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PROCEEDINGS OF THE 1973

LYNDON B. JOHNSON SPACE CENTER

ENDOCRINE PROGRAM CONFERENCE

Prepared by
Endocrine Laboratory
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16. Abstract

This document contains the proceedings of the 1973 Lyndon B. Johnson Space Center Endocrine Program Conference. Subjects covered include the following: biochemical changes during 28 days of space flight, modulating the pituitary-adrenal response to stress, the significance of biorhythms in space flight, the importance of the renin-angiotensin system in normal cardiovascular homeostasis, a progress report of stress-induced changes in corticosteroid metabolism, recent studies of physiological factors involved in the regulation of serotonin content and turnover in the brain, the role of brain biogenic amines in the control of pituitary-adrenocortical activity, application of the water immersion model to man by studies of acid-base homeostasis during simulated weightlessness, the present status of physiological studies and analysis of calcium homeostasis in the Apollo and Skylab Programs, and endocrine considerations in the red-cell-mass and plasma-volume changes of the Skylab 2 and 3 crews.

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LYNDON B. JOHNSON SPACE CENTER

ENDOCRINE PROGRAM CONFERENCE

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INTRODUCTION

By Carolyn S. Leach, Ph. D.

The fourth Endocrine Program Conference, held at the NASA Lyndon B. Johnson Space Center, was part of the continuing effort to fully understand and to evaluate the endocrine changes observed during previous manned missions and, thereby, to further understand the physiological adaptation of man to the space-flight environment. Investigators reported on work pertinent to the overall program developed in support of the Apollo missions and the long-duration Skylab flights. The goals of this program continue to be of utmost importance and, indeed, the relevance of these goals has been augmented as man further extends himself and his technology into space. For the purpose of review, these goals are restated here.

1. The establishment (and continuation) of an operational laboratory for immediate endocrinologic assays at the NASA Lyndon B. Johnson Space Center

2. The assembly of a group of endocrine experts who are qualified to advise on procedures and the interpretation of data

3. The advancement of the field of endocrinology by the application of analytical procedures that are low in sample-volume requirements but that are high in specificity

As in previous conferences, the attendees of the fourth annual NASA Lyndon B. Johnson Space Center Endocrine Program Conference consisted of those who were chosen to help conduct the program. Each participant presented a discussion of work in his area of specialization and related his contributions in support of the program, either with respect to studies or to the development of methodology. The tape-recorded transcripts of these presentations were submitted to the authors for editing and then were compiled into these proceedings.

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CONTENTS

1. BIOCHEMICAL OBSERVATION DURING 28 DAYS OF SPACE FLIGHT

By Carolyn S. Leach, Ph. D., and Paul C. Rambaut, Sc. D.

With the completion of the 28-day flight of Skylab 2, the sum of biochemical data on human reaction to the weightless environment was significantly extended both quantitatively and qualitatively. The biochemical studies can be divided into two broad categories. One group included the more routine blood studies similar to those used in everyday medical practice. The second category encompassed those analyses used to investigate more thoroughly the endocrinological and fluid changes first seen in the crewmembers following the Gemini, Apollo, and Soviet missions (refs. 1-1 and 1-2).

IN-FLIGHT CONDITIONS

One of the principal objectives of the Skylab Program was to demonstrate that man could live in a weightless environment for extended periods of time and also accomplish useful scientific work. To accomplish this objective, a 100-ton laboratory was inserted into low Earth orbit on May 14, 1973, from the NASA John F. Kennedy Space Center (KSC), Florida. About 2 weeks later, on May 25, three men in an Apollo command module were launched at 13:00 GMT from KSC. Approximately 10 minutes after lift-off, the Apollo command and service module was inserted into a 424- by 415-kilometer orbit and successfully rendezvoused and docked with the orbiting workshop. The first 24 hours of orbital flight were spent in preparing the damaged workshop for repairs. The second day was occupied in entering the workshop and setting up experimental equipment. With the exception of two extravehicular periods, the crew occupied the workshop for the duration of the mission, orbiting the Earth every 90 minutes. The workshop maintained a gas pressure of 32.4 to 34.6 kN/m^2 (4.70 to 5.02 psia), with a gas composition of approximately 70 percent oxygen, 30 percent nitrogen, and up to 2.5 percent carbon dioxide. Relative humidity ranged between 31 and 53 percent. The lighting in the workshop was 108 lm/m^2 (10 foot-candles). The conditioned work volume was 331 cubic meters (11 700 cubic feet). Because part of the heat shield was lost during the launch phase, ambient temperatures reached 330 K (135° F) inside the workshop but later subsided to 300 K (81° F) within 6 days following deployment of a sunshade.

The command module was reactivated on June 20 and undocked from the workshop at 09:40 GMT on day 29. It landed in the Pacific Ocean, 1300 kilometers southwest of San Diego, California, at 13:50 GMT on June 22, 1973. The total flight time of this first Skylab visit was 672 hours 49 minutes and 49 seconds. The crew underwent medical examinations onboard the recovery ship and returned to Houston on June 25 at 02:47 GMT (ref. 1-3).

PROCEDURES

Biomedical observations began 31 days before the mission with controlled dietary intake and complete urine and fecal collections. These procedures were continued throughout the flight and for 17 days postflight. Preflight and postflight urine collection was accomplished on a void-by-void basis. Following each collection, the specimens were cooled to 277 K (4° C). Each morning, the urine collected the previous day was received in its cooled state in the laboratory and was analyzed. The in-flight collections were performed with an automatic urine collection system designed to operate in a weightless environment. Urine was cooled during collection to approximately 277 K (4° C) and remained at this temperature for no more than 24 hours, at which time a sample of approximately 120 milliliters was frozen for return and postflight analysis.

Fasting blood samples were drawn five times during the 31-day preflight phase, four times during the flight, and about 1, 24, and 72 hours, and 14 days after recovery. For the analysis reported in this document, the blood volumes preflight and postflight were 25 milliliters, whereas the in-flight plasma volumes averaged 3 milliliters. Sodium ethylenediaminetetraacetic acid was used as the anticoagulant both in flight and on the ground.

Radionuclides were used to estimate the size of body compartments. These studies included the measurement of total body water, extracellular fluid, and exchangeable potassium.

The mean dietary intake of nitrogen (N), sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), and phosphorus (P) was kept within approximately ±2 percent using repetitive 6-day menu cycles. Menus for the preflight and postflight control phases were identical, except that a limited number of fresh foods were substituted for some of the flight food items. Whenever a particular food was not consumed, mineral supplements were provided to adjust the dietary intake of every element within the prescribed tolerance. In addition to elemental intakes, caloric intake was also relatively constant. The actual mean intakes (before, during, and after flight) for Na, K, calories, and water are given in table 1-I. Daily water intake was not controlled but was recorded.

Since considerable variation in the response of individual crewmembers has been observed in all space flights, each man served as his own control; that is, his in-flight and postflight data were compared with his preflight data. To facilitate interpretation, urine data have been grouped into periods of equal duration that coincide with the 6-day dietary cycle of repeating menus.

A change in the level of a plasma constituent is considered to have occurred when it differs by more than one standard deviation (SD) from the mean of the five preflight values. The analyses performed on the plasma and serum samples, with each substance and property quantitatively determined (an asterisk indicates determination was made during flight), are listed as follows.

Sodium*	Uric acid
Potassium*	Creatinine*

Calcium*	Total protein
Magnesium	Alkaline phosphatase
Chloride*	Serum glutamic oxaloacetic transaminase (SGOT) (aspartate aminotransferase)
Phosphorus*	Creatine phosphokinase (CPK)
Osmolality*	Lactic dehydrogenase (LDH)
Carbon dioxide	Glucose*
Cholesterol	Total bilirubin
Triglycerides	Human growth hormone (HGH)
Adrenocorticotropic hormone (ACTH)*	Thyroxine
Cortisol*	Thyroid-stimulating hormone (TSH)
Angiotensin I*	Testosterone
Aldosterone*	Parathormone*
Insulin*	Calcitonin
Blood urea nitrogen (BUN)	Vitamin D

The analyses performed on the 24-hour urine samples, with each substance and property quantitatively determined, are listed as follows.

Volume	Antidiuretic hormone (ADH)
Sodium	Aldosterone
Potassium	Cortisol
Chloride	Epinephrine
Osmolality	Norepinephrine
Calcium	Total 17-hydroxycorticosteroids (17-OHCS)
Phosphate	Total 17-ketosteroids
Magnesium	Uric acid
Creatinine	

Urine was obtained from each crewmember each day of flight with the exception of the first day. In-flight measures of urine volumes by pressure plate displacement assisted the crew physician in approximating the state of hydration of the crewmembers. However, because of sufficient variation between the postflight lithium analysis and the volume determined in flight, the lithium-dilution-derived answers were used for all biochemical analyses. A comparison between the pressure plate volume readings and the lithium-determined volumes for the three crewmembers over approximately 27 days in flight provided a correlation coefficient of 0.87.

RESULTS

The serum and plasma biochemical determination results for those measurements made on samples obtained in flight, as well as preflight and postflight, are presented in tables 1-II and 1-III. These data indicate that an increase in K, P, Ca, creatinine, insulin, cortisol, HGH, ACTH, aldosterone, and angiotensin I had occurred in flight. Decreases were observed in Na, chloride, and glucose. When the postflight results are compared with the preflight data, increases are observed in Ca, P, HGH, CPK, creatinine, glucose, insulin, aldosterone, osmolality, cholesterol, and total protein. Postflight decreases in ACTH, LDH, angiotensin I, alkaline phosphatase, and uric acid were also shown.

The results of the urine analyses summarized in table 1-IV reflect significant increases in Na, K, Ca, P, Mg, ADH, osmolality, aldosterone, and cortisol during the mission. In-flight decreases were observed in uric acid. Total 17-OHCS were either decreased or not changed from preflight values for the science pilot (SPT) and pilot (PLT). The commander (CDR) showed increases in three of five in-flight periods. The component 17-OHCS changes are given in table 1-V. Total 17-ketosteroids were generally increased. The 17-ketosteroid fractions responsible for this increase are indicated in table 1-VI, which gives preflight, in-flight, and postflight data for pregnanediol (PD), androsterone (AND), etiocholanolone (ETIO), dehydroepiandrosterone (DHEA), 11=0 AND, 11=0 ETIO, 11-OH AND, and 11-OH ETIO. Amino acids were measured in selected urine samples; the results are summarized in tables 1-VII to 1-IX.

DISCUSSION

With the advent of manned space flight and prolonged periods in null gravity, physiological changes were expected. Some changes were observed in the returning Mercury crewmembers, and further changes were observed in the Gemini and Apollo crewmembers (refs. 1-4 and 1-5). Because of the difficulty in collecting and preserving urine and blood samples in the weightless environment and because of the return weight of the samples, very few such samples were collected during the Gemini and Apollo missions. In the Skylab Program, however, a sufficient number of adequate samples were returned for an in-depth study of human biochemical responses to extended stays in space. These samples provided a basis for ascertaining the general biochemical response to flight

and the particular response of fluid/electrolyte and musculoskeletal metabolism. The regulation of these parameters together with the broader aspects of adaptation and metabolic processes were also studied.

