 3.23‡
40% casein diet	24.64 $\pm$ 1.68*	106 $\pm$ 8†	54.66 $\pm$ 6.08*
75% casein diet	21.05 $\pm$ 1.43*	88 $\pm$ 4	48.65 $\pm$ 3.90*

Male Sprague-Dawley rats were fasted overnight and allowed access to one of the test diets at 10 a.m. One hr later, they received an injection of RO4-4602 (800 mg/kg, i.p.). They were decapitated 30 min after injection. Blood was collected from the necks, and whole brains were analyzed for tyrosine [13] and DOPA [14, 15].

* $P < 0.01$ differs from fasted animals.

† $P < 0.001$ differs from fasted animals.

‡ $P < 0.05$ differs from fasted animals.

Table 6. Effect of varying the protein content of the diet on brain phenylalanine concentration

Treatment	Brain phenylalanine level ($\mu\text{g/g}$)
Fasted	13.12 $\pm$ 0.97
Protein-free diet	16.65 $\pm$ 0.51*
8% casein diet	18.41 $\pm$ 0.71†
18% casein diet	18.06 $\pm$ 0.71†
40% casein diet	21.95 $\pm$ 1.31†
75% casein diet	32.08 $\pm$ 0.67†

Male Sprague-Dawley rats were fasted overnight and allowed access to one of the test diets at 10 a.m. One hour later they received an injection of RO4-4602 (800 mg/kg, i.p.). They were killed 30 min later, and whole brains were analyzed for phenylalanine [18].

* $P < 0.01$ differs from fasted animals.

† $P < 0.001$ differs from fasted animals.

echol synthesis (obtained over a wide range of tyrosine concentrations [Fig. 1]) is not due to an effect of other neutral amino acids (besides phenylalanine and, possibly, leucine) on the activity of tyrosine hydroxylase (Tables 2 and 3); moreover, brain tyrosine concentrations probably affect catechol synthesis under normal conditions (i.e. after rats consume nutrients in proportions similar to those they are likely to eat naturally) (Tables 4 and 5).

The broad correlation between brain tyrosine level and catechol synthesis was clearly evident (Fig. 1) in animals acutely treated with PCPA, a drug widely used to inhibit tryptophan hydroxylase activity and serotonin synthesis [16]. Tagliamonte *et al.* [10] have observed decreased conversion of [^3H]tyrosine to [^3H]dopamine and [^3H]norepinephrine in PCPA-treated rats; the level of endogenous tyrosine and the specific activity of [^3H]tyrosine in the brain were also reduced. These authors attributed their findings to an impaired transport of tyrosine from plasma to brain.

Treatments with neutral amino acids, which also inhibit the transport of tyrosine into the brain, caused parallel reductions in brain catechol synthesis [9]. These reductions in synthesis cannot be attributed to changes in the kinetic properties of tyrosine hydroxylase (e.g. direct inhibition), inasmuch as the concentrations of neutral amino acids used to inhibit tyro-

sine transport had no effect on brain tyrosine hydroxylase activity *in vitro* (Table 3).

In choosing a 40 per cent protein meal for our original diet studies as a means for physiologically increasing brain tyrosine level, we made the following two assumptions:

(1) Added protein would cause a greater increase in plasma tyrosine than in other neutral amino acids because the protein contributes tyrosine both directly and indirectly—i.e. as phenylalanine, much of which is transformed to tyrosine in passing through the liver from the portal to the systemic circulation.

(2) Brain tyrosine levels change postprandially as a function of the ratio between plasma tyrosine level and the concentrations of competing neutral amino acids (i.e. just as brain tryptophan had been shown to vary with the plasma tryptophan/competitor ratio [7, 8]).

The latter assumption has been confirmed in studies by Fernstrom and Faller, who showed that for each of the six neutral amino acids tested (tyrosine, tryptophan, phenylalanine, leucine, isoleucine and valine), its brain concentration after food consumption could be predicted by the ratio of its plasma concentration to the sum of the five competitors [21].

In considering factors regulating catecholamine synthesis, investigators have often ignored the effect of substrate (i.e. tyrosine) concentration on the basis that its brain concentration would be well above the K_m , as determined *in vitro*. The problem of knowing what K_m value to compare *in vivo* brain tyrosine levels to is complicated by the fact that at least three different methods are used to assay tyrosine hydroxylase [2, 11], and that different investigators utilize different cofactors for these assays (e.g. the presumed natural cofactor, tetrahydrobiopterin, or the artificial cofactors, 6-methyltetrahydropteridine [6-DMPH₄] and 6,7-dimethyltetrahydropteridine [DMPH₄]). The apparent K_m values obtained for the substrates (tyrosine and oxygen) also vary depending upon the pterin used; for example, the tyrosine K_m for adrenomedullary tyrosine hydroxylase can vary by a factor of 10 depending on which cofactor is chosen [22]. Differences in the media used for homogenization and in the buffer systems employed in the assay mixture can also cause considerable differences in tyrosine hydroxylase activity, and in its affinity for its cofactors

and substrate [23]. Thus, from the presently available, often conflicting literature, it is very difficult to draw simple conclusions regarding the degree to which tyrosine hydroxylase is saturated *in vivo*.

Measurement of the *in vivo* tyrosine K_m by a method similar to that used by Carlsson for tryptophan and tryptophan hydroxylase [6] might provide a better index of the affinity of tyrosine hydroxylase for its substrate *in vivo* (even though interpretation of such data is complicated by the possibility that the tyrosine concentration in homogenates of whole brain is not equal to its concentration at the locus of tyrosine hydroxylase). Using this method [20], we calculated a K_m for whole brain. This value was approximately equal to the whole brain tyrosine concentration. Therefore, even in the normally fed rat, the enzyme is only about half saturated. Fasting or the administration of a neutral amino acid further reduces the saturation of the enzyme and, correspondingly, the catechol synthesis rate.

It is well established that the amount and the activity of tyrosine hydroxylase in a given tissue can control the rate at which that tissue converts tyrosine to DOPA. Thus, treatments that accelerate catecholamine release from adrenal medulla or nerve terminals (by increasing presynaptic inputs) rapidly activate existing tyrosine hydroxylase—either by decreasing the end-product inhibition of the enzyme [24] or by allosterically changing its affinity for its substrate and cofactor [25]. When prolonged, such treatments can also increase the amount of tyrosine hydroxylase enzyme protein in the tissue, probably by accelerating its synthesis [26, 27]. As demonstrated in this study, the substrate control of tyrosine hydroxylation probably serves a physiological function very different from the enzyme responses to enhanced neuronal activity: it allows neurotransmitter synthesis to be modulated by the plasma amino acid pattern, which in turn varies with food consumption, the actions of various hormones, and general metabolic state. Of course, it remains to be determined whether the changes in neuronal catecholamine synthesis (induced by modifying precursor levels or by other means) actually influence the quantities of neurotransmitter which are released into synapses and which act on postsynaptic receptors.

Acknowledgements—We gratefully acknowledge the excellent technical assistance of F. Larin and D. Taam. We also thank M. C. Scally and Dr. I. Ulus for help with the tyrosine hydroxylase assay and Dr. William Rand for help with the statistical calculations. These studies were supported in part by grants from the United States Public Health Service (AM-14228; AM-11237), the John A. Hartford Foundation, and the National Aeronautics and Space Administration (NGR-22-009-627). Ms. Gibson holds a

studentship from the Medical Research Council of Canada (100-11G-115).

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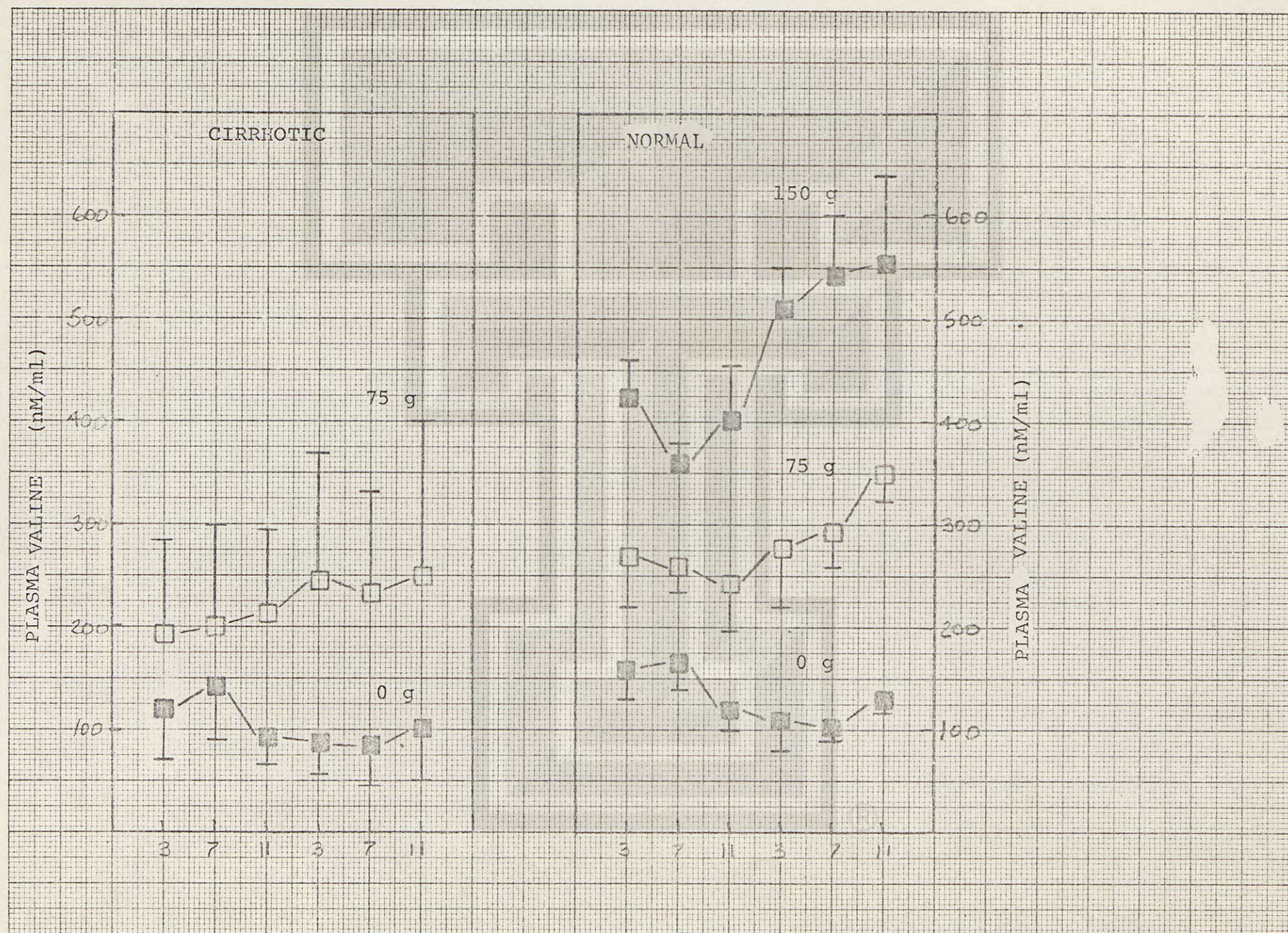
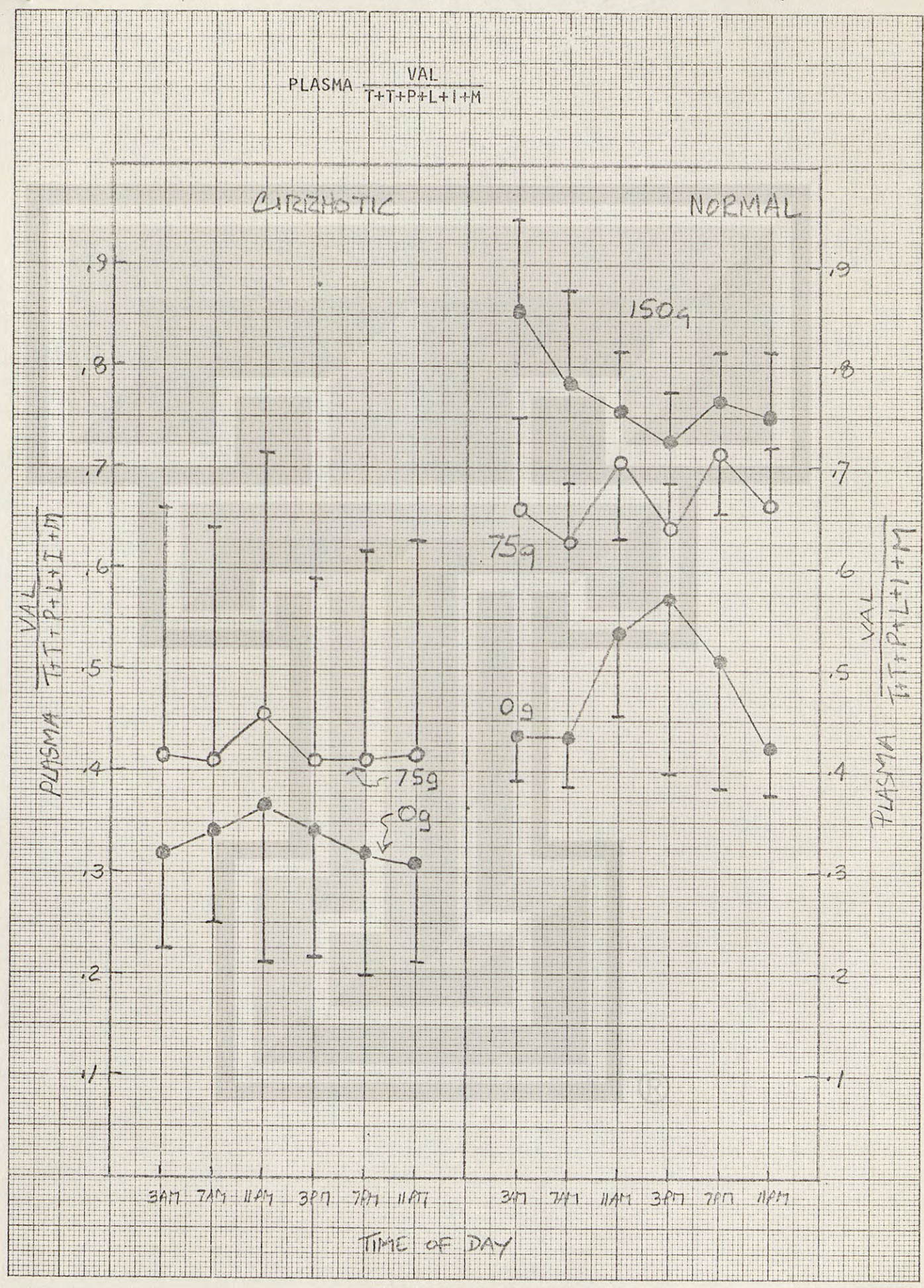


Fig. (1)

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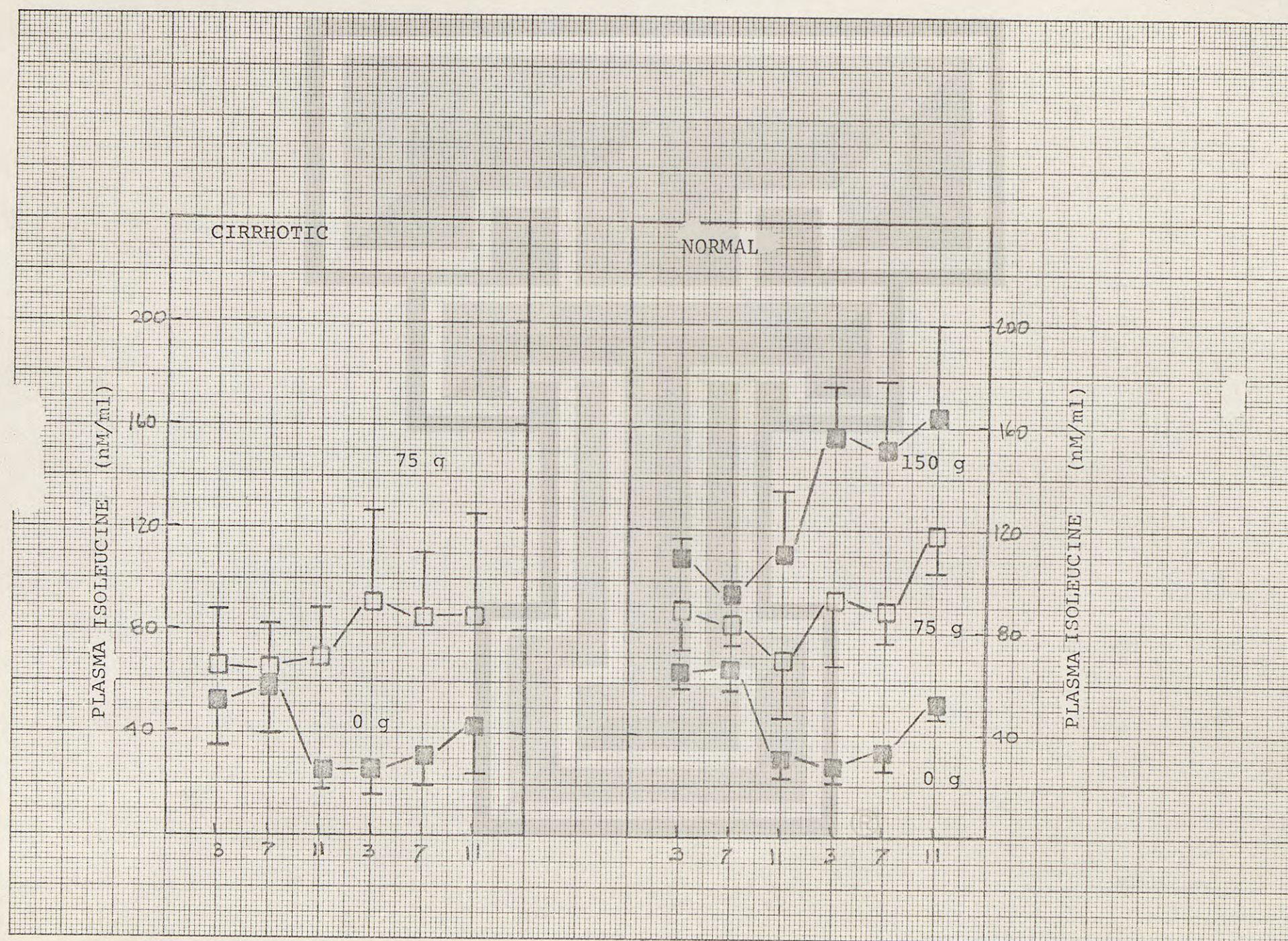
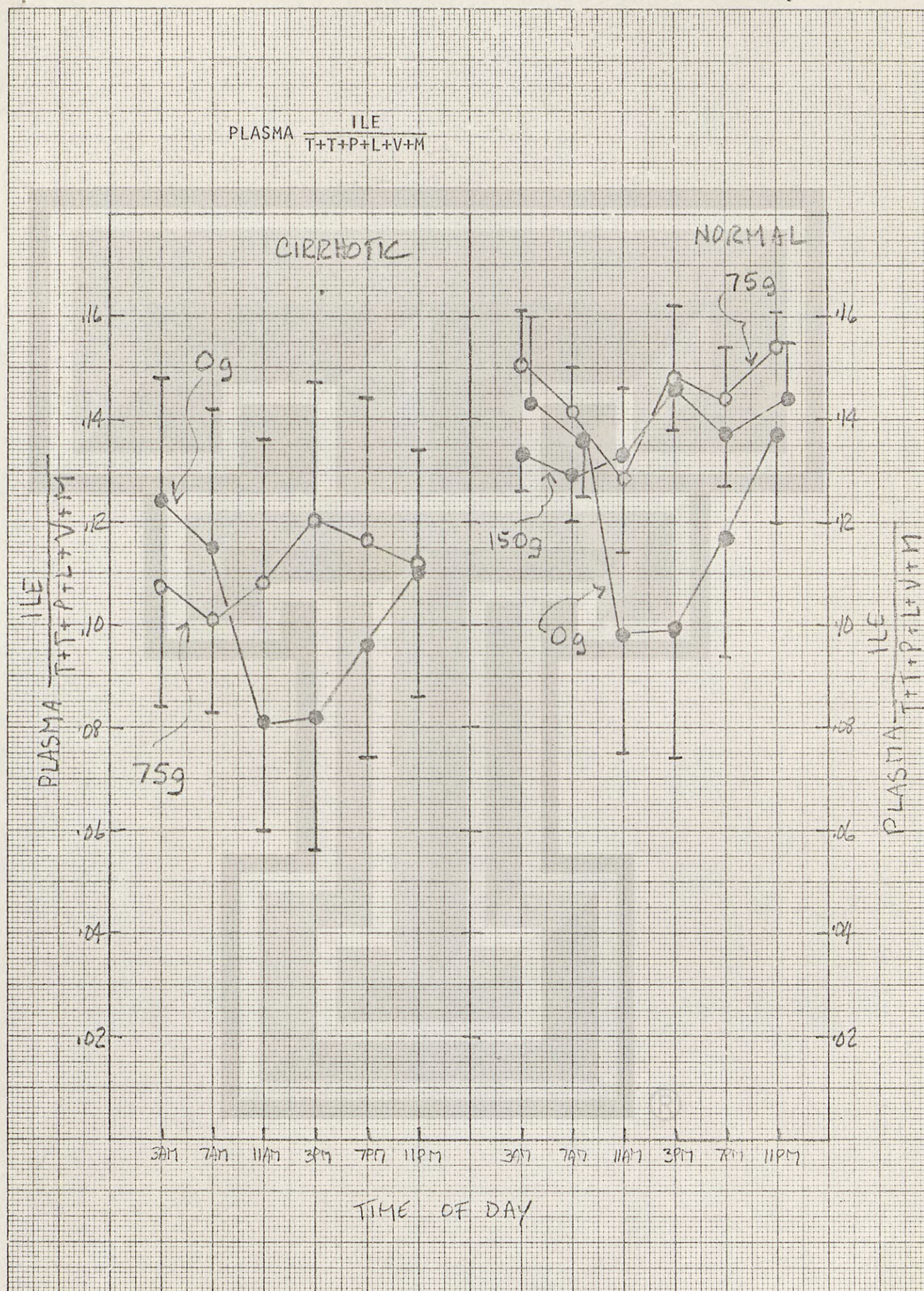


Fig. (2)



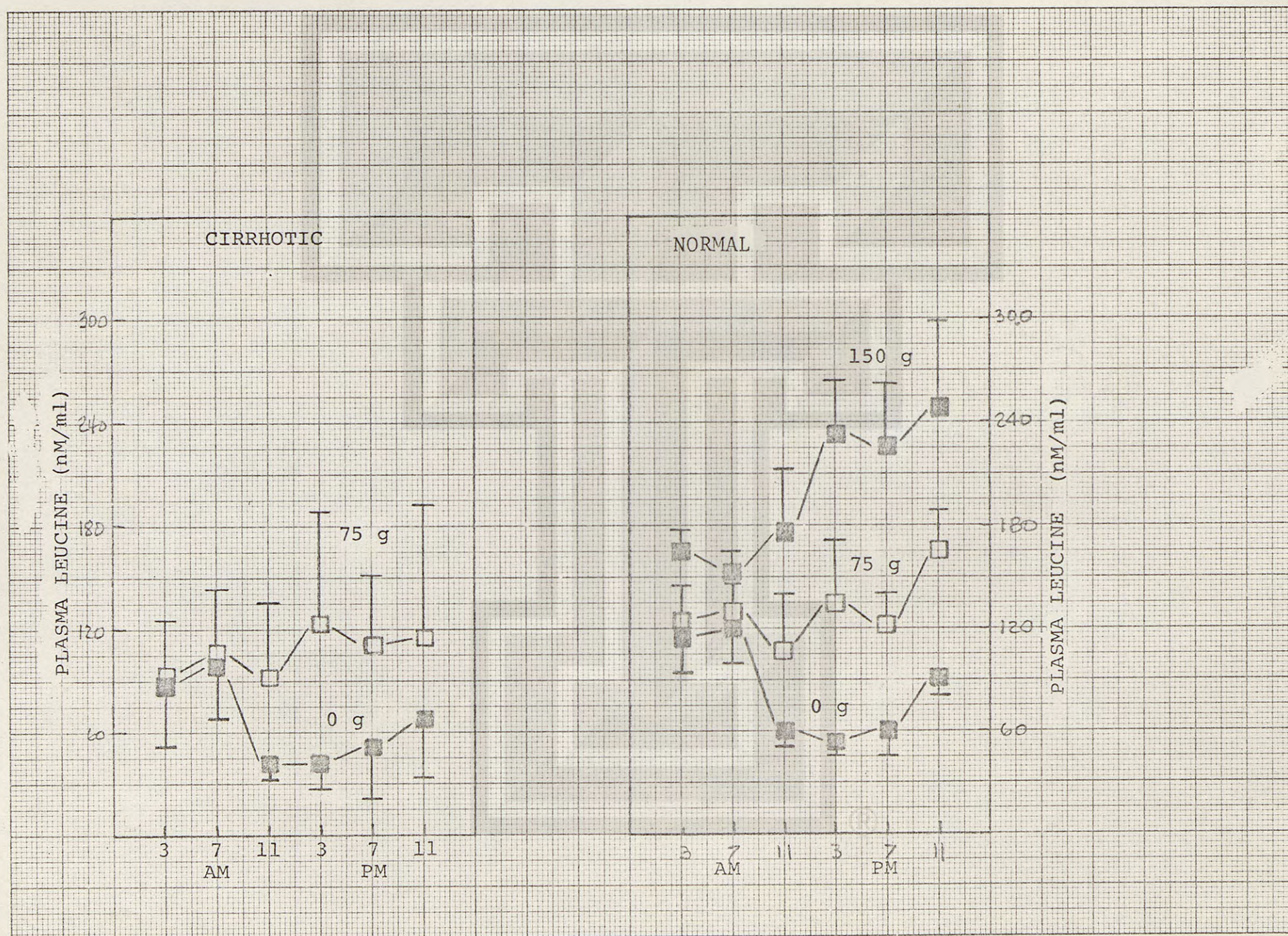
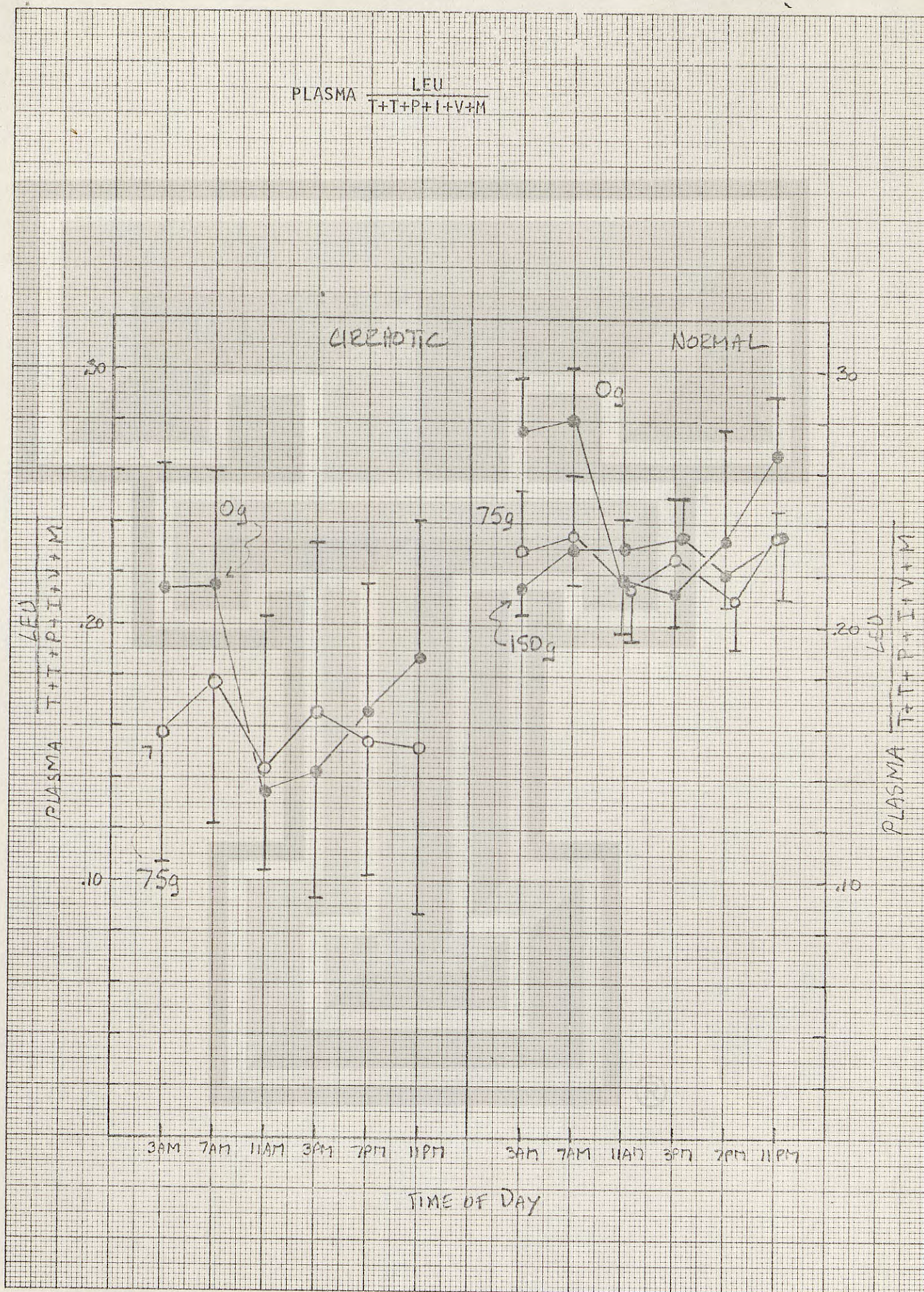