Of paramount interest were the crewmembers' responses during the initial period of flight, particularly in fluid and electrolyte balance. The elevated workshop temperature further augmented the initial reaction of the crew to weightlessness. During the 28-day mission, the CDR lost 1.9 kilograms, the SPT lost 3 kilograms, and the PLT lost 3.7 kilograms. Caloric intake and body compartment data have been interpreted to mean that, despite weight loss in all three crewmembers, adequate calories were consumed in flight. Loss of muscle mass and body fluids accounted for the weight loss (ref. 1-6).

A comparison between the preflight mean and the 6-day periods in flight and postflight reveals that all three crewmembers initially had decreased urine volumes that subsequently returned to or exceeded preflight values (fig. 1-1). The postflight urine volume for the SPT decreased. Although no significant in-flight diuresis was measured, it must be noted that collection of urine on the first day in flight was not performed, and the entire first period occurred during heat stress.

A pattern similar to the one observed for urine volume is also observed in the urinary ADH results given in figure 1-2. Significant increases in ADH occurred early in all crewmembers, and the elevations persisted for the CDR and PLT throughout the mission. The body fluid volumes shown in table 1-X indicate a decrease of about 3 percent in total body water and 3 percent in extracellular fluid; however, when the weight losses were taken into consideration, a proportionate increase in fluid was expressed as a percentage of body weight. These data indicate that the ADH mechanism was operative in water retention. The crewmembers did not show evidence of dehydration despite the excessive heat stress early in flight.

The urinary Na data (fig. 1-3) show slight losses early in flight for the CDR and SPT. All three crewmembers showed increased excretion during the remainder of the mission with significant decreases immediately postflight. This increase in urinary Na occurred at the same time that Na intake actually decreased from a preflight mean of 203 meq/day to an in-flight mean of 193 meq/day. Potassium intake remained constant (102 ± 2 meq/day preflight and 100 ± 2 meq/day in flight) during the control and flight phases. Urinary excretion of K (fig. 1-4) was increased throughout the mission for all three crewmembers with evidence of retention immediately postflight. This loss in K is confirmed by the decrease of 6 to 8 percent in total body exchangeable K (table 1-XI). However, plasma K results (table 1-II) do not reflect changes. A 20-percent postflight decrease in total body K has been observed by measurement of the total body K-40 after early Apollo flights.[1] Total body exchangeable K was measured on the Apollo 15 crewmembers and was found to be decreased about 13 percent postflight. The crewmembers of the Gemini 7 mission demonstrated positive K balance pre- and postflight with a negative balance during the mission.

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[1]Personal communication, J. V. Bailey.

The Gemini 7 results revealed increased urinary aldosterone excretion during the flight (ref. 1-7). Aldosterone was increased in the 24-hour urines of all three of the Skylab 2 crewmembers for the duration of the mission and immediately postflight (fig. 1-5). The levels reached for this period of time could certainly account for the urinary losses of K, but the data indicate the loss of Na even though aldosterone was increased. Results from the in-flight metabolic experiment on the 13-day Apollo 17 mission suggested similar responses from that crew (ref. 1-8). Several possibilities may account for these results. The data are suggestive of other mechanisms operative in the renal tubule proximal to the site of aldosterone action in the distal tubule. These mechanisms could involve humoral (ref. 1-9) or physical factors (ref. 1-10). Plasma aldosterone and renin activity changes indicate that there was an actual increase in production of aldosterone, probably by way of the renin-angiotensin mechanisms. This elevation could be produced by decreases in effective renal blood flow and carotid-artery or right-atrial pressure changes. Increased aldosterone secretion is the presumed cause of the K loss.

The high temperatures in the spacecraft must be considered when the data are evaluated because the effect of increased environmental temperature on the control of fluid and electrolytes is well documented (ref. 1-11). Changes in renal function could account for these changes. Postflight serum BUN did not change, and urinary creatinine remained relatively constant throughout the mission. However, plasma creatinine was slightly elevated during the mission and in two crewmembers postflight, as shown in figure 1-6.

Indicative of a significant stress response was the increase in urinary cortisol (fig. 1-7) during the mission and continuing postflight. The metabolic biochemical and exercise data must be viewed in light of the high cortisol excretion because this steroid plays a key role in the regulation of cellular metabolism. Plasma cortisol analysis (table 1-II) did not reflect a continuous high production of this hormone during the flight, and the postflight values were actually decreased in the CDR and PLT. These postflight results are comparable to the authors' earlier Apollo reports (ref. 1-12) and to the reports of Dlusskaia (ref. 1-13), who found similar results after Soyuz 9.

The total 17-OHCS showed either a decrease or no change from preflight values in two of the crewmembers. This occurred with changes shown in all four fractions. Pregnantriol is generally elevated, even when the other fractions are not changed or decreased.

The failure of the free cortisol increases to be reflected in total 17-OHCS is presently not explainable. Results similar to these were observed during the Gemini VII and the Apollo 16 and 17 flights. Metabolism of the adrenal steroids is believed to be implicated (ref. 1-14).

Total and ketosteroid fractions help to indicate specific areas of increases in the adrenal pathways of steroid production. Significant increases in all fractions were noted; however, the large increases in DHEA were specifically noteworthy. This compound is of adrenal origin, and increases in this compound were also observed during Apollo 17. In a pilot study performed on subjects deprived of adequate K intake, DHEA was significantly increased. These

results led to speculation concerning a role for DHEA in plasma K regulation (ref. 1-15).

The catecholamine excretion levels are variable, but both epinephrine (fig. 1-8) and norepinephrine (fig. 1-9) were generally decreased in the CDR during the mission. Postflight, both compounds were elevated significantly in all three crewmembers. Norepinephrine remained elevated for the duration of postflight testing. These compounds are secreted in response to physical and mental stress. In particular, increases in norepinephrine are often seen in response to challenges to the cardiovascular system. These data might be interpreted as part of the response of the cardiovascular system to a return to the one-g environment. Increases in norepinephrine excretion after bed rest have been reported and perhaps reflect an increase in vasomotor tone following reambulation (ref. 1-16).

These data are comparable to the results from the Gemini VII and Apollo missions, and contrast with the lack of changes observed on the crewmembers participating in the chamber simulation of Skylab (ref. 1-17). These findings are not indicative of immediate medical problems but emphasize the need to maintain good dietary intake. There is no indication from these results for the occurrence of a plateau in the biochemical responses to zero g within 28 days. Both an immediate response and a more prolonged adaptive response appear to be present.

The slight increases observed in plasma creatinine are indicative of decreases in creatinine clearance because urinary creatinine did not show parallel changes. These findings provide evidence for minor alterations in renal function in flight, a supposition also advanced by Balakhovskii and Natochin (ref. 1-2).

Among the earliest concerns for the effects of weightless flight were those involving losses of bone mineral. A complete metabolic study was conducted on this flight. The results showed significant losses of Ca, P, Mg, and N (ref. 1-18). The studies reported here extend the input/output balance experiment by providing data concerning plasma levels of musculoskeletal constituents. Plasma Ca and P increased during the flight in all three crewmembers. Plasma parathormone and 25-hydroxycholecalciferol were not significantly changed from preflight control values. Calcitonin was not detectable with the sensitivity of the assay; however, slight physiologic changes cannot be ruled out.

In humans, hypoalimentation stimulates HGH secretion. Hypoglycemia has a particularly rapid effect on HGH secretion. Human growth hormone, an insulin antagonist, raises blood glucose and plasma free fatty acids while lowering plasma amino acids. Growth hormone measurements were made together with measurements of insulin and glucose. Although plasma HGH levels were quite variable, significant elevation occurred during the first day in flight and the first days after recovery. Insulin and glucose were significantly decreased during the flight and increased after recovery. There was an increase in plasma cholesterol on recovery day. The constancy of the diets preflight, in-flight, and postflight would tend to preclude diet as a significant factor in

these changes immediately after flight, although losses in fat stores throughout the long missions may account for the mobilization of triglycerides after recovery.

The significant increases in thyroxine and the trend toward higher TSH levels correlate well with the decreases in cholesterol for 2 weeks following recovery. These data confirm earlier Apollo findings of an increased circulating free thyroxine after space flight (ref. 1-19). Similar findings were reported by the Soviets, who were able to correlate weight loss with cholesterol decreases. The Soviet workers suggested, without supportive data, that the thyroid gland might be implicated (ref. 1-2).

At recovery, blood glucose appears to be raised by the action of catecholamines, cortisol, and HGH, whereas the insulin is increased as a response to the elevated blood sugar. The in-flight decreases observed in both glucose and insulin have also been observed in bed rest. However, the decrease became significant at 28 days in space but did not become significant until 56 days in bed rest (ref. 1-20). The impaired tolerance to a glucose load that has been reported for exposure to bed rest was not measured in this study (ref. 1-21).

Total plasma protein as well as albumin increased on recovery day. Albumin decreased on the 3rd and 14th days after recovery, but not as much as total protein. This finding is inferential evidence that glycoproteins were increased immediately postflight. The cholesterol increase observed at recovery may also indicate an elevation in lipoproteins, particularly in high-density lipoproteins. Plasma volume increases as a result of water and electrolyte retention caused by the effects of gravity were recorded during this period. Thus, the decrease in albumin may have been dilutional rather than absolute. Unlike the Apollo results, triglycerides were elevated after flight until the 14th day postflight.

SUMMARY

The observations concerned with the biochemical reactions of the body to the stress of space flight included both endocrinological and metabolic measurements. This is the first comprehensive and integrated study of endocrinology and metabolism during prolonged space flight. Significant biochemical changes were observed that varied in magnitude and direction, but all disappeared shortly after return to Earth.

These changes are, for the most part, indicative of a successful adaptation by the body to the combined stresses of weightlessness. The transient nature of some of these changes, particularly in fluid and electrolyte metabolism, tends to support the conclusion that a new and stable condition of homeostasis has been achieved. In other areas, particularly those concerned with the metabolism of bone mineral, protein, and carbohydrates, unstable states appear to persist. It is unclear at this time in which form the ultimate sequelae of these changes will manifest themselves after flight has continued for much longer periods of time.

REFERENCES

1-1. Berry, Charles A.: Weightlessness. Bioastronautics Data Book, second ed. NASA SP-3006, 1973, pp. 349-415.

1-2. Balakhovskii, I.S.; and Natochin, Iu. V.: Obmen veshchestv v ekstremal'nykh usloviiakh kosmicheskogo poleta i pri ego imitatsii. Problemy Kosmicheskoi Biologii, vol. 22, Izdatel'stvo Nauka (Moscow), 1973.

1-3. Anon.: Skylab Mission Report First Visit. Rept. no. JSC-08414, NASA Lyndon B. Johnson Space Center, Aug. 1973.

1-4. Lutwak, Leo; Whedon, G. Donald; Lachance, Paul A.; Reid, Jeanne M.; and Lipscomb, Harry S.: Mineral, Electrolyte and Nitrogen Balance Studies of the Gemini-VII Fourteen-Day Orbital Space Flight. J. Clin. Endocrinol. Metab., vol. 29, no. 9, Sept. 1969, pp. 1140-1156.