Fig. (3)



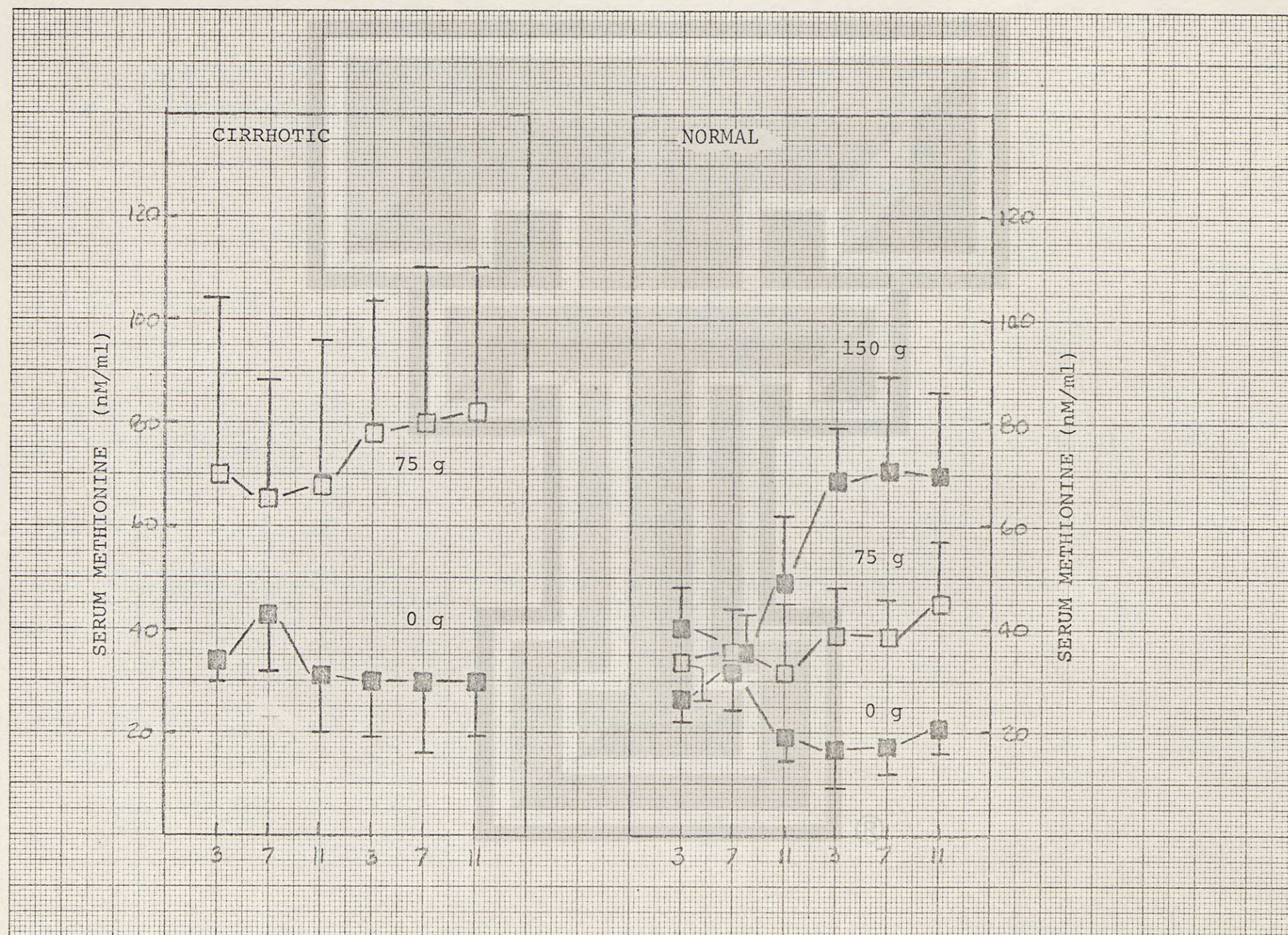
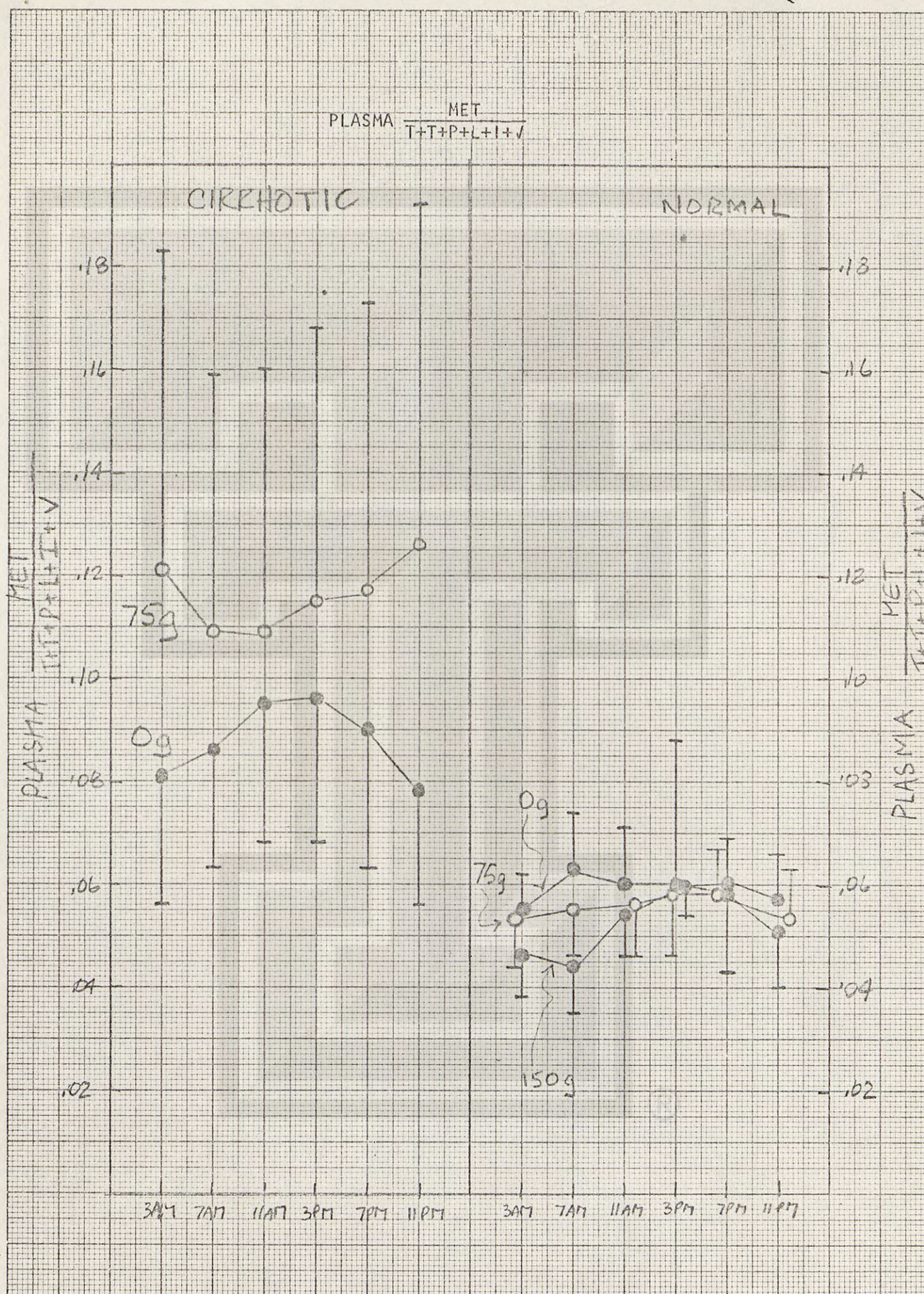


Fig (4)



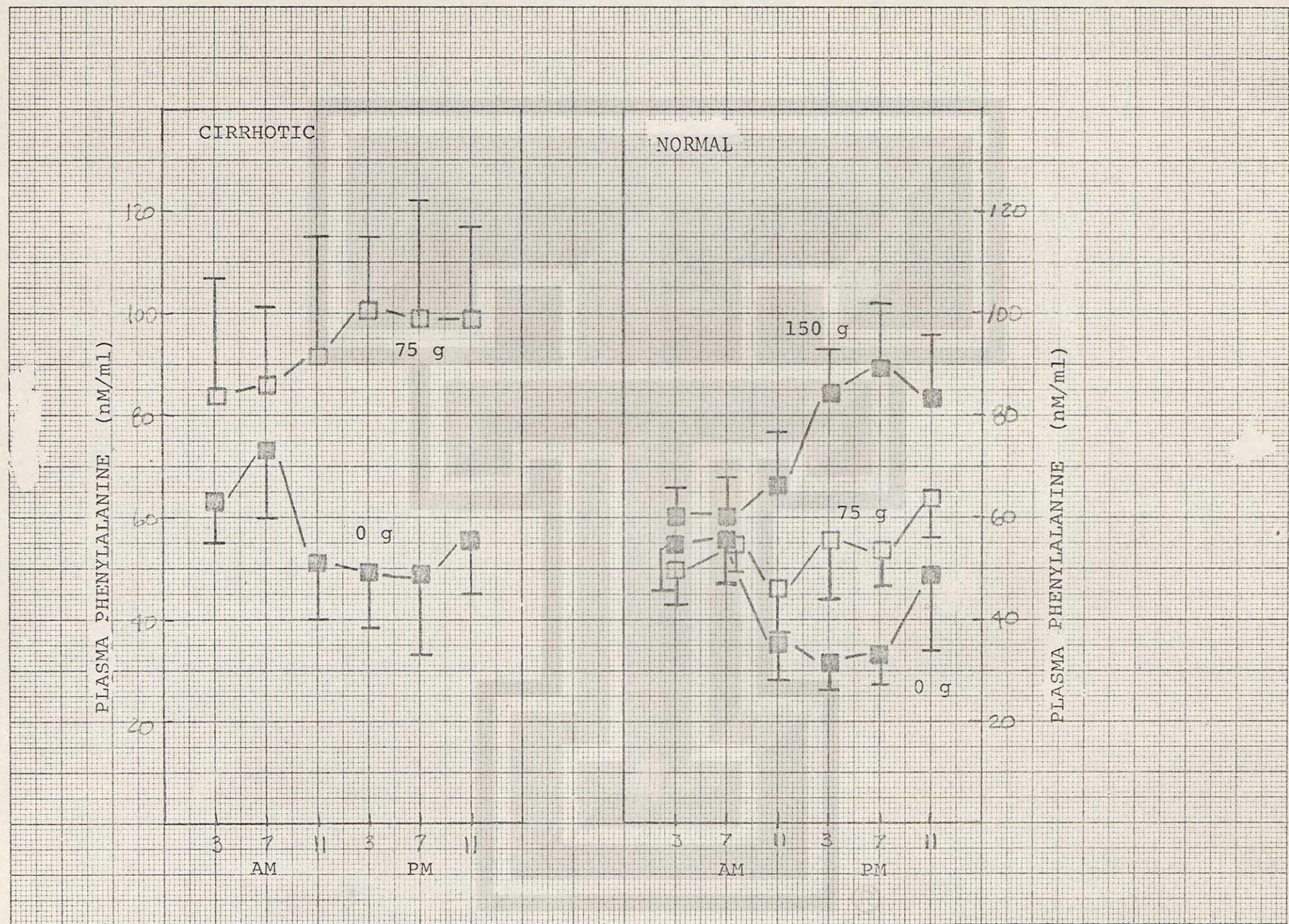
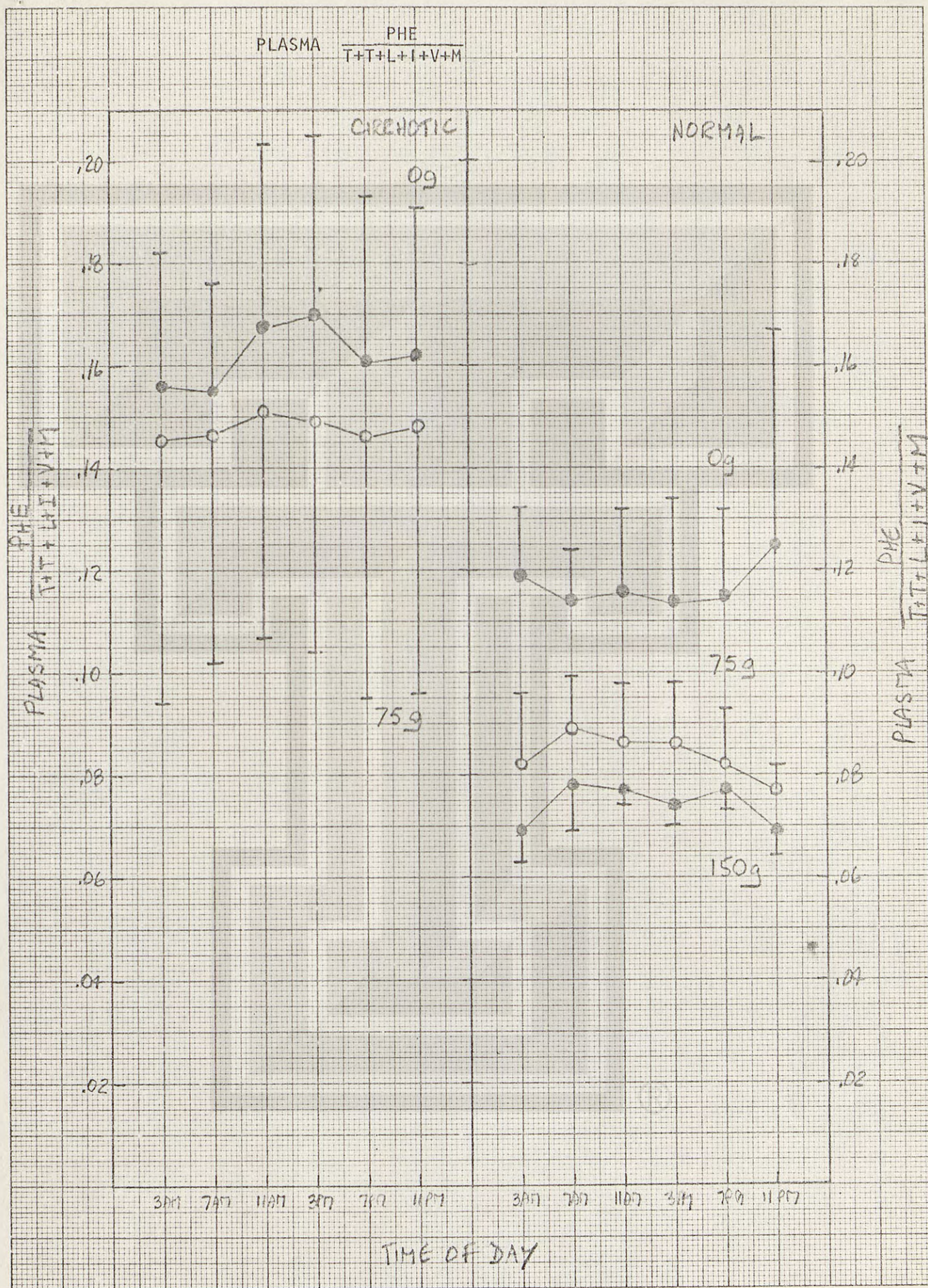


Fig. (5)



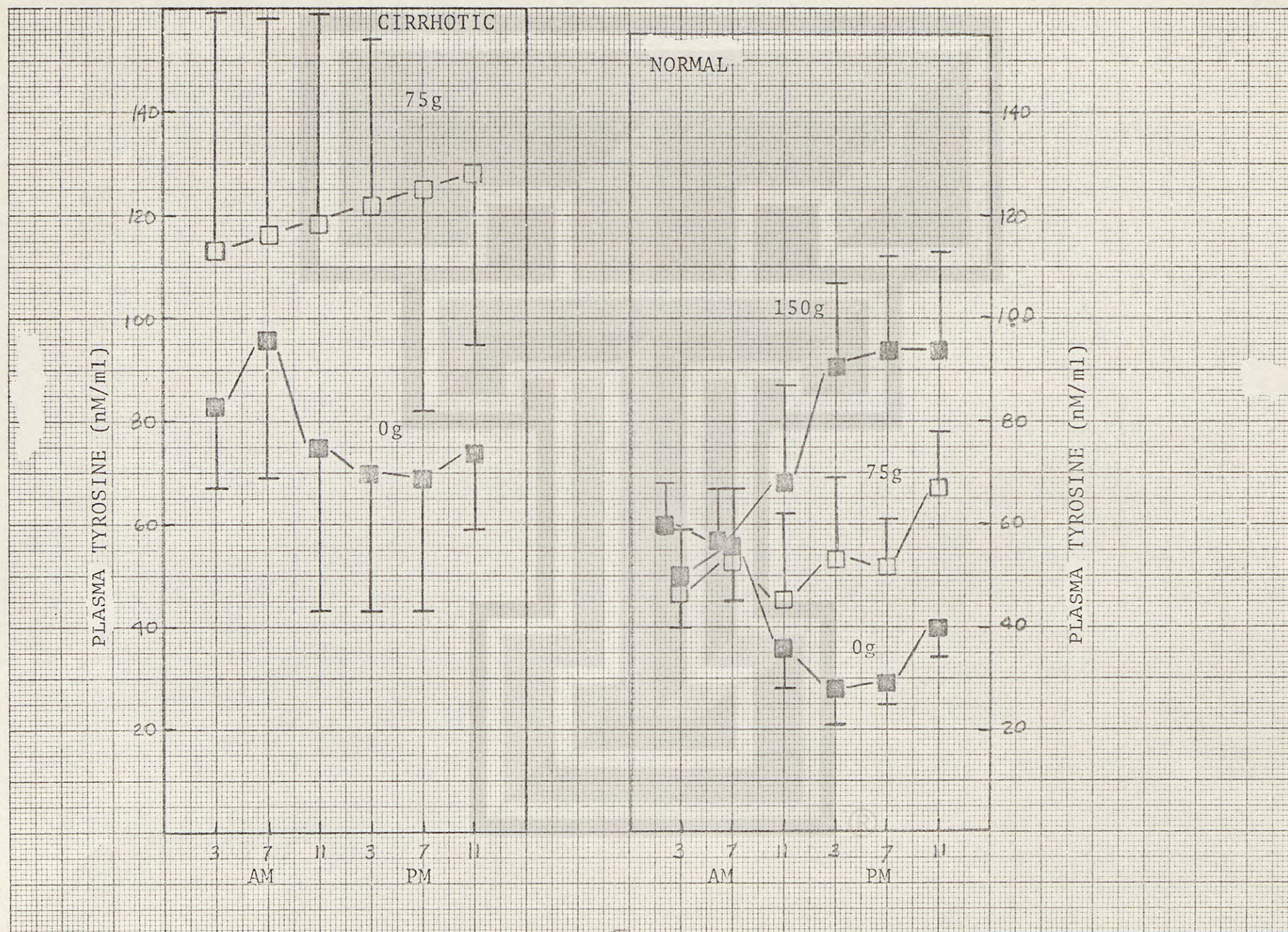
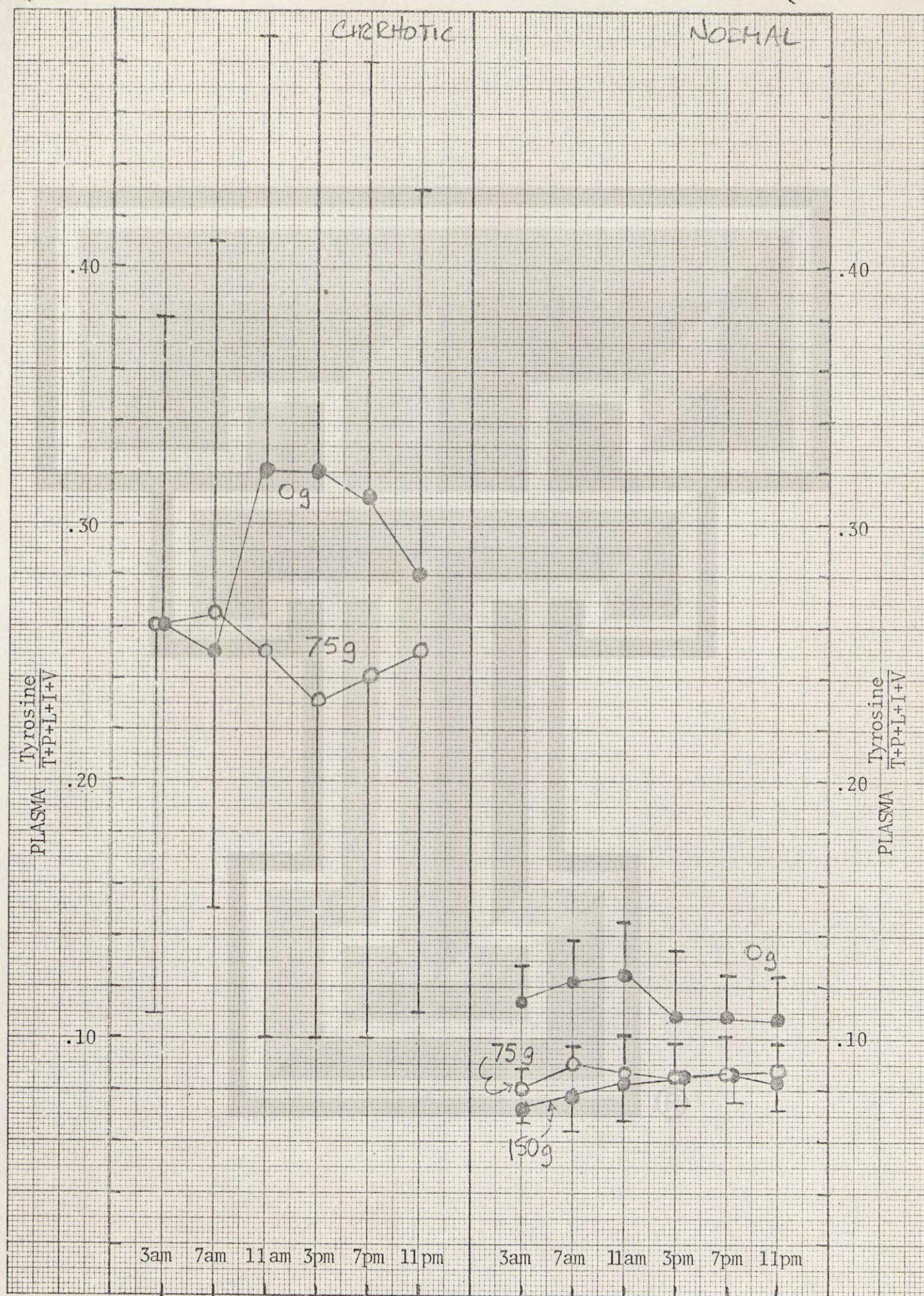
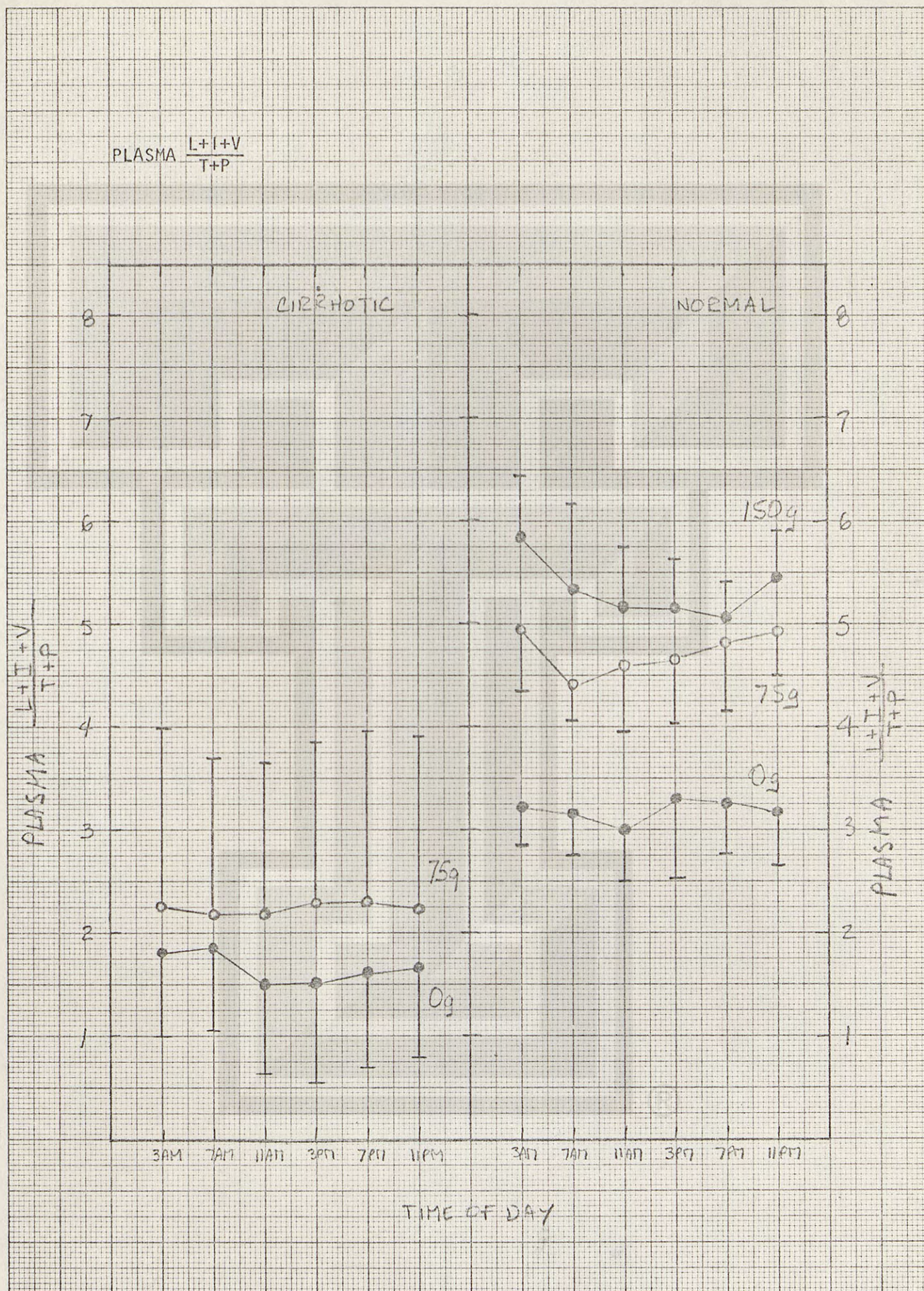


Fig. (6)