1-5. Leach, Carolyn S.; Johnson, Philip C.; and Alexander, W. C.: Endocrine, Electrolyte, and Fluid Volume Changes Associated With Apollo Missions. Biomedical Results of Apollo, NASA SP-368, 1975.

1-6. Whedon, G. Donald; Rambaut, Paul C.; and Smith, Malcolm C., Jr.: Mineral Balance — Experiment M071. Skylab Medical Experiments Altitude Test (SMEAT), NASA TM X-58115, 1973, pp. 7-1 through 7-12.

1-7. Leach, Carolyn S.: Review of Endocrine Results: Project Mercury, Gemini Program, and Apollo Program. In Proceedings of the 1970 Manned Spacecraft Center Endocrine Program Conference (Oct. 5 to 7, 1970), NASA TM X-58068, 1971, pp. 3-1 through 3-16.

1-8. Leach, Carolyn S.; Rambaut, Paul C.; and Johnson, Philip C.: Adrenocortical Responses of the Apollo 17 Crew Members. Aerospace Med., vol. 45, no. 5, May 1974, pp. 529-534.

1-9. Smith, Homer W.: Salt and Water Volume Receptors: An Exercise in Physiologic Apologetics. Am. J. Med., vol. 23, no. 4, Oct. 1957, pp. 623-652.

1-10. Schrier, R. W.; and De Wardener, H. E.: Tubular Reabsorption of Sodium Ion: Influence of Factors Other Than Aldosterone and Glomerular Filtration Rate. New Eng. J. Med., vol. 285, no. 22, Nov. 1971, pp. 1231-1243.

1-11. Strauss, Maurice B.: Body Water in Man. Little, Brown and Company (Boston), 1957.

1-12. Leach, Carolyn S.; and Campbell, Bonnalie O.: Hydrocortisone and ACTH Levels in Manned Spaceflight. Chronobiology, Lawrence E. Scheving, Franz Halberg, and John E. Pauly, eds., Igaku Shoin Ltd. (Tokyo), 1974, pp. 441-447.

1-13. Dlusskaia, I. G.; Vinogradov, L. A.; Noskov, V. B.; and Balakhovskii, I. S.: Effect of Hypodynamia and Other Spaceflight Factors on the Excretion of 17-hydroxycorticosteroids and Aldosterone. Kosmicheskaia Biologiia i Meditsina, vol. 7, no. 3, May-June 1973, pp. 43-48.

1-14. Leach, C. S.; and Johnson, P. C.: Steroid Metabolism in Stress. Paper presented at the annual meeting of the Aerospace Medical Association (Las Vegas, Nev.), 1973.

1-15. Leach, C. S.; Hyatt, K.; and Johnson, P. C.: Increased DHEA Excretion During a Low Potassium Diet. Clin. Res., vol. 21, 1973, p. 87.

1-16. Leach, C. S.; Hulley, S. B.; Rambaut, P. C.; and Dietlein, L. F.: The Effect of Bedrest on Adrenal Function. Space Life Sci., vol. 4, nos. 3/4, Sept./Dec. 1973, pp. 415-423.

1-17. Leach, Carolyn S.; and Rambaut, Paul C.: Bioassay of Body Fluids - Experiment M073. Skylab Medical Experiments Altitude Test (SMEAT), NASA TM X-58115, 1973, pp. 11-1 through 11-14.

1-18. Whedon, G. D.; Lutwak, L.; Reid, J.; Rambaut, P.; Whittle, M.; Smith, M.; and Leach, C.: Mineral and Nitrogen Balance Study: Results of Metabolic Observations on Skylab II. Astronautica Acta. (To be published.)

1-19. Sheinfeld, Maxim; Leach, Carolyn S.; and Johnson, Philip C.: Plasma Thyroxine Changes of the Apollo Crewmen. Aviat. Space Environ. Med., vol. 46, no. 1, 1975, pp. 47-49.

1-20. Vernikos-Danellis, Joan; Winget, Charles M.; Leach, Carolyn S.; and Rambaut, Paul C.: Circadian, Endocrine, and Metabolic Effects of Prolonged Bedrest: Two 56-Day Bedrest Studies. NASA TM X-3051, 1974.

1-21. Blotner, Harry: Effect of Prolonged Physical Inactivity on Tolerance of Sugar. Arch. Intern. Med., vol. 75, no. 1, Jan. 1945, pp. 39-44.

TABLE 1-I.- DIETARY PARAMETERS

(a) Commander

Menu cycles	Sodium, meq			Potassium, meq			Calories			Water, ml		
	Mean	±SD[a]	SE[b]	Mean	±SD	SE	Mean	±SD	SE	Mean	±SD	SE
Preflight intake												
1	173	28	16	76	17	10	2271	698	403	2153	178	103
2	204	18	7	99	8	3	2808	93	38	2635	337	138
3	205	15	6	99	7	3	2726	148	60	2511	398	162
4	194	28	11	100	7	3	2907	204	83	2535	427	174
5	213	65	27	93	12	5	2881	181	74	2353	260	106
6	203	42	21	100	11	5	3076	287	144	2580	350	175
In-flight intake												
1	189	17	7	97	12	5	2637	306	125	2842	580	237
2	196	8	3	102	6	3	2775	197	81	2374	222	91
3	200	11	4	103	6	2	2882	151	62	2412	148	60
4	202	8	3	103	6	2	2927	123	50	2379	154	63
5	193	23	12	104	5	2	2988	175	88	2413	231	115
Postflight intake												
1	211	29	12	107	12	5	3455	609	248	3047	830	339
2	211	16	6	98	7	3	3142	283	116	2533	286	117
3	215	20	9	102	8	4	3309	132	59	2716	961	430

[a]SD = Standard deviation.

[b]SE = Standard error.

TABLE 1-I.- DIETARY PARAMETERS - Continued

(b) Science pilot

Menu cycles	Sodium, meq			Potassium, meq			Calories			Water, ml		
	Mean	±SD	SE	Mean	±SD	SE	Mean	±SD	SE	Mean	±SD	SE
Preflight intake												
1	124	60	34	65	40	23	1858	767	443	1798	649	374
2	201	44	18	101	12	5	3222	301	123	2496	231	94
3	219	34	14	107	12	5	3244	157	64	2378	124	50
4	192	20	8	99	11	5	3092	228	93	2241	286	117
5	218	73	30	95	23	10	3334	505	206	2247	355	145
6	188	28	14	101	10	5	3036	231	115	2395	532	266
In-flight intake												
1	197	17	7	96	8	3	2761	251	103	2685	423	173
2	194	18	7	98	8	3	2920	163	66	2156	182	74
3	197	20	8	103	5	2	3114	187	76	2183	191	78
4	197	21	8	103	5	2	3079	133	54	2309	256	104
5	195	22	11	99	2	1	2994	191	96	2194	253	127
Postflight intake												
1	173	42	17	79	15	6	2619	398	163	2361	374	153
2	207	42	17	97	9	4	3089	108	44	2533	248	101
3	196	44	20	90	15	7	2976	227	101	2289	833	373

TABLE 1-I.- DIETARY PARAMETERS - Concluded

(c) Pilot

Menu cycles	Sodium, meq			Potassium, meq			Calories			Water, ml		
	Mean	±SD	SE	Mean	±SD	SE	Mean	±SD	SE	Mean	±SD	SE
Preflight intake												
1	101	82	48	48	34	20	1692	1096	633	2443	994	574
2	200	6	2	95	12	5	3125	112	46	4254	424	173
3	196	8	3	96	11	5	3010	152	62	4496	592	242
4	199	6	2	95	12	5	3072	152	62	4210	484	198
5	225	39	16	95	16	7	3245	260	106	3671	553	226
6	207	18	9	96	17	9	3112	171	86	3640	639	320
In-flight intake												
1	184	37	15	89	21	8	2614	420	171	3541	1093	446
2	189	17	7	100	14	6	2843	255	104	4411	384	157
3	189	18	7	103	14	6	2871	176	72	3741	534	218
4	185	12	5	103	14	6	2877	131	53	4154	697	284
5	184	17	8	100	13	6	2874	209	104	4190	1134	567
Postflight intake												
1	198	3	1	105	15	6	3384	425	174	4332	595	243
2	196	7	3	92	11	4	3086	89	36	3897	508	207
3	194	7	3	92	15	7	3156	202	90	4973	805	360

TABLE 1-II.- SERUM/PLASMA RESULTS

Sample schedule (a)	Sodium, meq/liter	Potassium, meq/liter	Chloride, meq/liter	Creatinine, mg percent	Glucose, mg percent	Osmolality, milliosmols/liter
Commander						
F-21	--	--	--	--	--	293
F-14	137	4.25	96.8	1.06	80.3	288
F-7	138	4.48	99.4	1.14	80.1	291
F-1	134	4.33	96.3	1.14	72.2	285
F-1A	140	4.13	97.5	1.19	81.9	289
Preflight mean ± SD	137 ± 3	4.29 ± .14	97.5 ± 1.4	1.13 ± .05	78.6 ± 4.4	289 ± 3
MD4	138	4.49	98.1	1.18	80.6	286
MD6	131	4.24	100.8	1.18	82.3	286
MD13	140	3.96	96.4	1.15	81.7	286
MD27	137	4.38	95.7	1.25	80.1	286
R+0	138	4.36	94.8	1.30	95.1	286
R+1	138	4.50	94.8	1.12	89.3	284
R+4	136	4.48	94.6	1.15	90.9	295
R+14	--	--	--	--	--	297
R+67	--	--	--	--	--	286
Science pilot						
F-21	--	--	--	--	--	285
F-14	141	4.50	100.2	1.23	92.5	288
F-7	134	4.69	100.9	1.18	90.7	290
F-1	143	4.28	99.0	1.20	89.1	286
F-1A	135	4.39	98.2	1.18	88.4	286
Preflight mean ± SD	138 ± 4	4.46 ± .17	99.6 ± 1.2	1.19 ± .02	90.2 ± 1.8	287 ± 2
MD4	140	4.28	99.6	1.26	90.5	286
MD6	140	4.27	99.6	1.23	88.4	288
MD13	138	4.62	97.2	1.26	87.5	285
MD27	137	4.46	96.0	1.38	83.2	284
R+0	134	4.19	96.8	1.18	91.4	287
R+1	138	3.90	96.8	1.43	101.9	286
R+4	135	4.22	96.8	1.25	92.5	289
R+14	--	--	--	--	--	288
R+67	--	--	--	--	--	290
Pilot						
F-21	--	--	--	--	--	287
F-14	143	3.96	98.3	1.31	88.9	288
F-7	143	4.52	99.6	1.31	92.7	289
F-1	141	4.22	98.6	1.36	88.0	284
F-1A	139	3.98	100.6	1.31	85.0	299
Preflight mean ± SD	142 ± 2	4.17 ± .26	99.3 ± 1.0	1.32 ± .02	88.7 ± 3.2	289 ± 6
MD4	139	4.32	96.9	1.33	89.1	290
MD6	--	--	--	--	--	--
MD13	141	4.12	97.2	1.25	90.9	289
MD27	135	4.48	97.0	1.30	83.7	288
R+0	135	4.16	97.4	1.46	100.4	292
R+1	137	4.44	96.6	1.33	87.3	288
R+4	138	4.38	97.5	1.44	93.4	302
R+14	--	--	--	--	--	287
R+67	--	--	--	--	--	289

[a] F = flight, MD = mission day, R = recovery. (For example, F-21 is 21 days before flight.)