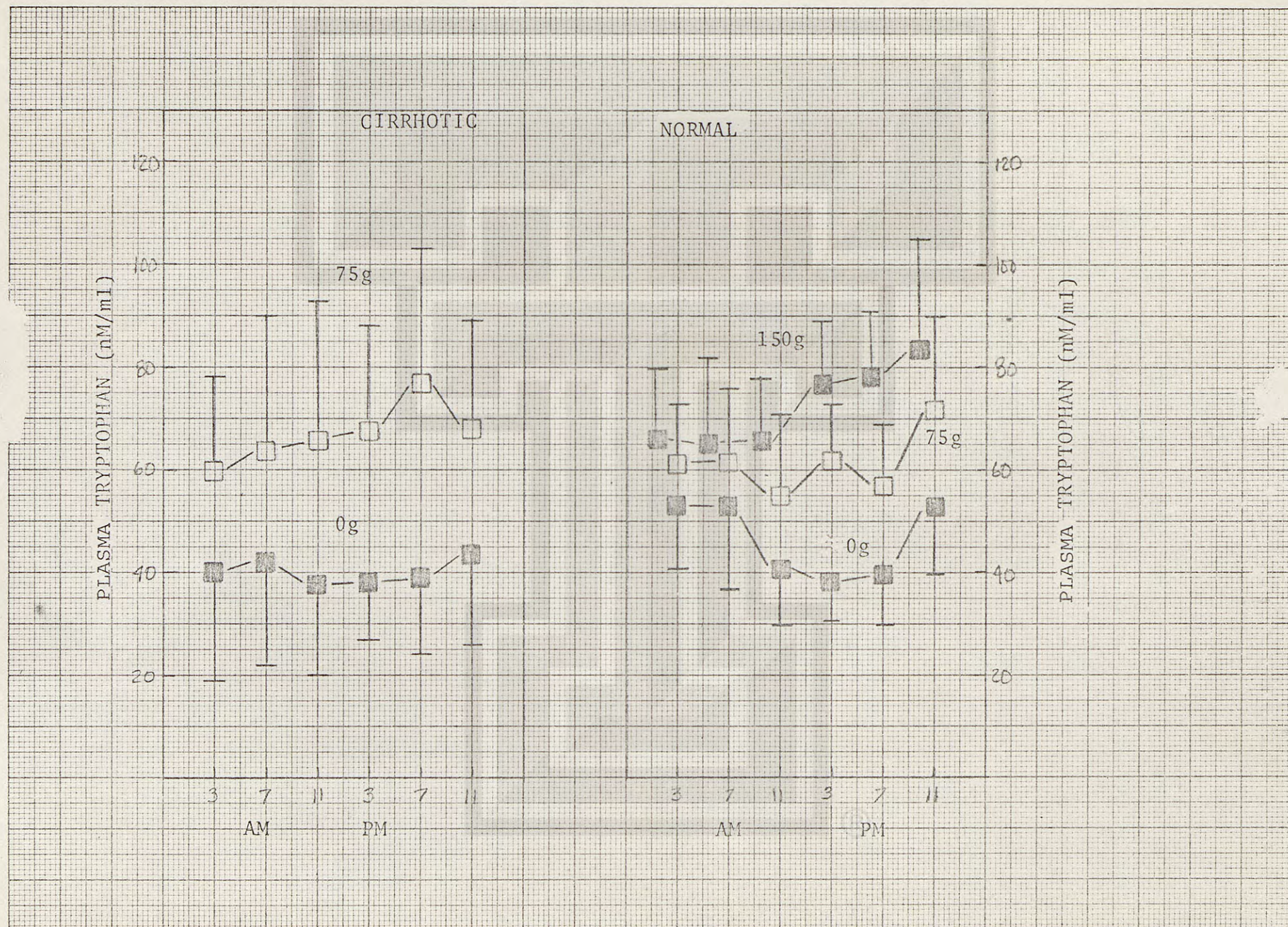


Fig. (7)

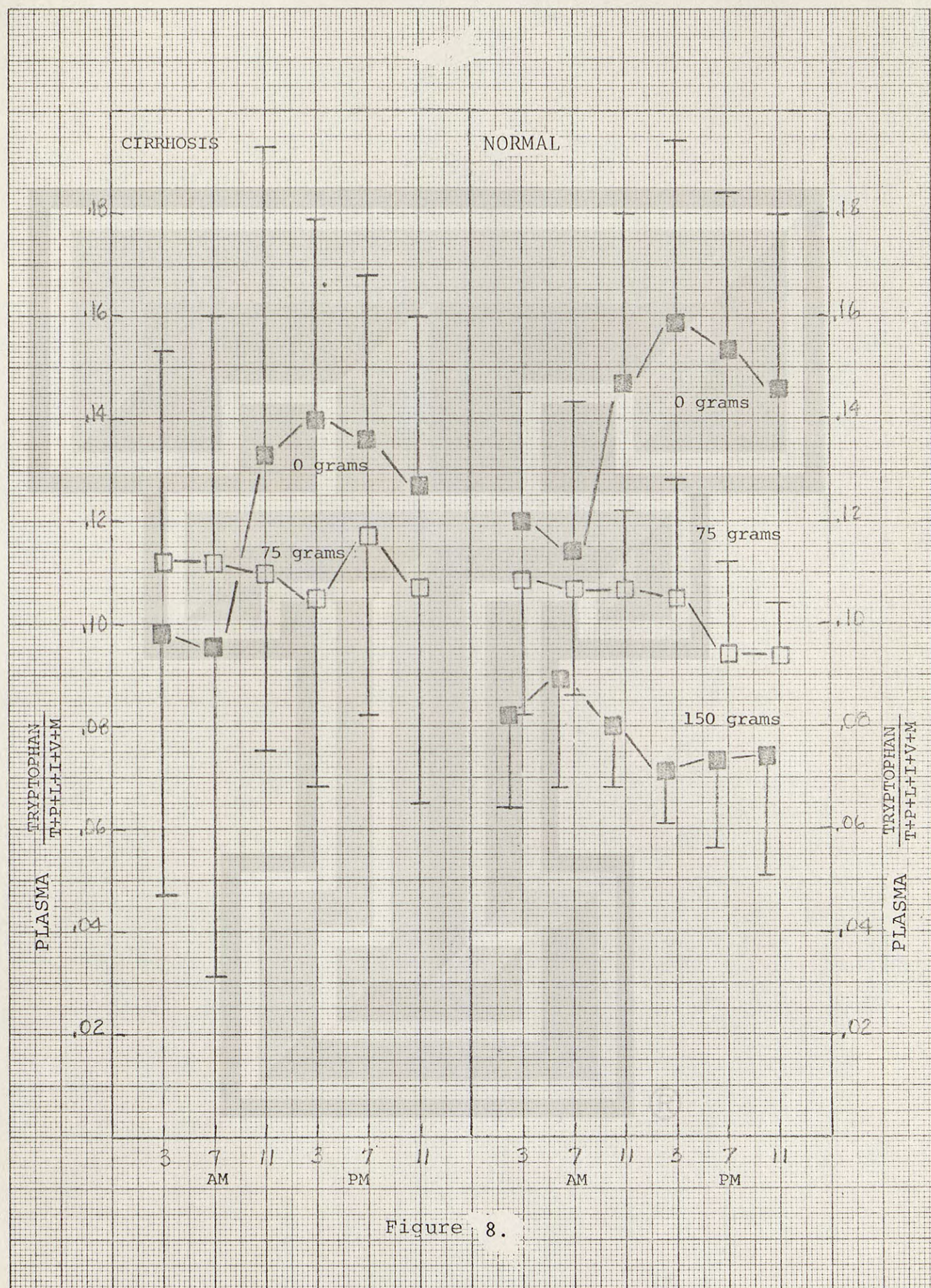
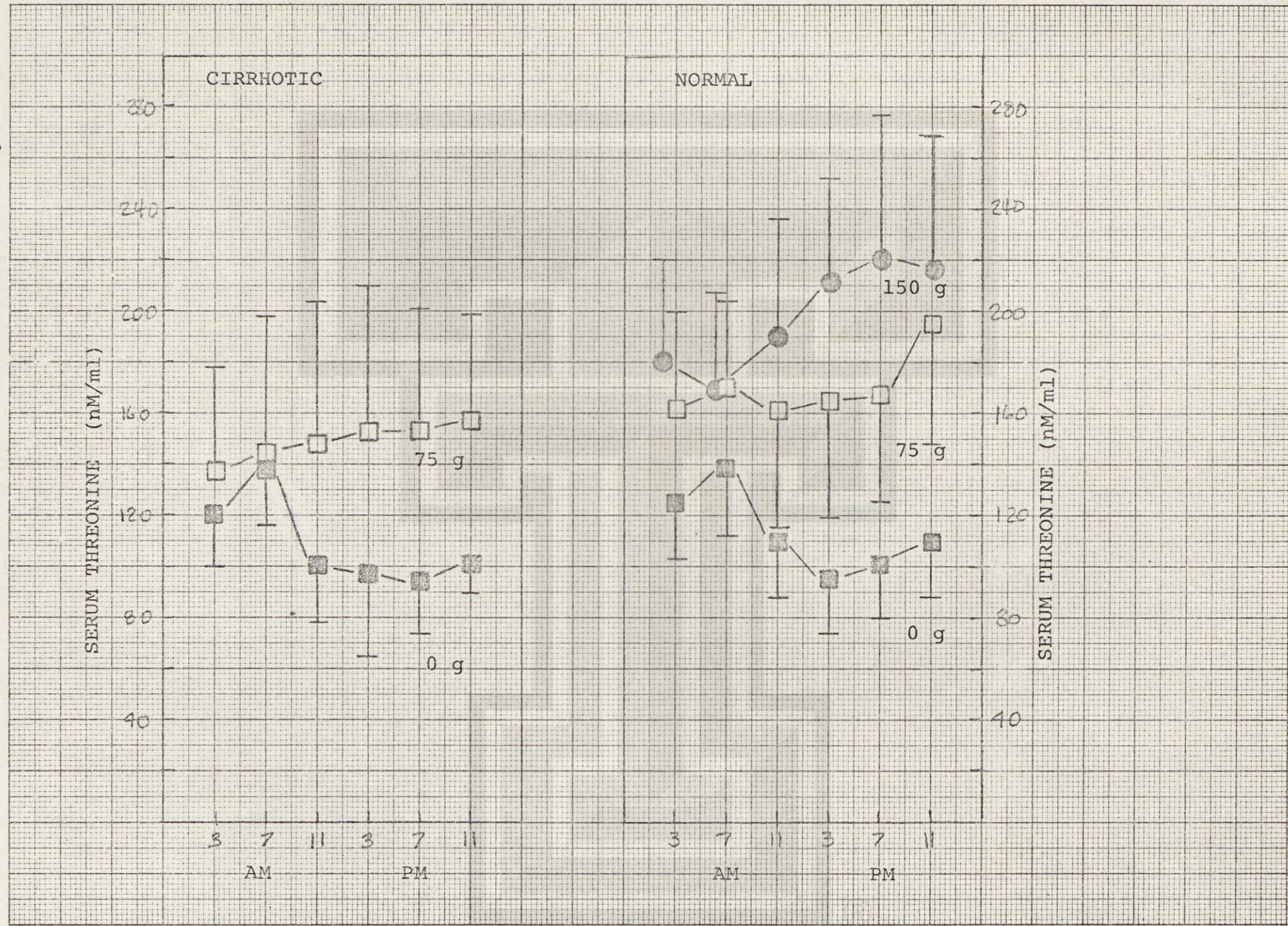
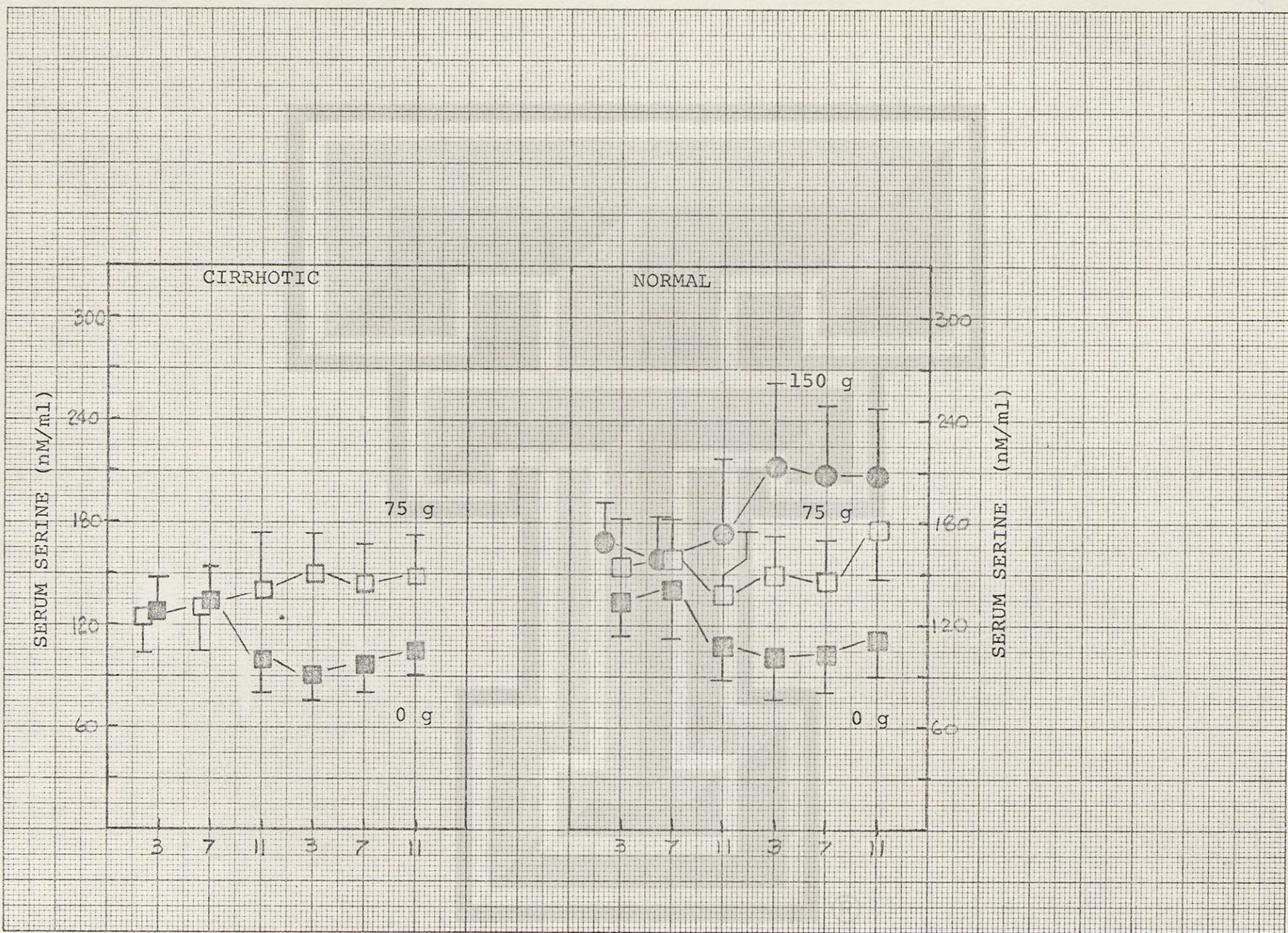
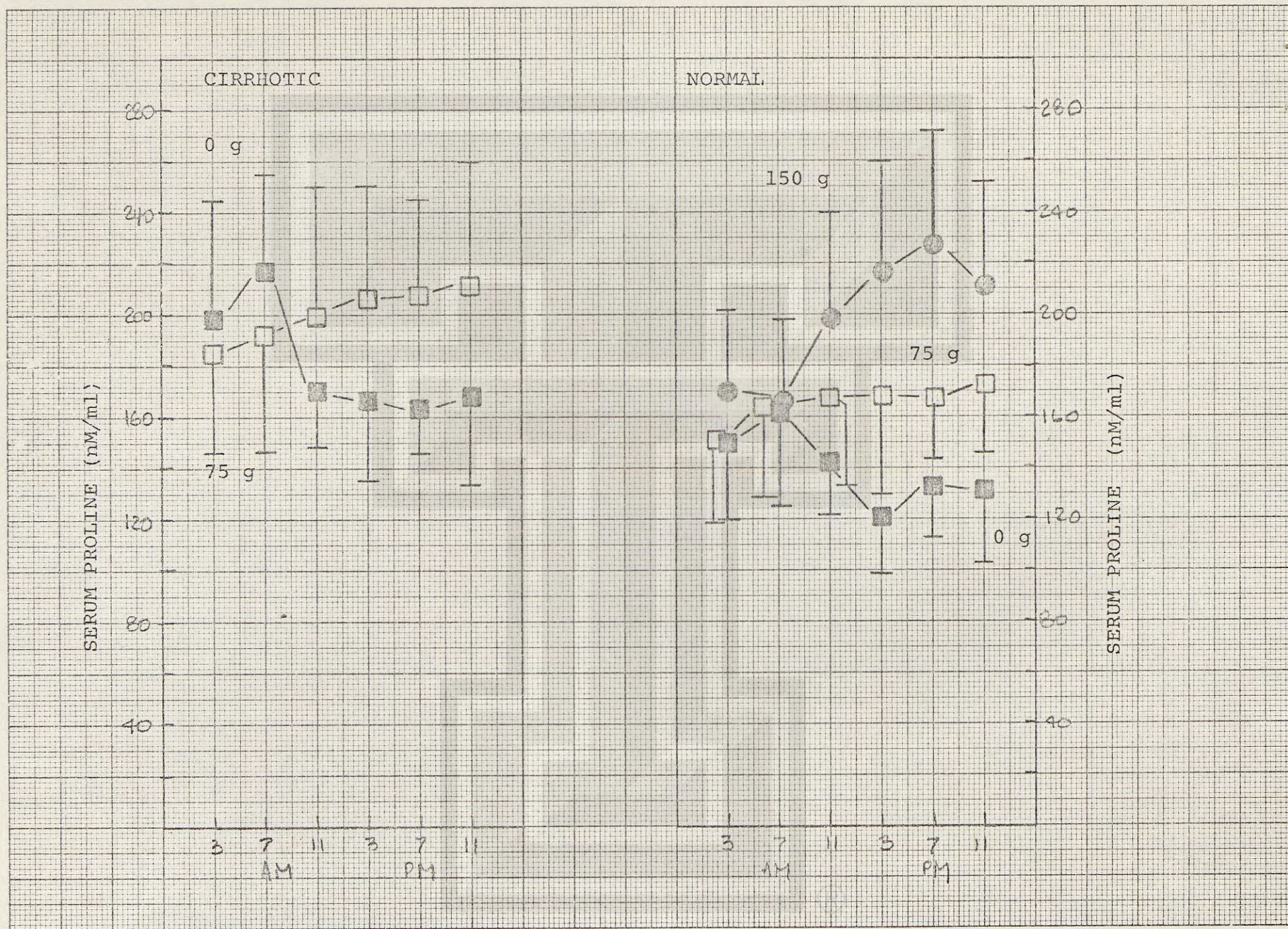
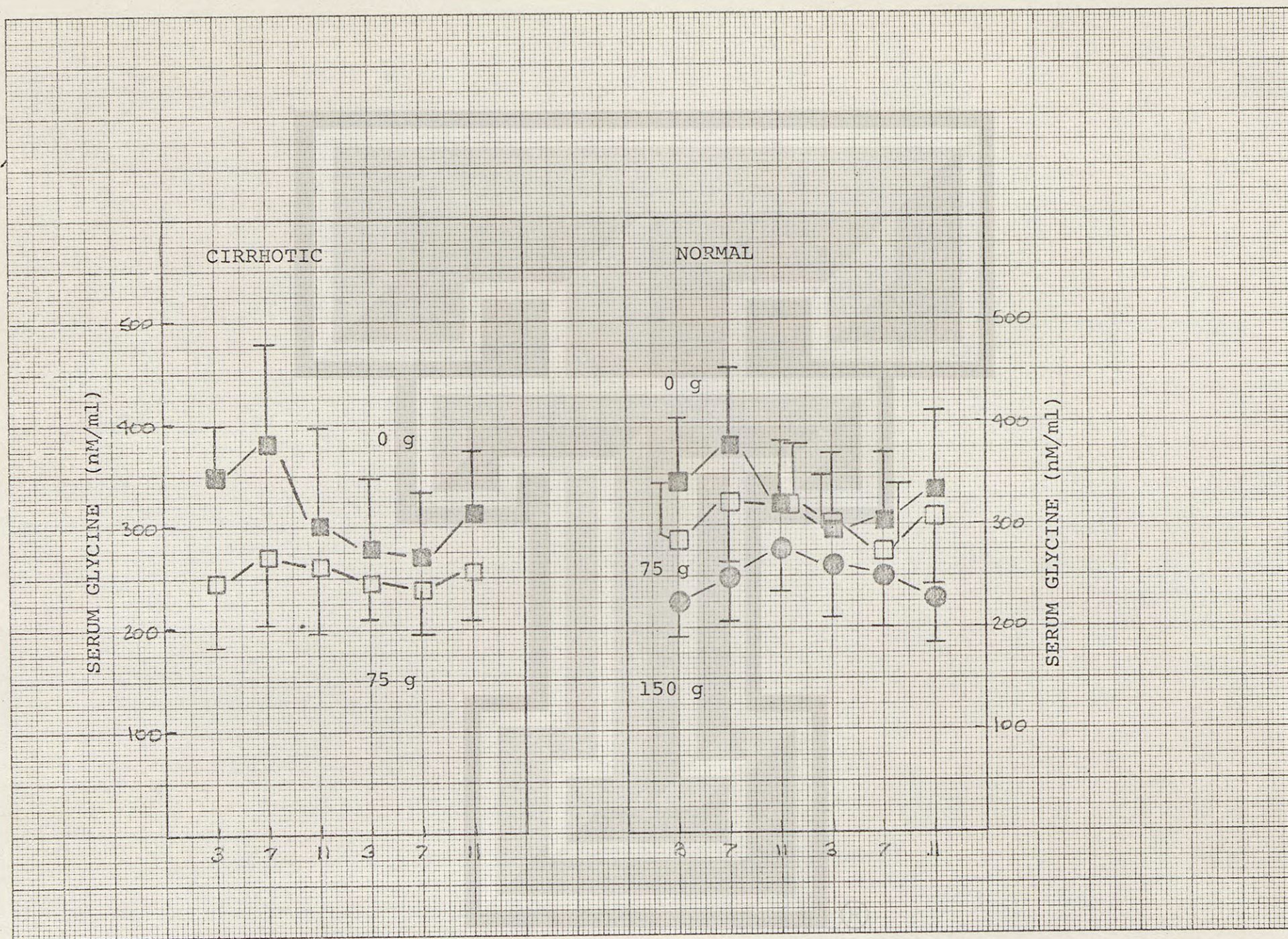