(PREFLIGHT, IN-FLIGHT, AND POSTFLIGHT COMPARISONS)

Calcium, mg percent	Phosphate, mg percent	Cortisol, µg/100 ml	Angiotensin I, ng/ml/hr	Aldosterone, µg/100 ml	ACTH, pg/ml	Insulin, µU/ml	HGH, ng/ml	Parathormone, ng/ml
Commander								
--	--	14.0	0.29	66	19.8	14	1.0	10.6
9.8	4.06	11.2	.55	390	24.4	14	.4	10.6
9.9	3.81	16.0	.34	106	24.0	12	1.5	13.8
9.6	4.09	13.0	1.13	480	64.3	21	.8	10.9
10.0	4.15	18.2	1.65	480	26.7	18	.8	18.0
9.8 ± .2	4.02 ± .14	14.5 ± 2.7	.79 ± .58	304 ± 203	31.8 ± 18.3	16.0 ± 4	.9 ± .4	13.0 ± 3.0
10.4	4.74	20.4	.83	530	--	18	2.4	--
10.3	4.27	20.6	1.17	620	58.7	24	.4	10.7
10.6	4.03	20.6	.30	418	70.1	24	1.4	17.0
10.8	4.52	22.0	.40	500	--	20	.4	10.5
10.1	4.58	6.0	.33	250	8.3	20	2.6	18.0
10.8	3.44	8.6	.76	290	9.5	24	5.8	18.0
10.6	3.62	19.8	.18	560	18.9	10	2.6	18.0
--	--	12.2	.29	100	47.3	12	1.3	10.0
--	--	11.8	.44	92	22.1	16	.8	11.5
Science pilot								
--	--	10.8	1.03	34	22.7	14	1.0	12.0
10.0	4.27	8.8	1.94	210	35.1	14	1.0	11.8
9.8	4.24	16.4	1.39	96	34.7	19	1.1	10.2
10.0	4.65	11.4	2.42	255	70.6	18	.4	10.1
9.8	4.34	18.4	2.34	295	51.1	19	1.1	18.0
9.9 ± .1	4.37 ± .18	13.2 ± 4.1	1.82 ± .60	178 ± 110	42.8 ± 18.5	17 ± 3	.9 ± .3	12.4 ± 3.2
10.2	4.34	9.0	2.33	180	--	24	1.8	10.8
9.8	4.89	14.8	3.47	350	61.7	22	1.8	16.6
10.5	4.80	13.4	2.07	380	15.2	29	1.4	17.4
10.4	4.40	8.2	.97	135	59.3	31	1.4	--
9.7	4.27	21.0	.46	640	33.8	16	5.8	18.0
10.4	3.72	18.4	5.30	680	26.3	22	8.0	18.0
10.0	3.93	15.4	.82	480	25.7	16	2.6	18.0
--	--	11.0	.45	62	57.5	16	2.0	12.0
--	--	10.2	.22	140	47.0	22	.8	10.8
Pilot								
--	--	6.0	2.85	62.5	18.1	16	1.3	11.4
9.6	3.87	10.8	.22	170	34.9	15	1.3	11.3
9.9	3.75	22.0	.45	103	26.9	16	.4	12.3
10.0	3.53	14.4	.94	360	56.9	20	1.7	10.8
9.6	3.68	14.0	.14	225	29.4	12	1.1	18.0
9.8 ± .2	3.70 ± .14	13.4 ± 5.8	.92 ± 1.12	184.1 ± 116	33.2 ± 14.5	16 ± 3	1.2 ± .5	12.8 ± 3.0
10.3	4.83	11.8	1.43	370	34.0	26	1.0	19.0
--	--	--	--	--	--	--	--	--
10.4	4.40	13.8	.90	260	36.9	22	1.0	16.5
10.5	4.68	17.0	.90	440	18.2	26	1.0	14.0
10.2	4.55	11.0	.63	530	.33	24	3.2	18.0
10.2	3.59	9.2	1.13	410	1.80	29	.8	18.0
10.3	3.99	13.6	.20	300	24.0	28	.8	18.0
--	--	9.8	.10	36	49.2	30	.4	10.0
--	--	10.6	.07	115	34.2	13	.8	14.5

TABLE 1-III.- SERUM/PLASMA RESULTS (PREFLIGHT-POSTFLIGHT COMPARISONS)

(a) Sample schedule 1

Sample schedule	Cholesterol, mg percent	SGOT, mU/ml	BUN, mg percent	Uric acid, mg percent	Alkaline phosphatase, IU	Mg, mg percent	Total Bilirubin, mg percent	CPK, IU	LDH, mU/ml	Triglyceride, mg percent	Carbon dioxide, meq/liter	Albumin, gm percent	Total protein, gm percent
Commander													
F-21	210	14	20	5.4	29	2.2	0.6	49	156	93	24.0	4.4	6.6
F-7	198	14	24	5.0	38	2.2	.4	58	251	72	24.5	4.4	6.4
F-1	216	14	18	4.7	39	2.0	.4	54	196	97	26.0	4.5	6.5
F-1A	220	15	18	5.2	36	2.1	.5	55	240	111	23.5	4.2	6.4
Preflight mean ± SD	211 ± 10	14 ± 1	20 ± 3	5.1 ± .3	36 ± 5	2.1 ± .1	.5 ± .1	54 ± 4	211 ± 44	93 ± 16	24.5 ± 1.1	4.4 ± .1	6.5 ± .1
ASAP[a]	222	7	19	5.0	25	2.0	.2	60	138	179	23.5	4.2	6.7
R+1	222	12	18	4.9	27	2.0	.5	66	157	81	25.5	4.4	7.2
R+4	207	13	18	4.8	26	2.0	.4	40	243	64	24.0	4.1	6.6
R+14	198	14	21	4.7	33	2.2	.4	37	170	82	24.0	4.1	6.6
R+67	218	10	20	5.8	20	2.1	.5	53	133	66	22.0	4.0	6.5
Science pilot													
F-21	132	10	23	8.2	20	1.8	1.3	27	141	56	26.0	3.6	5.7
F-7	177	15	21	8.9	30	2.0	1.1	67	240	68	26.0	4.3	6.5
F-1	200	9	16	6.9	31	1.9	.9	38	196	95	28.0	4.5	6.5
F-1A	215	13	17	8.4	30	1.9	1.1	82	211	128	22.5	4.3	6.4
Preflight mean ± SD	181 ± 3	12 ± 3	19 ± 3	8.1 ± .9	28 ± 5	1.9 ± .1	1.1 ± .2	54 ± 25	197 ± 42	87 ± 32	24.9 ± 2.6	4.2 ± .4	6.3 ± .4
ASAP[a]	199	10	22	6.5	18	1.8	1.4	40	211	106	24.5	4.1	6.5
R+1	217	14	20	7.4	21	2.0	2.7	40	157	74	24.0	4.4	7.2
R+4	188	12	19	7.6	19	8.9	1.2	51	222	54	22.0	4.0	6.4
R+14	176	10	18	6.9	24	9.0	1.1	31	120	117	25.0	4.1	6.1
R+67	151	7	18	8.0	26	8.9	.7	21	134	50	24.0	3.2	5.7
Pilot													
F-21	116	10	16	6.2	16	2.0	0.7	24	112	82	25.0	3.5	6.4
F-7	147	12	14	7.0	39	2.2	.4	42	209	--	24.5	4.5	7.0
F-1	153	10	14	6.6	22	2.0	.7	37	175	75	30.5	4.3	6.7
F-1A	167	17	13	7.8	23	2.2	.7	55	177	139	23.0	4.2	6.8
Preflight mean ± SD	146 ± 22	12 ± 3	14 ± 1	6.9 ± 7	25 ± 10	2.1 ± .1	.6 ± .2	40 ± 13	168 ± 41	99 ± 35	25.8 ± 3.3	4.1 ± .4	6.7 ± .3
ASAP[a]	168	12	14	5.9	12	2.0	.6	48	136	61	23.5	4.3	7.1
R+1	159	14	14	5.8	13	2.0	.6	57	131	61	26.5	4.1	7.1
R+4	152	12	12	6.4	14	2.1	.4	43	222	49	25.0	3.9	6.8
R+14	164	13	12	6.6	19	2.1	.5	29	196	69	25.0	4.0	6.6
R+67	155	10	15	6.6	20	2.0	.5	53	133	66	21.0	3.6	6.7

[a]ASAP = as soon as possible.

TABLE 1-III.- SERUM/PLASMA RESULTS (PREFLIGHT-POSTFLIGHT COMPARISONS) - Concluded

(b) Sample schedule 2

Sample schedule	Triiodothyronine, relative percent uptake	Thyroxine, µg/100 ml	Vitamin D, ng/ml	Calcitonin, ng/ml	TSH, µU/ml
Commander					
F-21	34.2	5.0	56.7	0.3	2
F-14	33.8	6.1	38.0	.3	2
F-7	34.2	6.0	36.3	.3	2
F-1	36.2	7.5	45.3	.3	6
F-1A	35.0	6.6	27.0	.21	4
Preflight mean ± SD	34.7 ± 1.0	6.2 ± .9	40.7 ± 11.1	.28 ± .04	3 ± 2
R+0	33.5	7.2	20.5	.21	12
R+1	31.2	9.3	29.5	.21	10
R+4	32.7	7.7	49.0	.21	4
R+14	33.8	4.5	--	.52	--
R+67	33.8	6.7	30.0	.7	--
Science pilot					
F-21	34.6	6.9	28.5	0.3	1
F-14	32.3	6.3	24.7	.3	4
F-7	30.8	11.5	37.0	.3	2
F-1	33.1	6.8	33.3	.3	2
F-1A	35.0	8.2	15.5	.21	7
Preflight mean ± SD	33.2 ± 1.7	7.9 ± 2.1	27.8 ± 8.3	.28 ± .04	3 ± 2
R+0	32.3	8.9	16.9	.21	25
R+1	31.5	12.8	35.0	.21	15
R+4	36.2	13.1	36.3	.21	6
R+14	33.1	6.3	58.0	.21	--
R+67	37.3	7.8	28.0	.7	--
Pilot					
F-21	36.0	8.0	28.3	0.3	1
F-14	31.5	8.3	24.7	.3	2
F-7	33.8	9.4	27.0	.3	0
F-1	35.0	8.0	25.7	.3	1
F-1A	35.4	9.3	--	.38	1
Preflight mean ± SD	34.3 ± 1.8	8.6 ± .7	26.4 ± 1.6	.32 ± .04	1 ± .7
R+0	35.0	8.5	--	.40	8
R+1	33.5	10.5	52.5	.47	6
R+4	35.0	8.8	32.7	.40	6
R+14	33.1	5.7	38.5	.32	--
R+67	35.0	7.0	92.0	.7	--

TABLE 1-IV.- TWENTY-FOUR-HOUR URINE RESULTS

(a) Commander

Variable (a)	Preflight mean ± SE	In-flight period					Postflight period		
		1	2	3	4	5	1	2	3
Sodium, meq/TV	151 ± 7	143 ± 18	165 ± 20	152 ± 11	165 ± 9	169 ± 16	140 ± 12	177 ± 17	133 ± 18
Potassium, meq/TV	72 ± 3	89 ± 7	73 ± 6	91 ± 7	91 ± 4	84 ± 9	72 ± 5	80 ± 8	76 ± 2
Chloride, meq/TV	147 ± 8	132 ± 18	153 ± 24	147 ± 12	154 ± 7	150 ± 13	147 ± 11	163 ± 20	133 ± 8
Osmolality, milliosmols	672 ± 43	918 ± 48	649 ± 81	678 ± 40	667 ± 25	776 ± 16	560 ± 41	559 ± 40	697 ± 22
Uric acid, mg/TV	936 ± 29	855 ± 77	849 ± 62	870 ± 32	807 ± 19	825 ± 73	967 ± 98	957 ± 100	827 ± 59
Creatinine, mg/TV	1708 ± 46	1858 ± 55	1855 ± 116	1663 ± 136	1765 ± 72	1750 ± 73	1935 ± 78	2016 ± 97	1650 ± 70
Calcium, meq/TV	11.7 ± 0.4	13.3 ± 0.8	18.9 ± 1	21.8 ± 0.9	23.6 ± 2	23.0 ± 1	20.8 ± 2	14.4 ± 2	12.2 ± 1
Phosphate, mg/TV	1001 ± 24	1457 ± 74	1211 ± 83	1365 ± 50	1371 ± 62	1387 ± 21	1229 ± 106	1127 ± 87	941 ± 116
Magnesium, meq/TV	10.3 ± 0.3	11.4 ± 0.9	11.8 ± 1	11.4 ± 0.6	11.0 ± 0.7	10.7 ± 0.5	10.3 ± 0.8	11.1 ± 1.4	9.4 ± 0.9
Hydrogen ion, ml 0.1 N NaOH/TV	253 ± 18	—	--	--	--	--	335 ± 60	232 ± 40	222 ± 17
Cortisol, µg/TV	58.6 ± 4	55.1 ± 4	81.2 ± 9	80.5 ± 8	80.3 ± 6	89.1 ± 16	84.9 ± 14	78.6 ± 11	60.8 ± 4
Aldosterone, µg/TV	17.7 ± 3	59.3 ± 5	54.9 ± 6	51.1 ± 6	36.5 ± 6	46.2 ± 8	25.8 ± 10	13.8 ± 2	13.7 ± 3
ADH, mU/TV	75.6 ± 6	151.8 ± 9	109.2 ± 15	93.1 ± 3	82.4 ± 6	80.3 ± 14	78.0 ± 14	85.9 ± 14	91.3 ± 24
Epinephrine, µg/TV	43.0 ± 3	20.5 ± 7	29.4 ± 4	28.5 ± 4	27.1 ± 3	33.1 ± 5	42.8 ± 8	44.2 ± 9	35.5 ± 8
Norepinephrine, µg/TV	51.4 ± 14	26.1 ± 1	39.6 ± 8	40.9 ± 5	47.6 ± 4	55.3 ± 6	113.5 ± 16	96.6 ± 10	81.4 ± 2
Total 17-OHCS, mg/TV	5.19 ± 0.36	7.25 ± 1.11	7.19 ± 0.50	6.86 ± 0.15	5.62 ± 0.33	5.16 ± 0.45	5.48 ± 0.67	4.91 ± 1.02	4.48 ± 0.68
Total 17-keto-steroids, mg/TV	5.43 ± 0.44	5.83 ± 0.44	6.49 ± 0.02	7.66 ± 0.86	8.63 ± 0.52	7.09 ± 0.61	6.26 ± 0.16	4.85 ± 0.65	5.11 ± 0.13

[a]TV = total volume.

TABLE 1-IV.- TWENTY-FOUR-HOUR URINE RESULTS - Continued

(b) Science pilot

Variable	Preflight mean ± SE	In-flight period					Postflight period		
		1	2	3	4	5	1	2	3
Sodium, meq/TV	149 ± 9	124 ± 8	172 ± 16	145 ± 12	161 ± 7	176 ± 11	79 ± 22	169 ± 12	185 ± 7
Potassium, meq/TV	72 ± 4	83 ± 9	83 ± 3	84 ± 6	94 ± 6	115 ± 14	47 ± 4	63 ± 6	85 ± 10
Chloride, meq/TV	132 ± 10	109 ± 10	147 ± 17	145 ± 12	150 ± 5	168 ± 11	69 ± 22	170 ± 18	191 ± 5
Osmolality, milliosmols	942 ± 34	1289 ± 40	1090 ± 25	964 ± 46	799 ± 48	837 ± 84	775 ± 52	632 ± 68	831 ± 85
Uric acid, mg/TV	871 ± 26	1208 ± 173	987 ± 66	869 ± 28	793 ± 41	973 ± 120	825 ± 44	824 ± 22	863 ± 50
Creatinine, mg/TV	2056 ± 42	2001 ± 34	2009 ± 117	1966 ± 198	1900 ± 45	2282 ± 241	1943 ± 121	2002 ± 38	2139 ± 101
Calcium, meq/TV	6.8 ± 0.3	8.1 ± 0.8	11.6 ± 0.8	11.8 ± 0.6	10.8 ± 0.9	12.7 ± 0.9	9.3 ± 0.7	7.7 ± 0.8	9.9 ± 1.1
Phosphate, mg/TV	1083 ± 48	1476 ± 51	1357 ± 60	1287 ± 62	1178 ± 86	1475 ± 100	1014 ± 54	1047 ± 56	1155 ± 28
Magnesium, meq/TV	8.9 ± 0.3	11.6 ± 0.3	12.0 ± 0.7	11.1 ± 0.3	10.2 ± 0.8	11.8 ± 0.5	7.7 ± 0.5	8.8 ± 0.5	10.2 ± 0.9
Hydrogen ion, ml 0.1 N NaOH/TV	276 ± 15	—	—	—	—	—	293 ± 18	254 ± 25	305 ± 101
Cortisol, µg/TV	43.0 ± 4	57.1 ± 5	66.6 ± 3	62.2 ± 5	58.4 ± 5	70.8 ± 11	65.2 ± 17	59.5 ± 4	57.1 ± 5
Aldosterone, µg/TV	13.2 ± 2	47.5 ± 4	49.1 ± 6	50.7 ± 4	25.1 ± 3	62.2 ± 9	28.7 ± 11	9.7 ± 2	5.1 ± 0.6
ADH, mU/TV	43.7 ± 5	65.9 ± 9	36.6 ± 4	34.9 ± 4	22.7 ± 2	28.8 ± 8	54.3 ± 26	22.2 ± 3	26.7 ± 2
Epinephrine, µg/TV	23.0 ± 2	12.5 ± 3	27.6 ± 4	38.9 ± 6	24.0 ± 4	25.0 ± 5	34.9 ± 10	29.3 ± 4	25.4 ± 17
Norepinephrine, µg/TV	51.6 ± 3	59.4 ± 6	61.5 ± 4	51.4 ± 10	57.9 ± 3	52.8 ± 11	103.3 ± 6	96.5 ± 14	89.8 ± 8
Total 17-OHCS, mg/TV	6.09 ± 0.35	6.04 ± —	7.02 ± 0.24	4.68 ± 1.02	4.50 ± 0.37	5.10 ± 0.48	5.76 ± 0.72	5.72 ± 0.82	10.96 ± 5.36
Total 17-keto-steroids, mg/TV	7.61 ± 0.24	6.55 ± —	8.62 ± 0.23	8.77 ± 0.23	9.29 ± 0.83	8.63 ± 1.05	6.94 ± 0.67	7.12 ± 0.74	8.14 ± 0.41

TABLE 1-IV.- TWENTY-FOUR-HOUR URINE RESULTS - Concluded

(c) Pilot

Variable	Preflight mean ± SE	In-flight period					Postflight period		
		1	2	3	4	5	1	2	3
Sodium, meq/TV	154 ± 8	139 ± 13	140 ± 9	164 ± 19	171 ± 10	152 ± 16	115 ± 23	157 ± 10	148 ± 16
Potassium, meq/TV	72 ± 3	76 ± 8	78 ± 7	89 ± 9	91 ± 5	92 ± 9	74 ± 8	70 ± 6	72 ± 5
Chloride, meq/TV	148 ± 11	114 ± 8	232 ± 72	144 ± 15	184 ± 48	171 ± 36	126 ± 24	130 ± 8	130 ± 13
Osmolality, milliosmols	331 ± 12	706 ± 109	417 ± 62	414 ± 48	371 ± 26	417 ± 72	328 ± 33	321 ± 25	261 ± 13
Uric acid, mg/TV	985 ± 15	811 ± 31	996 ± 52	1034 ± 92	897 ± 29	867 ± 36	812 ± 72	940 ± 112	873 ± 48
Creatinine, mg/TV	2227 ± 37	2146 ± 78	2381 ± 229	2100 ± 201	2175 ± 35	1885 ± 324	2217 ± 33	2189 ± 37	2197 ± 89
Calcium, meq/TV	7.2 ± 0.4	12.5 ± 1.3	18.2 ± 1.0	21.1 ± 1.3	21.5 ± 0.9	20.0 ± 0.9	16.0 ± 0.8	11.9 ± 0.5	12.2 ± 0.8
Phosphate, mg/TV	1157 ± 32	1475 ± 62	1385 ± 106	1667 ± 140	1603 ± 65	1504 ± 63	997 ± 59	1144 ± 51	1045 ± 65
Magnesium, meq/TV	9.6 ± 0.4	10.1 ± 0.5	12.4 ± 0.5	12.3 ± 1.0	12.4 ± 0.5	12.4 ± 0.4	9.6 ± 0.6	10.6 ± 0.6	10.1 ± 0.6
Hydrogen ion, ml 0.1 N NaOH/TV	221 ± 15	--	--	--	--	--	213 ± 82	227 ± 20	227 ± 31
Cortisol, µg/TV	59.0 ± 5	82.1 ± 13	121.2 ± 16	109.6 ± 16	103.0 ± 8	111.2 ± 6	84.3 ± 12	76.3 ± 4	81.9 ± 8
Aldosterone, µg/TV	9.4 ± 0.7	42.8 ± 2	45.7 ± 10	25.0 ± 9	23.2 ± 1	51.4 ± 16	33.2 ± 16	7.9 ± 1	6.0 ± 2
ADH, mU/TV	23.0 ± 2	54.5 ± 11	35.4 ± 4	43.4 ± 5	42.9 ± 2	36.9 ± 2	47.6 ± 20	30.7 ± 2	30.6 ± 1
Epinephrine, µg/TV	25.7 ± 3	11.8 ± 4	21.9 ± 4	27.8 ± 3	26.7 ± 6	32.9 ± 7	27.3 ± 6	29.2 ± 8	4.4 ± 3
Norepinephrine, µg/TV	70.4 ± 5	63.7 ± 7	80.3 ± 9	67.5 ± 5	78.0 ± 8	94.6 ± 19	117.6 ± 16	118.4 ± 12	107.8 ± 12
Total 17-OHCS, mg/TV	6.50 ± 0.64	8.75 ± 1.47	6.19 ± 0.99	3.96 ± 0.49	6.08 ± 0.28	6.99 ± 1.61	5.49 ± 0.33	4.89 ± 0.77	4.44 ± 1.02
Total 17-keto-steroids, mg/TV	10.41 ± 0.42	13.38 ± 1.40	12.23 ± 0.83	12.70 ± 1.80	14.49 ± 0.39	12.65 ± 1.11	10.05 ± 0.97	10.12 ± 1.24	11.26 ± 0.57