Figure 8.









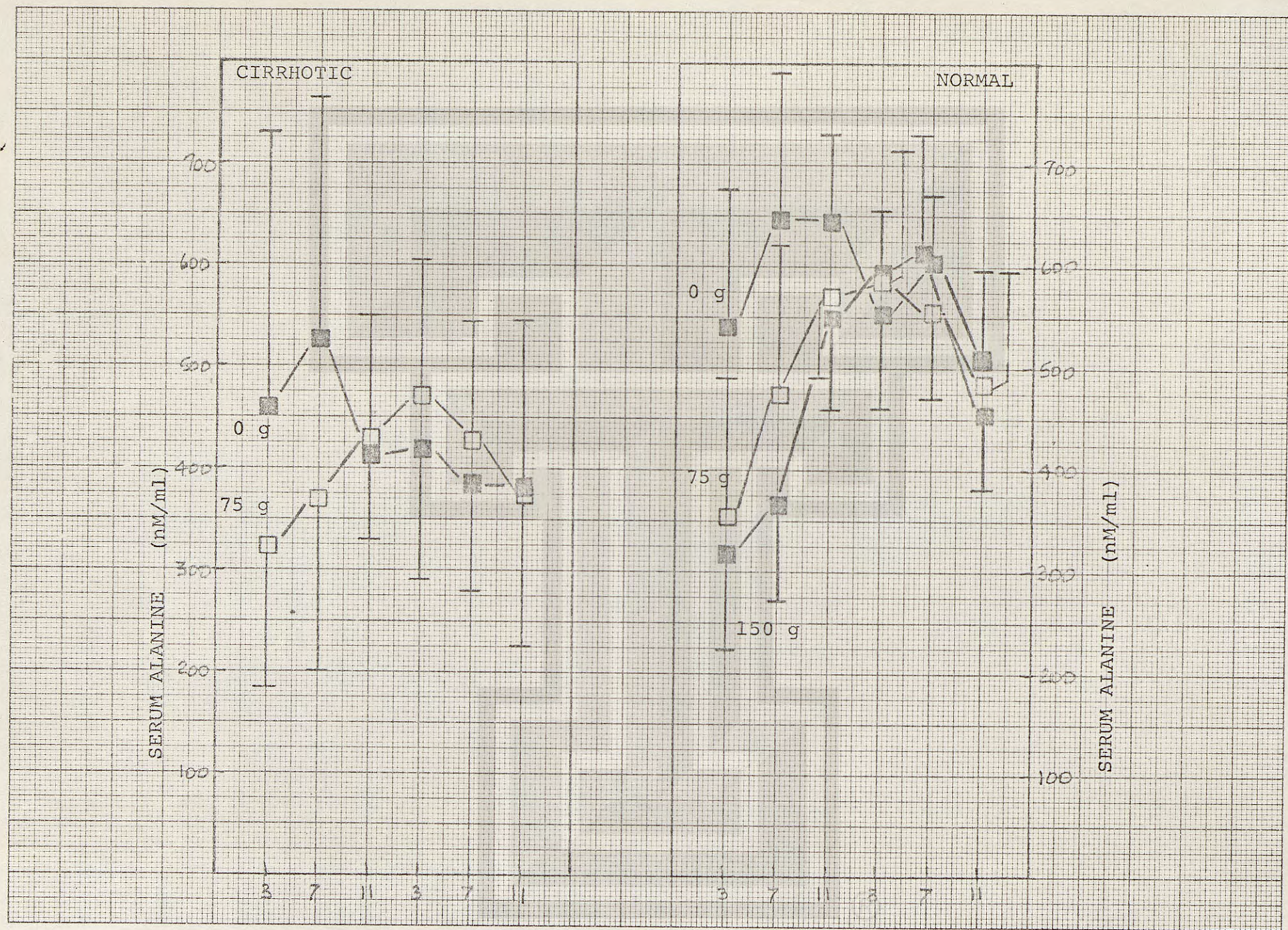


TABLE 2 -- EFFECTS OF A SINGLE CHOLINE CHLORIDE OR LECITHIN CONTAINING MEAL ON SERUM
LEVELS OF GLUCOSE, INSULIN, CORTISOL, AND PROLACTIN IN NORMAL SUBJECTS

	HOURS AFTER MEAL INGESTION					
	0	1/2	1	4	8	12
GLUCOSE (mg %)						
Control	87 $\pm$ 10	84 $\pm$ 25	96 $\pm$ 17	97 $\pm$ 09	89 $\pm$ 18	----
Choline	70 $\pm$ 21	115 $\pm$ 45	102 $\pm$ 38	85 $\pm$ 11	90 $\pm$ 14	88 $\pm$ 09
Lecithin	83 $\pm$ 18	110 $\pm$ 27	92 $\pm$ 34	91 $\pm$ 19	88 $\pm$ 17	85 $\pm$ 18
INSULIN (uU/ml)						
Control	18.1 $\pm$ 4.7	48.8 $\pm$ 17.0	33.8 $\pm$ 17.0	17.5 $\pm$ 5.0	17.3 $\pm$ 4.0	----
Choline	10.4 $\pm$ 1.3	53.7 $\pm$ 29.3	45.0 $\pm$ 16.4	14.6 $\pm$ 6.3	12.7 $\pm$ 4.4	11.0 $\pm$ 2.5
CORTISOL (ug %)						
Control	14.5 $\pm$ 3.7	12.2 $\pm$ 3.0	11.1 $\pm$ 2.7	9.7 $\pm$ 5.0	11.0 $\pm$ 7.1	----
Choline	14.0 $\pm$ 2.5	15.1 $\pm$ 3.7	11.8 $\pm$ 4.7	8.4 $\pm$ 5.7	6.0 $\pm$ 2.3	----
Lecithin	16.1 $\pm$ 3.6	15.8 $\pm$ 4.8	10.6 $\pm$ 4.0	8.9 $\pm$ 4.9	6.8 $\pm$ 1.2	----
PROLACTIN (ng/ml)						
Choline	10.1 $\pm$ 2.0	8.4 $\pm$ 2.0	8.6 $\pm$ 2.2	7.7 $\pm$ 1.4	7.8 $\pm$ 1.5	9.0 $\pm$ 2.1
Lecithin	9.2 $\pm$ 1.8	8.4 $\pm$ 2.3	8.4 $\pm$ 1.8	7.5 $\pm$ 1.3	8.4 $\pm$ 2.1	9.2 $\pm$ 2.5

Data are expressed as the mean value $\pm$ standard deviation of the mean.

Level, Skatue

PERFORMANCE ANALYSIS

I. Introduction

This report contains the results of a five day examination of problems relative to performance. The goal of the effort was to develop an approach for providing criteria for determining an index of performance for man-machine systems in an operational situation. Although the contents of this report are quite general, it is believed that an approach has been established for determining an index of performance, and a program for carrying out this approach has been outlined. The program suggested considers an overall analytical and experimental program for establishing fundamental requirements, an experimental flight test program for utilization of the Biological Research and Medical Operations aircraft, and a flight test program which can be applied on a noninterference basis to the Test Pilot School syllabus.

II. Objectives

The objectives for this effort were established as--optimum performance of man-machine systems in conducting an overall mission. To achieve these objectives a series of tasks were defined.

III. Tasks

Task I - Define Performance

Task II - Define Performance of a Man-machine System

Task III - Define Performance of Machine

Task IV - Define Performance of Man

Task V - Define Performance Index

Task VI - Define Responsibility

Task VII - Define Mission

Task VIII - Define Performance Measurement

IV. Technical Discussion

Task I - Performance can be defined as functional effectiveness, or the relationship of Output, Input, and Deterioration.

$$P = f(O), f(I), f(D)$$

Optimum performance can be defined as a condition when minimum input functions result in maximum output functions, with a minimum of deterioration.

$$P_{\max} = f(O_{\max}), f(I_{\min}), f(D_{\min})$$

Functions as used here are defined as actions necessary to satisfy the objectives.