TABLE 1-V.- TOTAL AND FRACTIONATED 17-HYDROXYCORTICOSTEROIDS

(a) Commander

Schedule	Fraction, mg/24 hr				Total, mg/24 hr
	Pregnantriol	Tetrahydros	Tetrahydrocortisol	Tetrahydrocortisone	
Preflight data					
Preflight mean ± SE	0.46 ± 0.04	0.09 ± 0.02	1.20 ± 0.13	3.63 ± 0.24	5.19 ± 0.36
Period:					
1	.45 ± .01	--	1.23 ± .21	3.41 ± .78	7.25 ± 1.11
2	.52 ± .01	--	--	3.67 ± .06	7.19 ± .50
3	.32 ± .06	--	1.39 ± .38	4.07 ± .88	6.86 ± .15
4	.49 ± --	--	.75 ± --	3.27 ± --	5.22 ± .33
5	--	--	--	--	5.16 ± .45
In-flight data					
Period:					
1	0.80 ± 0.10	--	1.76 ± 0.26	4.69 ± 0.78	--
2	.88 ± .10	--	1.89 ± .18	4.43 ± .27	--
3	1.07 ± .22	--	1.82 ± .49	3.96 ± .45	--
4	.77 ± .07	.03 ± .01	1.34 ± .09	3.49 ± .21	--
5	.63 ± .04	.11 ± --	1.34 ± .20	3.15 ± .24	--
Postflight data					
Period:					
1	0.69 ± 0.06	0.07 ± 0.01	1.49 ± 0.22	3.27 ± 0.39	5.48 ± 0.67
2	.72 ± .13	--	1.14 ± .31	3.05 ± .62	4.91 ± 1.02
3	.62 ± .09	--	1.06 ± .16	2.80 ± .66	4.48 ± .68

(b) Science pilot

Schedule	Fraction, mg/24 hr				Total, mg/24 hr
	Pregnantriol	Tetrahydros	Tetrahydrocortisol	Tetrahydrocortisone	
Preflight data					
Preflight mean ± SE	0.79 ± 0.06	0.03 ± 0.01	1.61 ± 0.11	3.68 ± 0.24	6.09 ± 0.35
Period:					
1	1.01 ± .15	.05 ± .01	1.86 ± .16	4.43 ± .56	6.04 ± --
2	.70 ± .02	.02 ± --	1.48 ± .08	3.60 ± .20	7.02 ± .24
3	.85 ± .06	.02 ± .00	1.95 ± .06	3.91 ± .14	4.68 ± 1.02
4	.73 ± .04	--	1.27 ± .20	3.34 ± .54	4.49 ± .37
5	.55 ± --	--	1.38 ± --	2.57 ± --	5.10 ± .48
In-flight data					
Period:					
1	0.68 ± --	--	1.58 ± --	3.79 ± --	--
2	1.10 ± .11	--	1.69 ± .04	4.24 ± .41	--
3	.93 ± .07	--	1.20 ± .28	3.22 ± .53	--
4	.83 ± .05	--	.94 ± .08	2.73 ± .24	--
5	1.03 ± .12	--	1.27 ± .11	2.80 ± .29	--
Postflight data					
Period:					
1	0.85 ± 0.09	0.06 ± 0.005	1.57 ± 0.18	3.28 ± 0.50	5.76 ± 0.72
2	1.11 ± .18	.03 ± --	1.39 ± .18	3.22 ± .46	5.72 ± .82
3	1.77 ± .75	.16 ± --	2.55 ± 1.24	6.58 ± 3.42	10.96 ± 5.36

TABLE 1-V.- TOTAL AND FRACTIONATED 17-HYDROXYCORTICOSTEROIDS - Concluded

(c) Pilot

Schedule	Fraction, mg/24 hr				Total, mg/24 hr
	Pregnantriol	Tetrahydros	Tetrahydrocortisol	Tetrahydrocortisone	
Preflight data					
Preflight mean ± SE	0.72 ± 0.06	--	1.56 ± 0.23	4.31 ± 0.38	6.50 ± 0.64
Period:					
1	.84 ± .10	--	2.05 ± .16	4.57 ± .38	8.75 ± 1.47
2	.74 ± --	--	1.55 ± --	5.05 ± --	6.19 ± .99
3	.73 ± .00	--	1.38 ± .29	4.75 ± .41	3.96 ± .49
4	.58 ± .18	--	1.01 ± .77	3.12 ± 1.23	6.08 ± .28
5	--	--	--	--	6.99 ± 1.61
In-flight data					
Period:					
1	0.79 ± 0.01	--	2.20 ± 0.52	5.72 ± 1.01	--
2	.78 ± .10	--	1.44 ± .28	3.97 ± .68	--
3	.67 ± .06	--	.67 ± .15	2.62 ± .29	--
4	.76 ± .04	--	1.42 ± .09	3.90 ± .16	--
5	.89 ± .05	--	1.97 ± .82	4.13 ± .80	--
Postflight data					
Period:					
1	0.77 ± 0.05	--	1.26 ± 0.08	3.47 ± 0.23	5.49 ± 0.33
2	.76 ± .07	--	1.06 ± .24	3.07 ± .57	4.89 ± .77
3	.91 ± .06	--	1.11 ± .25	2.92 ± .68	4.44 ± 1.02

TABLE 1-VI.- TOTAL AND FRACTIONATED 17-KETOSTEROIDS

(a) Commander

Schedule	Fraction, mg/24 hr								Total, mg/24 hr
	PD	AND	ETIO	DHEA	11=O AND	11=O ETIO	11-OH AND	11-OH ETIO	
Preflight data									
Preflight mean ± SE	0.63 ± 0.03	1.01 ± 0.09	2.20 ± 0.12	0.61 ± 0.08	0.30 ± 0.11	0.42 ± 0.04	0.19 ± 0.04	0.41 ± 0.05	5.43 ± 0.44
Period:									
1	.55 ± .06	.69 ± .18	1.48 ± .12	.34 ± .08	--	.30 ± .05	.07 ± .01	.31 ± .06	5.83 ± .44
2	.70 ± .08	.96 ± .22	2.22 ± .22	.62 ± .21	.30 ± .25	.49 ± .10	.27 ± .14	.46 ± .11	6.49 ± .02
3	.68 ± .07	1.15 ± .22	2.30 ± .25	.80 ± .20	.31 ± .01	.43 ± .08	.23 ± .08	.50 ± .15	7.66 ± .86
4	.51 ± .11	1.22 ± .13	2.62 ± .24	.55 ± .4	--	.47 ± .06	--	.31 ± .08	8.63 ± .52
5	.66 ± .03	1.06 ± .24	2.65 ± .11	.58 ± .04	--	.40 ± .14	--	.42 ± .13	7.09 ± .61
In-flight data									
Period:									
1	0.70 ± 0.13	1.08 ± 0.06	2.25 ± 0.21	1.07 ± 0.05	0.03 ± 0.02	0.37 ± 0.06	0.12 ± --	0.31 ± 0.07	--
2	.67 ± .06	1.64 ± .13	2.39 ± .19	1.27 ± .17	.05 ± --	.35 ± .05	.22 ± .02	.35 ± .05	--
3	.67 ± .08	2.03 ± .17	2.95 ± .27	1.26 ± .33	--	.53 ± .05	--	.30 ± .07	--
4	.75 ± .06	1.94 ± .15	3.88 ± .25	1.40 ± .21	.15 ± --	.49 ± .07	.31 ± .15	.28 ± .06	--
5	.74 ± .08	1.44 ± .13	2.94 ± .23	1.07 ± .19	--	.46 ± .08	.16 ± .04	.43 ± .13	--
Postflight data									
Period:									
1	0.68 ± 0.05	1.27 ± 0.05	2.58 ± 0.06	0.82 ± 0.09	--	0.53 ± 0.06	0.14 ± 0.03	0.31 ± 0.05	6.26 ± 0.16
2	.57 ± .15	.98 ± .18	2.27 ± .30	.52 ± .09	--	.54 ± .16	.14 ± .11	.21 ± --	4.85 ± .65
3	.59 ± .04	.93 ± .06	2.00 ± .13	.45 ± .07	--	.78 ± .03	.18 ± --	.29 ± .04	5.11 ± .13

(b) Science pilot

Schedule	Fraction, mg/24 hr								Total, mg/24 hr
	PD	AND	ETIO	DHEA	11=O AND	11=O ETIO	11-OH AND	11-OH ETIO	
Preflight data									
Preflight mean ± SE	0.34 ± 0.03	1.57 ± 0.08	4.86 ± 0.17	0.47 ± 0.06	0.04 ± 0.02	0.17 ± 0.03	0.10 ± 0.01	0.25 ± 0.05	7.61 ± 0.24
Period:									
1	.37 ± .07	1.76 ± .24	4.34 ± .18	.61 ± .22	--	.12 ± .04	.13 ± --	.16 ± .03	6.55 ± --
2	.30 ± .02	1.53 ± .10	5.05 ± .19	.44 ± .05	--	.11 ± .02	--	.17 ± .03	8.62 ± .23
3	.35 ± .02	1.67 ± .22	5.79 ± .42	.46 ± .02	.05 ± --	.15 ± .02	.08 ± .01	.25 ± .05	8.77 ± .23
4	.24 ± .02	1.56 ± .18	4.55 ± .46	.31 ± .14	.01 ± --	.13 ± --	--	.16 ± --	9.29 ± .83
5	.25 ± .00	1.52 ± .29	5.00 ± .15	.20 ± .02	--	.15 ± .04	--	.14 ± .03	8.63 ± 1.05
In-flight data									
Period:									
1	0.29 ± --	1.19 ± --	3.89 ± --	0.89 ± --	--	0.31 ± --	--	0.20 ± --	--
2	.30 ± .01	2.32 ± .09	4.99 ± .06	.82 ± .02	--	.40 ± --	--	--	--
3	.30 ± .03	2.25 ± .15	5.09 ± .24	.72 ± .09	.13 ± --	.35 ± .06	--	.14 ± --	--
4	.33 ± .03	2.46 ± .38	5.34 ± .40	.93 ± .14	.14 ± .04	.28 ± .02	--	--	--
5	.31 ± .02	2.15 ± .26	5.53 ± .77	.63 ± .03	--	--	--	--	--
Postflight data									
Period:									
1	0.30 ± 0.05	1.27 ± 0.14	4.15 ± 0.35	0.24 ± 0.06	--	0.40 ± 0.12	0.20 ± --	0.34 ± 0.11	6.94 ± 0.67
2	.20 ± .04	1.68 ± .21	4.42 ± .54	.48 ± .08	--	.29 ± .01	.26 ± .04	.22 ± .02	7.12 ± .74
3	.29 ± .04	2.31 ± .06	4.86 ± .28	.53 ± .05	--	.10 ± --	.26 ± --	.10 ± --	8.14 ± .41