Input functions are defined as the processes for deriving information and energy from the environment necessary for performing a task.

Output functions are the processes for imparting energy to the environment in order to accomplish a task.

Corrected { Deterioration is the cost in energy to the system due to its inefficiency or its inability to meet the demands of a task.

Since we cannot achieve absolute perfection in any endeavor, it can be axiomatically stated that the outputs of any system will always be less than the inputs. It can further be stated that there will always be some amount of deterioration to the system because of increased entropy.

From the above, an expression for performance can be written as:

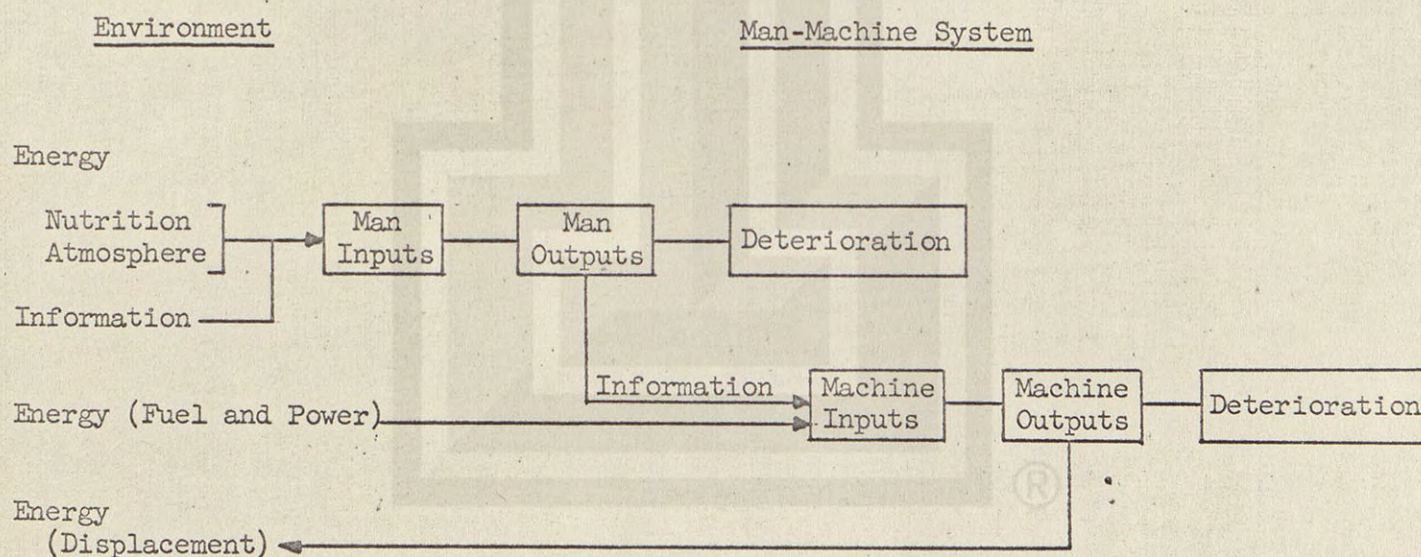
$$\text{Performance} = \text{Input} - \text{Output} - \text{Deterioration}$$

When performance is considered in terms of energy, deterioration can be defined as the cost in energy to restore the system to its initial state, since deterioration within the system necessitates maintenance and repair. In this instance the expression would be written as:

$$\text{Performance} = \text{Input Energy} - \text{Output Energy} + \text{Restoration Energy}$$

Task II - Performance of a man-machine system is the sum of the performance of the man and the performance of the machine as shown in figure 1.

Figure 1



Thus performance for the system can be written as:

$$\text{Performance}_{\text{man-machine system}} = (\text{Inputs}_{\text{man}} - \text{Outputs}_{\text{man}} - \text{Deterioration}_{\text{man}}) \\ + (\text{Inputs}_{\text{machine}} - \text{Output}_{\text{machine}} - \text{Deterioration}_{\text{machine}})$$

Task III - From the above then, performance of the machine in terms of energy can be written as:

$$\text{Performance}_{\text{machine}} = \text{Input Energy}_{\text{machine}} - \text{Output Energy}_{\text{machine}} \\ + \text{Energy to restore}_{\text{machine}}$$

Task IV - Performance of the man is:

$$\text{Performance}_{\text{man}} = \text{Input Energy}_{\text{man}} - \text{Output Energy}_{\text{man}} + \text{Energy to restore}_{\text{man}}$$

Task V - In order to measure performance it is necessary to establish an index of performance which can be defined as the ratio between required inputs, required outputs, and allowable deterioration, and actual inputs, actual outputs, and actual deterioration.

$$\text{Performance Index} = \frac{\text{Inputs Required} - \text{Outputs Required} - \text{Allowable Deterioration}}{\text{Actual Inputs} - \text{Actual Outputs} - \text{Actual Deterioration}}$$

In terms of measurable energy -

$$\text{Performance Index} = \frac{\text{Required Energy Input} - \text{Required Energy Output} + \text{Required Restoration Energy}}{\text{Actual Energy Input} - \text{Actual Energy Output} + \text{Actual Restoration Energy}}$$

Task VI - Recent tests have shown variations of certain physiological parameters relative to the nature of the task being performed. ~~for task~~ considered. It is believed that these parameters varied as a result of increasing and decreasing the responsibilities of the subjects. An expression for Responsibility in terms of Performance can be derived

as follows:

$$\text{Responsibility} = \frac{\text{Assigned Duties}}{\text{Capability}}$$

Capability can be considered as Performance Index relative to time, or:

$$\text{Capability} = \text{Performance Index} \times \text{Time}$$

$$\therefore \text{Responsibility} = \frac{\text{Assigned Duties}}{\text{Performance Index} \times \text{Time}}$$

When the assigned duties are increased due to additional tasks being necessary because of malfunctions of the system or systems within a mission, there is an increase in responsibility of the man-machine system. If the performance index is reduced due to an increase in required output necessitating greater inputs, or an increase in deterioration, the responsibility is further increased, i.e.:

$$\text{System Responsibility} = \frac{\text{Assigned Duties} + \text{Changes}}{\text{Performance Index} \times \text{Time}} = \text{Strain if } > 1$$

(If > 1 - Stress producing)
(If ≤ 1 - Stress producing)

As the assigned duties become greater than 1 or if the Performance Index becomes less than 1, a stress producing condition would be generated with an increase in responsibility as well as strain. In terms of energy,

System Responsibility = $\frac{\text{System Req. Input Energy} - \text{System Req. Output Energy} + \text{Allowable System Restoration Energy} + \text{Changes}}{\text{Time}}$

+ Time $\frac{\text{Man Req. Input Energy} - \text{Man Req. Output Energy} + \text{Man Allowable Restoration Energy}}{\text{Mans Actual Input Energy} - \text{Mans Actual Output Energy} + \text{Mans Actual Restoration Energy}}$

+ Time $\frac{\text{Mach Req. Input Energy} - \text{Mach Req. Output Energy} + \text{Mach Allowable Restoration Energy}}{\text{Mach Actual Input Energy} - \text{Mach Actual Output Energy} + \text{Mach Actual Restoration Energy}}$

Thus it can be seen that in the physiological experiments previously referred to, when the assigned duties were increased making the numerator of the expression greater than 1, there was an increase in man's required output forcing an increase in man's actual output causing the performance index to become less than 1 resulting in strain on the man which was manifested by an increase in the physiological parameter being investigated. Specifically the change in the task examined required more information into the man and more work out. Although these conclusions are self evident, the use of the expression for Performance Index provides a means for getting closer to the cause of stress producing conditions, and an approach to the problem of performance measurement. For example it seems reasonable that measurement of variations of the energy inputs and outputs of man relative to his actual responsibilities would be indicative of his actual performance.

Task VII - In order to determine means for measuring performance it is first necessary to examine the nature of the tasks to be conducted. Since the present efforts of the Office of Biological Research and Medical Operations are directed toward aircraft performance, flight missions were selected for examination.

Although there are a great variety of aircraft missions, they can all be generally classified as consisting of part or all of the following phases:

Takeoff, rendezvous, navigation, strike (or the action required to meet the mission objective), rejoin (which is the same as rendezvous), return (which is the same as navigation), and landing.

With the exception of total time and rates of change, each of the phases are identical relative to the equations of motion.

Each phase is carried out with six degrees of freedom.

Each phase consists of moving the c.g. of the vehicle from some point A to some point B.

Generalized equations for each phase describe the dynamics of displacement from A to B, and B may be relative to either a fixed or moving coordinate system.

There are two basic inputs to the system, i.e., information, and energy (fuel). There is only one output of the system, i.e., displacement of the c.g.

The information required consists of angular displacement about the three axes, which when integrated over time along with information relative to thrust, produces linear displacement.

Thus the basic functions of the man in the system is to provide changes in roll; pitch, yaw, and thrust.

In the case of an aircraft, the system output is dependent upon the output of man supplying inputs to the machine to produce the desired displacement. The output of the man is in the form of muscular activity to a set of pitch, roll, yaw, and thrust controls. These outputs are information to the machine. Energy is consumed by the man in relaying the information to the machine through the controls, and energy is consumed by the machine in providing direction and amplitude of thrust.

Thus the output of man is muscular activity. The output of the machine is movement of the c.g. by direction and amplitude of thrust. The output of the system is spatial displacement.

The energy inputs to man are in the form of nutrition and atmosphere. The information inputs to man consist of the position of the machine with respect to the six degrees of freedom and time, relative to some planned operational profile. Since the energy available in the machine is limited with respect to gravity and time, additional information is required relative to energy or thrust management, and the Vg envelope.

Since the machine is capable of operating in an environment hostile to the man, information to the man is also required relative to those environmental parameters which may exceed the man's limitations.

Task VIII

The factors to be considered in performance measurements are the man, the machine, the man-machine combination, and environment.

Man-Machine

The performance of the man-machine combination is determined by the ratio of the profile actually achieved to the profile required to accomplish a given task or mission, and the amount of deterioration which occurred while conducting the task or mission.

Machine

Machine performance is the ratio of the achievable output of the machine relative to the inputs by man to the

actual output-input relationship achieved during the conduct of a task or mission, and the deterioration which occurred while conducting the task or mission.

Man

The man's performance is the ratio of this capability to control the machine relative to his inputs to his actual output relative to input, and his deterioration from conducting a given task or mission.

Measurement

A measure of man-machine performance can be accomplished by devices for recording the following:

Pitch

Roll

Yaw

Thrust (computed)

Velocity

Acceleration

Altitude

Rate of climb

Time

Geographical displacement

These measurements can be compared with the prescribed mission in order to determine the time and magnitude of deviations.

Machine performance can be measured in a variety of ways such as recording input and output of subsystems and components. Performance as well as deterioration can be determined by correlating the recorded data with a measured baseline.

Man's performance can be measured by such techniques as deviations in physiological parameters, time, motion, and force studies of muscular outputs, along with variations of information inputs.

A baseline can be established through application of these measurements while the individual is performing a task such as driving an automobile under varying conditions. By recording physiological changes during both passive and active operations, the level of increase or decrease of the physiological parameters can be established for performing tasks which are relatively normal.

The performance baseline can further be established by measuring the physiological parameter variations utilizing information inputs requiring a minimum of integration along with a carefully controlled optimal environment.

A second area of consideration would consist of recording variations in environmental parameters such as O_2 supply, toxicants, temperature, etc.

Deviations from the baseline physiological measurements

during conduct of a task or mission will give some measure of man's performance. A comparison of actual inputs to the machine relative to those required for the mission will also indicate performance. A summation of both will indicate system performance.

	Baseline	Performance
Man	Muscle output vs. task Optimal info inputs Optimal env. Physiological variations from passive to active operation of terrestrial tasks	<u>Outputs required</u> Actual outputs Variation of physio-parameters from baseline Variations of outputs and physio parameters with env. changes and input changes
Machine	Input vs. output Strain gages Instrument readings or sensors Freq. and amp. correlation	<u>Outputs required</u> Actual outputs Strain imposed Variations from baseline parameters
Man-Machine	Pitch Roll Yaw Thrust Velocity Altitude Time Geo. positions	<u>Mission profile</u> Actual profile Variations of baseline parameters

Man-Machine deviations = Man deviations + Machine deviations

Proposed Program

The analysis to date suggest, a series of experiments and studies, the results of which should provide data to solidify the relationships of man and machine relative to performance. To carry out the experiments and studies, three separate programs are suggested as follows:

Program I -

This is an extension or continuation of the present physiological measurement program. The major objective of this additional effort would be to attempt to establish baselines for man, machine, man-machine combination, and environment. It could be conducted in either the Biological Research and Medical Operations aircraft, the Test Pilot School aircraft, or both.

The tasks to be considered are as follows:

Task 1 - BF-1, 17-8 BRMO

Establish "normal" ranges of man's physiological measurements. - passive.

Task 2 - BF-1, 17-9, TPS, BRMO

Establish "normal" ranges of man's physiological measurements - terrestrial tasks (automobile). The difference between tasks 1 and 2 should represent "normal" variations of the physiological measurements while performing "normal" tasks.

Task 3 - BB-5 BRMO

Establish "normal" ranges of machine subsystems and components - (actual flight)

Task 4 - BF-1 BRMO

Establish allowable deviations during performance of a task for man-machine combination - (mission analysis baseline)

Task 5 - BB-5 BRMO

Establish allowable deviations of environment during a task.

Program II -

This program would consist of further measurement of the same parameters as in Program I with the exception that the inputs and assigned duties would be varied. It is hoped that the results from this program will assist in more clearly determining the relationships of man and machine to performance by correlating the results with the previously established baselines.

Task 1 - BB-5 BRMO

Continue flight qualification of physiological measurements systems.

Task 2 - BB-5 BRMO

Establish variations of physiological parameters when shifting location of subject from front to back seat.

Task 3 - BB-5 BRMO, TPS

Establish variations of physiological parameters when flying under the hood and contact, and actual instrument flight relative to contact.

Task 4 - BB-5 BRMO, TPS

Establish variations of physiological parameters relative to tasks of varying degrees of additional duties. Comparison of the variations would be made between takeoff and landing, air intercept and ground attack, piloting and radio navigation, routine and scramble missions, etc. Comparisons would also be made during simulated emergency conditions such as flame out, control boost loss, subsystem failures such as instruments, hydraulics, etc.

Task 5 - BB-5 BRMO

Establish variations of physiological parameters when varying the type of display on the instrument panel.

Task 6 - BB-5 BRMO

Establish variations of physiological parameters when varying the type of controls. (Central vs. side stick etc.)

Program III

The third program is designed to conduct longer range studies relative to various aspects of the performance analysis.