TABLE 1-VI.- TOTAL AND FRACTIONATED 17-KETOSTEROIDS - Concluded

(c) Pilot

Schedule	Fraction, mg/24 hr								Total, mg/24 hr
	PD	AND	ETIO	DHEA	11=O AND	11=O ETIO	11-OH AND	11-OH ETIO	
Preflight data									
Preflight mean ± SE	0.39 ± 0.02	3.05 ± 0.09	4.28 ± 0.18	1.47 ± 0.14	0.36 ± 0.23	0.64 ± 0.04	0.33 ± 0.03	0.59 ± 0.06	10.41 ± 0.42
Period:									
1	.42 ± .03	2.78 ± .11	3.75 ± .41	1.35 ± .31	.14 ± .04	.58 ± .03	.28 ± .06	.55 ± .10	13.38 ± 1.41
2	.37 ± .02	3.25 ± .14	4.53 ± .27	1.36 ± .21	—	.73 ± .05	.35 ± .06	.41 ± .03	12.23 ± .83
3	.38 ± .02	3.43 ± .17	4.46 ± .36	1.74 ± .18	.80 ± —	.66 ± .13	.37 ± .05	.56 ± .08	12.70 ± 1.80
4	.33 ± .06	3.09 ± .25	4.58 ± .60	1.58 ± .41	—	.62 ± .05	.38 ± .05	.51 ± .02	14.49 ± .39
5	.28 ± .04	2.50 ± .02	4.23 ± .74	.49 ± .11	—	.38 ± .04	.15 ± —	—	12.65 ± 1.11
In-flight data									
Period:									
1	0.41 ± 0.04	3.37 ± 0.42	4.42 ± 0.38	3.51 ± 0.55	—	0.75 ± 0.05	0.45 ± 0.04	0.73 ± 0.15	—
2	.40 ± .07	3.62 ± .22	4.50 ± .28	2.91 ± .24	—	.54 ± .15	.51 ± .01	—	—
3	.37 ± .02	3.93 ± .37	5.17 ± .88	2.43 ± .42	—	.72 ± .09	.37 ± —	—	—
4	.40 ± .02	3.66 ± .11	6.41 ± .21	2.12 ± .15	—	.94 ± .20	.63 ± .05	.49 ± .04	—
5	.34 ± .04	3.42 ± .19	6.08 ± .62	1.45 ± .08	—	.75 ± .04	.60 ± .10	—	—
Postflight data									
Period:									
1	0.38 ± 0.01	2.80 ± 0.21	4.52 ± 0.40	0.87 ± 0.30	—	0.90 ± 0.06	0.33 ± 0.08	0.42 ± 0.01	10.05 ± .97
2	.36 ± .03	3.03 ± .29	4.24 ± .67	.94 ± .10	.05 ± —	.82 ± .17	.15 ± .03	.41 ± .03	10.12 ± 1.24
3	.39 ± .08	3.46 ± .22	4.32 ± .10	1.43 ± .16	—	.88 ± .14	.55 ± .12	.54 ± .10	11.26 ± .57

TABLE I-VII.- FREE AMINO ACID ANALYSIS FOR THE COMMANDER

[Each value represents milligrams collected per 24 hr]

Variable	Sample schedule													
	F-28	F-21	F-11	F-1	F-1A	Preflight mean ± SE	MD 11	MD 17	MD 20	MD 26	R+1	R+6	R+11	R+16
Phosphoserine	21.32	20.17	27.20	17.50	14.92	20.22 ± 2.07	29.07	25.10	19.63	24.41	38.49	37.43	24.14	24.46
Phosphoethanolamine	9.52	8.68	7.38	8.90	8.32	8.56 ± 0.35	11.22	11.75	8.78	9.78	16.19	21.06	13.35	14.04
Taurine	168.52	79.04	224.29	167.80	248.63	177.66 ± 29.26	218.93	214.80	142.13	172.20	472.09	207.41	161.88	195.28
Aspartic acid	10.31	7.77	8.13	7.81	27.46	12.30 ± 3.82	11.24	5.17	6.39	9.55	12.95	10.58	10.05	16.94
Threonine	23.14	24.12	26.12	20.31	12.53	21.25 ± 2.37	22.96	18.60	18.15	21.88	32.84	42.78	24.48	23.54
Serine	54.08	51.25	52.20	43.21	47.48	49.64 ± 1.93	53.06	49.28	41.22	47.71	63.84	66.27	51.65	50.18
Glutamine/asparagine	104.94	110.27	108.40	85.76	84.11	98.70 ± 5.69	117.08	103.03	87.73	103.76	169.25	143.98	120.49	122.73
Glutamic acid	5.73	3.81	5.00	4.59	3.21	4.47 ± 0.44	8.72	5.32	4.65	5.09	8.00	7.35	6.48	14.03
Glycine	132.91	112.45	135.37	103.38	111.13	119.05 ± 6.36	126.18	135.50	119.67	118.22	199.50	148.33	142.09	118.42
Alanine	38.52	41.84	47.01	33.10	35.76	39.25 ± 2.42	43.51	40.33	37.82	38.70	53.10	55.87	42.17	45.63
α-aminoadipic acid	12.94	8.86	14.97	13.14	12.14	12.41 ± 1.00	25.15	13.13	19.84	17.81	33.93	19.21	19.69	30.64
α-amino-n-butyric acid	2.13	2.04	1.95	1.40	1.10	1.72 ± 0.20	2.54	1.36	1.04	1.73	5.25	0.83	1.15	4.86
Valine	4.23	4.32	3.09	2.07	3.64	3.47 ± 0.41	11.50	3.00	3.49	3.37	5.42	15.37	2.42	3.79
Half cystine	9.86	7.46	11.91	9.54	15.11	10.78 ± 1.29	40.21	23.24	21.18	26.27	28.23	11.68	19.22	18.70
Cystathionine	24.89	24.46	29.08	26.16	6.25	22.17 ± 4.06	24.25	9.93	19.21	12.40	33.11	32.79	38.75	22.25
Methionine	14.07	5.00	5.97	5.02	7.12	7.44 ± 1.70	13.47	24.71	10.96	15.63	16.65	18.26	15.05	10.69
Isoleucine	3.52	3.86	3.25	1.76	7.17	3.91 ± 0.89	8.87	6.10	7.37	6.40	10.76	9.20	7.74	6.45
Leucine	9.19	8.68	8.50	8.19	8.27	8.57 ± 0.18	13.35	11.54	11.01	9.60	16.54	19.86	10.57	10.57
Tyrosine	23.87	22.83	22.37	22.01	13.93	21.00 ± 1.80	28.33	22.08	25.46	23.43	37.89	33.81	21.92	19.63
Phenylalanine	11.65	10.51	7.79	7.91	5.61	8.69 ± 1.07	12.97	10.22	21.95	17.45	30.03	16.97	0.53	8.42
β-alanine	27.59	6.39	5.59	7.02	1.61	9.64 ± 4.58	6.27	6.89	31.41	10.40	24.42	7.25	4.49	8.75
β-aminoisobutyric acid	6.07	4.13	3.47	2.81	3.81	4.06 ± 0.55	3.05	2.66	2.90	2.91	5.67	6.48	4.97	6.99
Hydroxylysine	5.14	LD[a]	0.77	0.61	1.83	1.67 ± 0.92	0.95	1.91	2.04	4.04	5.61	4.15	3.10	1.42
Ornithine	7.73	0.98	1.07	0.67	2.01	2.49 ± 1.33	1.18	2.08	1.45	1.50	1.69	2.60	2.18	1.40
Lysine	27.54	2.28	75.77	93.23	7.72	41.3 ± 18.34	37.06	23.74	42.70	—	57.52	74.04	41.21	29.04
1-methylhistidine	82.12	43.52	62.58	41.64	258.38	97.65 ± 40.85	167.43	194.23	137.74	—	154.13	311.97	191.06	446.74
Histidine	113.79	84.12	44.91	77.79	116.41	87.40 ± 13.13	110.97	128.29	106.67	—	178.02	175.02	142.17	69.79
3-methylhistidine	82.12	43.52	62.58	41.64	82.77	62.53 ± 8.92	76.27	107.08	95.57	—	121.22	161.92	123.63	102.78
Anserine	43.08	8.30	LD	LD	29.59	16.19 ± 8.63	LD	93.64	1.07	0.83	11.44	80.03	53.09	37.80
Creatinine	3257.96	2324.23	1153.72	2859.07	3233.11	2565.62 ± 391.33	1716.33	3330.02	1275.16	1947.52	3172.62	5958.32	3868.87	3448.10
Carnosine	LD	LD	LD	LD	56.95	11.39 ± 11.39	67.47	111.65	94.26	79.70	149.84	110.54	108.90	65.60
Arginine	2.72	LD	LD	LD	LD	0.54 ± 0.54	LD	LD	3.24	2.15	2.40	4.64	4.73	2.70

[a]LD means less than detectable at sample level.