Some tasks which might be considered are as follows:

1. Continue Performance Analysis in order to more clearly define the problem (detail).
2. Determine energy expended from muscular activity for a given task.
3. Determine energy expended from decision making and calculations during a given task.

4. Determine energy expended by the machine during a given task.
5. Determine energy input requirements for man including nutrition and atmosphere.
6. Determine information input requirements for man to perform any task.
7. Determine energy input requirements for the machine.
8. Determine information input requirements for the machine (control).
9. Determine operational environment requirements for man and machine.
10. Investigate different methods for measuring and recording physiological functions. (This would be directed to variations relative to cause rather than effect.)
11. Investigate different methods for measuring and recording machine functions (cause).
12. Investigate different methods for measuring and recording man-machine combination actual trajectory or displacement.

MEDICAL SCIENCES PROGRAM

MINIMUM PROGRAM

- Current Program Minus the Two Non-Problem Oriented RTOP's
- Delete RTOP's:
 - 199-21 Supporting Biomedical Research (ARC) -218K
 - 199-23 Supporting Biomedical Research (JSC) -218K
- Minimum Program = 6890K (7326-436)

Impact:

Loss of task to develop Shuttle Payload Science Management plan and SL-1 science support.

Loss of Task to define, plan, and provide science Data support for SL-1, and to develop a science data plan for ILSSE implementation.

Loss of task to purchase ground based computer hardware for Life Sciences data requirements for SL-1 and ILSSE.

Loss of MEDICS, an integrated biomedical computerized data system utilized for clinical and research data by the Life Sciences laboratories at JSC for the past 8-10 years.

Loss of an effort to develop a new bioinstrumentation technique for analyzing urinary proteins and protein subunits with a high level of discrimination.

Loss of efforts to determine adrenal regulating mechanisms as influenced by stress, hypo- and hypergravic effects on insulin regulation of carbohydrate metabolism, fat metabolism studies, nutritional control of central nervous system function, neuroregulation of circulation, and viral epidemiology in isolated small populations.

At the present time, the Shuttle payload and payload data support functions cannot be accommodated anywhere else in the budget, and the lead time is essential. MEDICS is a vital element in the function of JSC's Life Sciences research and clinical activities and has no other funding source at this time. Significant state-of-the-art advancements by highly competent investigators have in the past put NASA at the "cutting edge" of new technology, a role which should be maintained and strengthened. Finally, endocrinology, metabolism, and epidemiology are not included, per se, in the problem oriented RTOP's. This work serves as a valuable substrate of fundamental research having potential application to several problem areas and which will result in well-founded payload experiments.



SCALE DEFINITIONS FOR THE
INFLUENCE STYLE QUESTIONNAIRE (ISQ)

INFLUENCE STYLE QUESTIONNAIRE: SCALE DEFINITIONS

There are four basic influence styles:

- REWARD AND PUNISHMENT (R&P)
- PARTICIPATION AND TRUST (P&T)
- COMMON VISION (CV)
- ASSERTIVE PERSUASION (AP)

Each influence style is made up of several specific influence behaviors. In total, there are ten influence behaviors.

In the pages that follow you will find:

- a) a short narrative description of each influence style and the behaviors included in it,
- b) examples of each type of influence behavior, and
- c) the questions from the Influence Style Questionnaire used to collect data on your use of each influence behavior.

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REWARD AND PUNISHMENT (R&P)

Reward and Punishment is the use of pressures and incentives to control others' behavior. Rewards may be offered for compliance; and punishment or deprivation may be threatened for non-compliance. Naked power may be used, or more indirect and veiled pressures may be exerted through the use of status, prestige, and formal authority.

This influence style is characterized by "contingency management": letting others know clearly what they must do to get what they want and avoid negative consequences. It includes most bargaining and negotiating behavior.

Both Reward and Punishment and Assertive Persuasion involve agreeing and disagreeing with others. The difference is that in Assertive Persuasion one agrees or disagrees with another's proposal because it is more or less effective, correct, accurate or true. In using Reward and Punishment, on the other hand, the judgment of right or wrong is an evaluation based on a moral or social standard, a regulation, or an arbitrary performance standard. The person making the evaluation sets himself up as the judge, rather than appealing to logic or reason.

There are three aspects of Reward and Punishment:

1. Evaluating,
2. Prescribing Goals and Expectations,
3. Incentives and Pressures.

EVALUATING (E)

Evaluating involves praise and criticism, approval and disapproval, and the moral judgement of right and wrong. You are **evaluating** when you let others know how well they are meeting your standards or requirements. For example, the following statements are evaluating:

"That's no good!"

"What a good idea!"

"It wouldn't be fair to take that approach."

"I'm glad you have finally decided to come round to our way of thinking on this."

"That's a very generous proposal."

"That's not very loyal of you."

The following ISQ questions were used to assess your use of Evaluation:

1. He gives the impression of sitting in judgment on others.
11. She lets others know when they do not meet her standards.
21. He points out others' mistakes.
31. She makes moral judgments about what others do or say.
41. He lets others know whether his requirements are being met.
51. She is quick to give credit for good work.
61. He shows his approval when others perform up to his standards.

PRESCRIBING GOALS AND EXPECTATIONS (PGE)

When **prescribing goals and expectations**, you let others know exactly what is required and expected of them. You set clear standards on how you will judge the performance of others. For example, the following statements prescribe goals or expectations:

- "We ought to be able to better that figure."
- "You would have to come up with some good arguments to convince me of that."
- "We've got to finish our work by six o'clock."
- "We must come up with some better ideas."
- "I don't think we ought to go that far."
- "I'd appreciate it if we could get together on this."

The following ISQ questions assessed your use of Prescribing Goals and Expectations:

- 2. He uses moral imperatives such as "should," "ought" and "must."
- 12. She is quick to make her own wishes and desires known to others.
- 22. He verbalizes standards which he thinks others ought to meet.
- 32. She lets people know the standards by which their performance will be judged.
- 42. He is very explicit about what he wants or requires from others.
- 52. She expresses strong ideas about how things should be done.
- 62. He lets others know, directly or indirectly, exactly what he expects from them.

INCENTIVES AND PRESSURES (I&P)

Applying **incentives and pressures** involves offering rewards for compliance and threats of punishment or deprivation for non-compliance. It may involve the use of naked power or the more indirect, veiled pressure of status, prestige, and formal authority. It specifies the positive or negative consequences of behaving, or failing to behave, in a prescribed manner. For example:

- "If you take that line, you'll find it hard to get an agreement from us."
- "You would get more cooperation from me if you listened to our proposals."
- "Give us the supplies, and we'll have the job done on time."
- "We can't do that, according to the rules."
- "As Chairman, I'm going to ask that we take these points in order."
- "If I have to, I shall use my authority to make sure it gets done."
- "All right, I'll raise my offer in order to get it settled."

The following ISQ questions were used to assess your use of Incentives and Pressures:

3. When he wants something, he does not hesitate to pressure others into giving it.
13. She uses her authority to get things done.
23. He makes it clear what he is willing to give in return for what he wants.
33. She offers bargains or deals to get what she wants from others.
43. He uses veiled or open threats to get others to comply.
53. She promises rewards to get others to do what she wants.
63. He deals with differences in aims and interests by bargaining and negotiation.

PARTICIPATION AND TRUST (P&T)

Unlike Assertive Persuasion and Reward and Punishment, where influence is exerted by **pushing** others to accept ideas or to behave in desirable ways, the use of the **Participation and Trust** influence style **pulls** others toward what is desired or required by **involving them**. By actively listening to and involving others, an influencer increases their commitment to the task, and follow-up and supervision become less critical.

There are three aspects to Participation and Trust:

1. **Personal Disclosure,**
2. **Recognizing and Involving Others,**
3. **Testing and Expressing Understanding.**

PERSONAL DISCLOSURE (PD)

People who use **personal disclosure** openly accept their limitations of knowledge and resources; there is no defensive attitude about mistakes or weaknesses. By this example, others feel accepted for what they are, too, and do not need to compete for attention and control. Personal disclosure builds trust, and with it willingness to be influenced. Examples of personal disclosure are statements such as:

"Frankly, I'm not quite sure what to do next."

"I'm not at my best in that sort of situation."

"I guess I wasn't thinking clearly when I came up with that idea."

"Up till now, I haven't been very open about what we really wanted."

"Our record-keeping isn't good enough to answer that point."

The following ISQ questions were used to measure Personal Disclosure:

- 4. He admits his own mistakes and errors.
- 14. She admits her lack of knowledge and expertise in a situation.
- 24. He is open about his personal hopes and fears.
- 34. She accepts criticism without becoming defensive.
- 44. He acknowledges when he is confused, uncertain or doesn't have the answer.
- 54. She volunteers information of a personal nature.
- 64. He reveals his personal feelings.

RECOGNIZING AND INVOLVING OTHERS (RIO)

Recognizing and involving others involves drawing out and listening carefully to the contributions of others, and building on and extending those ideas, rather than countering with alternative proposals. It is being quick to give credit for others' ideas and suggestions and being willing to delegate responsibility. By skillfully recognizing and involving others, the influencer insures that they work to solve the problem and are committed to the result, rather than resisting influence or blocking a solution. For example:

- "How would you go about solving such a problem?"
- "I'm thinking about Bill's earlier objections on that point."
- "If we did it Mike's way, we could probably also simplify the procedure."
- "What you said makes me think we might be able to get it done sooner."
- "I'd be happy to delegate that to Joe and Bill to come up with an answer."
- "I'll trust your information and experience in this area."
- "Has anyone any further ideas to offer?"
- "Maybe we could each give a brief rundown of our experience with this process."

The following ISQ questions were used to measure Recognizing and Involving Others:

- 5. He encourages people to come up with their own solutions to problems.
- 15. She gives other people credit for their ideas.
- 25. He helps others to get a hearing.
- 35. She delegates full responsibility for important tasks.
- 45. He treats other people as being worthy of his trust and confidence.
- 55. She looks for ways to use other peoples' ideas and suggestions.
- 65. He makes other people feel that they have something of value to contribute.

TESTING AND EXPRESSING UNDERSTANDING (TEU)

Testing and expressing understanding is an extension of recognizing and involving others. By rephrasing or "playing back" what others have said, the accuracy of communication is checked and the other person feels that his or her ideas are valued. Communicating understanding and acceptance of another's ideas lets others know they have been listened to, and increases their willingness to listen to your ideas and to be influenced by them. Some examples of testing and expressing understanding:

"As I understand it, what you want is ..."

"Are you saying that you think we should be more cautious on this point?"

"I can understand that it would be difficult for you to give way after all you've put into this."

"It must be rather upsetting to find yourself in such a position."

"If I were in your situation, I suppose that would make me rather angry."

"Are we all saying we think we've done enough work on this?"

The following ISQ questions are used to measure Testing and Expressing Understanding:

6. He expresses sympathy to others when they have difficulties.
16. If others become angry or upset, she listens with understanding.
26. He shows tolerance and acceptance of others' feelings.
36. She listens carefully when people express views which are different from her own.
46. During a discussion he summarizes or paraphrases what others have said to make certain they have been heard.
56. She lets others know that she understands what they are trying to say.
66. He clarifies what others have just said before he responds or states his own ideas.

COMMON VISION (CV)

This influence style aims to identify a common vision of the future for a group and to strengthen the group members' belief that through their collective and individual efforts, the vision can become reality. The appeals are to the emotions and values of others, activating their personal commitment to private hopes and ideals, and channeling that energy into work toward a common purpose.

There are two aspects of Common Vision:

1. Articulating Exciting Possibilities,
2. Generating a Shared Identity.

ARTICULATING EXCITING POSSIBILITIES (AEP)

People who **articulate exciting possibilities** both imagine and communicate enthusiasm about possible outcomes of seemingly routine as well as unusual projects or challenges. Using images and metaphors, they kindle excitement within others and help them imagine a better future, too. Examples of this behavior are:

- "Here's an idea which really turns me on!"
- "If we can just solve this problem, it will be easy from here on in."
- "Wouldn't it be exciting if ..."
- "With just a bit more effort, we'll have this little obstacle knocked in the head."
- "With a new product like this, we're going to beat every previous sales record."
- "The effort and creativity we've put into this is bound to win the respect of the judges."

The following ISQ questions are used to measure Articulating Exciting Possibilities:

- 7. When others become uncertain or discouraged, his enthusiasm carries them along.
- 17. She brings others to see the exciting possibilities in a situation.
- 27. His way of speaking conveys a sense of excitement to others.
- 37. Her enthusiasm is contagious.
- 47. He puts into words the hopes and wishes which others may feel.
- 57. In persuading others, she appeals to their values, emotions and feelings.
- 67. He uses emotionally charged language to generate enthusiasm for a task.

GENERATING A SHARED IDENTITY (GSI)

Generating a shared identity, an individual appeals to common values and hopes in others, and helps them feel the strength which comes from being part of a unified group. The emphasis is on what we can accomplish if we all work together to achieve common goals and ideals. Examples of the kind of statements which generate a shared identity are:

- "With the talent we've got in this room, we've just got to come up with a winner."
- "We all have a lot more to gain by cooperating than we do by going our separate ways."
- "When I look at all we have in common, I can't see why we squabble about these minor points."
- "The thing which makes us different from the rest is that we all listen to each other in this group."
- "After all we've been through together and all the effort each one of us has put in, let's not give up now!"

The following ISQ questions are used to measure Generating a Shared Identity:

8. When working with others, he communicates his belief in the value and importance of the common task.
18. She heightens others' awareness of the benefits of pulling together as one.
28. He causes people he works with to be more aware of the aims and goals they have in common.
38. She fosters an esprit de corps where others feel a sense of common purpose.
48. He helps others to see how they can achieve more by working together.
58. She helps people she works with to find common values and aims which strengthen their commitment to one another.
68. He generates a feeling of "We're in this thing together ..." among the people with whom he works.

ASSERTIVE PERSUASION (AP)

This style of influencing others is characterized by the use of logic, facts, opinions, and ideas to persuade others. The basis for agreement and approval is the soundness of the other person's reasoning. It is a "push" style (like R&P) because you "force" others to accept your view or conclusion by the logic of your arguments.

Assertive Persuasion has two aspects:

1. **Proposing,**
2. **Reasoning For and Against.**

PROPOSING (P)

People who use proposing behavior are usually highly verbal and articulate. They are forward with their ideas, proposals, and suggestions and they are not afraid of others' reactions to them. Often they ask questions in order to present their own position on matters. They are persistent and energetic in persuading others. Some examples of proposing behavior are statements such as:

"I think we should make an agenda first."

"Do you think it might work better if we reversed the last two steps?"

"If that's the situation, then I think we'd do better to quit."