TABLE 1-VIII.- FREE AMINO ACID ANALYSIS FOR THE SCIENCE PILOT

[Each value represents milligrams collected per 24 hr]

Variable	Sample schedule													
	F-28	F-21	F-11	F-1	F-1A	Preflight mean ± SE	MD 11	MD 17	MD 20	MD 26	R+1	R+6	R+11	R+16
Phosphoserine	22.09	12.84	16.33	21.37	17.64	18.05 ± 1.70	17.96	33.01	15.13	14.74	16.20	15.56	19.10	13.72
Phosphoethanolamine	7.50	7.49	5.93	7.97	11.48	8.07 ± 0.92	8.46	16.99	9.25	10.00	11.72	9.94	11.10	6.71
Taurine	434.42	145.12	116.43	73.38	462.20	246.31 ± 83.37	383.90	258.41	112.81	146.52	557.89	112.09	122.99	95.70
Aspartic acid	9.11	5.04	3.65	4.54	6.26	5.72 ± 0.95	10.00	14.53	5.44	4.77	10.93	5.02	15.90	7.62
Threonine	19.34	21.42	14.80	13.80	26.10	19.09 ± 2.25	24.66	40.25	16.47	15.10	13.88	25.34	17.61	11.51
Serine	33.49	40.27	27.01	28.51	46.59	35.17 ± 3.68	33.46	75.44	30.29	27.72	23.61	24.61	35.06	22.79
Glutamine/asparagine	67.10	102.09	64.05	71.88	117.40	84.50 ± 10.66	89.28	137.68	68.33	59.78	42.24	81.60	83.56	58.14
Glutamic acid	8.08	5.02	2.68	3.78	5.11	4.93 ± 0.90	3.45	8.54	5.46	4.15	3.78	2.54	4.99	4.02
Glycine	42.70	56.02	41.90	41.05	61.19	48.57 ± 4.18	44.22	97.17	41.45	39.92	27.21	40.33	39.09	29.17
Alanine	28.26	36.82	26.83	29.19	37.82	31.78 ± 2.30	29.69	60.72	29.35	24.17	21.15	29.86	28.52	19.23
α-aminoadipic acid	9.54	10.35	4.27	6.94	11.78	8.58 ± 1.33	12.56	18.50	8.54	10.34	3.26	6.86	13.78	7.39
α-amino-n-butyric acid	2.53	1.55	1.25	1.36	2.06	1.75 ± 0.24	1.32	2.22	1.51	1.31	0.70	0.16	0.65	0.72
Valine	5.87	5.83	1.96	2.50	4.67	4.17 ± 0.82	3.62	5.96	2.53	2.20	11.24	9.87	3.48	1.90
Half cystine	7.74	9.63	11.12	15.20	36.52	16.04 ± 5.26	13.50	56.67	26.07	27.91	7.68	6.44	17.17	15.65
Cystathionine	26.68	26.18	19.38	24.63	11.73	21.72 ± 2.81	7.71	16.93	23.01	7.96	8.64	19.49	35.02	10.99
Methionine	6.28	7.44	5.27	5.98	8.72	6.74 ± 0.61	9.41	20.78	10.36	10.22	6.99	10.17	12.87	5.71
Isoleucine	4.26	5.28	3.80	4.98	5.46	4.76 ± 0.31	3.71	9.25	4.87	4.59	6.38	5.55	3.88	1.89
Leucine	9.01	9.41	5.92	7.44	10.51	8.46 ± 0.80	8.30	16.04	8.11	7.52	10.25	12.91	7.91	5.45
Tyrosine	26.39	26.66	16.36	17.87	25.78	22.61 ± 2.26	22.37	40.29	19.39	18.98	16.92	13.80	12.67	11.63
Phenylalanine	14.13	11.25	8.11	9.60	14.86	11.59 ± 1.29	16.92	33.53	16.40	16.09	14.67	6.65	7.04	9.73
β-alanine	0.85	4.56	0.58	3.98	1.05	2.20 ± 0.85	14.11	7.78	4.40	0.46	13.79	0.29	0.42	8.02
β-aminoisobutyric acid	0.69	5.54	3.61	9.64	5.96	5.09 ± 1.47	3.98	7.78	3.37	3.33	9.84	3.53	3.48	4.68
Hydroxylysine	4.91	0.71	LD	LD	2.61	1.65 ± 0.94	2.69	9.01	1.28	11.69	0.61	2.33	2.06	4.81
Ornithine	3.96	3.07	1.44	2.12	8.28	3.77 ± 1.20	4.72	7.63	2.58	5.59	0.57	1.91	1.86	2.33
Lysine	58.41	136.01	68.38	55.49	190.94	101.85 ± 26.70	184.20	191.76	105.12	89.72	29.17	172.16	107.08	51.15
1-methylhistidine	120.62	164.36	43.09	47.77	99.34	95.04 ± 22.82	57.15	105.93	93.65	59.53	63.21	121.19	62.70	44.97
Histidine	93.94	89.14	27.23	23.84	165.24	79.88 ± 25.96	131.16	200.47	70.94	65.91	37.57	83.26	81.56	65.19
3-methylhistidine	66.77	53.17	37.76	35.00	155.27	69.59 ± 22.17	124.05	221.41	92.27	85.55	66.00	114.62	121.15	110.40
Anserine	20.44	8.12	LD	LD	18.27	9.37 ± 4.35	LD	1.65	0.40	0.16	LD	1.72	28.52	1.11
Creatinine	2710.70	2372.21	1539.66	2550.18	3324.78	2499.51 ± 288.60	1862.51	2921.71	1248.94	2250.32	2047.29	3729.08	3208.59	3835.30
Carnosine	LD	LD	LD	LD	91.82	18.36 ± 18.36	157.87	142.16	34.50	60.59	42.09	58.31	40.47	11.49
Arginine	3.76	LD	LD	LD	5.84	1.92 ± 1.22	LD	10.64	3.70	1.99	2.03	2.76	2.55	3.50

TABLE 1-IX.- FREE AMINO ACID ANALYSIS FOR THE PILOT

[Each value represents milligrams collected per 24 hr]

Variable	Sample schedule													
	F-28	F-21	F-11	F-1	F-1A	Preflight mean ± SE	MD 11	MD 17	MD 20	MD 26	R+1	R+6	R+11	R+16
Phosphoserine	8.24	10.58	23.12	10.68	31.84	16.89 ± 4.56	20.66	32.17	17.79	27.08	23.04	24.85	18.64	29.89
Phosphoethanolamine	4.07	9.30	11.13	4.16	17.25	9.18 ± 2.45	9.52	16.62	13.47	15.21	12.13	15.90	11.39	16.18
Taurine	50.27	64.52	75.76	45.08	308.31	108.79 ± 50.17	154.55	139.96	119.61	116.28	199.53	57.53	39.98	201.76
Aspartic acid	1.83	5.00	4.93	3.24	6.96	4.39 ± 0.87	5.22	5.93	10.07	4.56	10.88	4.26	7.17	8.79
Threonine	5.71	19.28	19.70	10.90	25.96	16.31 ± 3.57	17.06	19.30	20.74	11.79	16.57	27.34	10.73	20.45
Serine	10.78	40.33	41.09	23.57	58.47	34.85 ± 8.16	37.73	48.91	45.08	36.16	21.30	39.65	31.54	51.81
Glutamine/asparagine	43.37	98.08	73.22	46.72	126.23	77.52 ± 15.70	86.01	101.56	99.12	69.98	75.27	96.51	70.90	96.60
Glutamic acid	1.00	3.48	4.07	2.14	6.97	3.53 ± 1.01	2.96	6.29	6.24	5.48	2.62	3.06	5.21	7.03
Glycine	28.26	93.40	95.03	71.83	167.39	91.18 ± 22.54	100.33	131.43	116.45	93.44	82.51	95.55	77.87	157.63
Alanine	9.13	30.68	35.70	25.66	50.08	30.25 ± 6.67	36.18	48.15	43.63	32.32	27.72	33.48	25.39	50.58
α-aminoadipic acid	2.72	5.41	6.51	2.46	22.77	7.97 ± 3.78	11.66	15.73	12.40	12.40	12.94	10.69	10.80	12.71
α-amino-n-butyric acid	0.65	1.59	1.42	0.30	1.19	1.03 ± 0.22	1.27	2.47	2.39	2.18	0.38	0.29	LD	LD
Valine	1.81	3.87	1.97	1.50	5.78	2.99 ± 0.81	9.48	3.96	3.25	3.10	15.24	12.48	LD	1.12
Half cystine	3.79	5.33	9.15	4.66	26.39	9.86 ± 4.23	13.54	28.62	20.02	18.53	4.62	5.50	LD	LD
Cystathionine	8.60	19.79	26.55	3.53	12.16	14.13 ± 4.08	24.07	40.49	36.86	31.48	23.88	31.02	44.48	53.79
Methionine	1.81	4.80	7.67	8.47	11.21	6.79 ± 1.61	12.14	22.22	12.32	15.59	12.97	15.54	13.69	18.91
Isoleucine	1.31	4.06	5.75	3.26	8.90	4.66 ± 1.28	9.16	15.87	10.34	7.50	8.24	12.10	3.94	19.49
Leucine	3.52	8.99	10.16	6.05	15.03	8.75 ± 1.95	13.11	16.10	11.38	13.03	12.81	16.25	10.09	20.95
Tyrosine	11.31	19.93	17.52	10.91	26.37	17.21 ± 2.88	16.68	21.20	19.34	16.00	15.24	17.99	13.05	21.10
Phenylalanine	5.35	7.72	7.77	4.35	10.95	7.23 ± 1.14	7.66	11.71	11.08	9.02	7.38	8.71	6.98	9.43
β-alanine	2.57	0.93	0.60	0.71	3.81	1.72 ± 0.63	5.92	0.28	5.53	0.64	0.84	LD	LD	0.91
β-aminoisobutyric acid	7.44	31.00	16.15	8.01	22.87	17.09 ± 4.49	13.96	14.47	15.01	15.47	9.44	21.11	12.31	17.20
Hydroxylysine	0.11	LD	LD	LD	0.08	0.04 ± 0.02	LD	LD	2.17	1.92	1.11	1.31	0.35	0.87
Ornithine	0.39	LD	0.07	LD	2.97	0.69 ± 0.58	0.32	LD	0.70	0.83	0.56	1.06	0.78	0.87
Lysine	14.39	132.72	70.00	103.56	49.00	73.93 ± 20.64	19.60	152.84	4.29	72.12	33.26	45.40	30.37	30.89
1-methylhistidine	46.12	47.62	62.67	99.56	145.02	80.20 ± 18.85	49.39	143.91	178.47	198.43	101.56	138.94	63.65	106.61
Histidine	22.87	47.10	34.33	60.96	213.57	75.77 ± 35.03	93.00	76.20	114.39	86.52	83.57	125.44	78.04	108.75
3-methylhistidine	17.02	24.98	32.87	73.14	159.19	61.44 ± 26.28	82.58	209.33	126.71	104.89	78.80	146.07	107.25	136.91
Anserine	LD	LD	LD	LD	29.98	6.00 ± 6.00	LD	LD	LD	31.25	0.65	LD	32.04	64.32
Creatinine	508.36	1185.13	923.79	226.53	3120.80	1192.92 ± 509.50	1544.27	2949.49	2174.17	1927.09	2884.94	3356.30	2569.64	2402.51
Carnosine	LD	LD	LD	LD	31.96	6.39 ± 6.39	60.10	159.72	61.42	76.57	48.65	39.07	6.56	81.66
Arginine	LD	LD	LD	6.70	LD	1.34 ± 1.34	LD	LD	LD	LD	LD	LD	LD	LD

TABLE 1-X.- BODY FLUID VOLUMES

(a) Total body water

Schedule	Volume, liters	Volume/kg body weight, ml/kg
Commander		
Preflight mean ± SD	41.6 ± 0.4	670 ± 2
Recovery day	40.8	677
13 days postflight	41.2	678
Science pilot		
Preflight mean ± SD	48.8 ± 0.6	626 ± 13
Recovery day	48.0	649
13 days postflight	49.1	655
Pilot		
Preflight mean ± SD	52.2 ± 0.2	643 ± 4
Recovery day	50.0	658
13 days postflight	50.0	647

TABLE 1-X.- BODY FLUID VOLUMES - Concluded

(b) Extracellular fluid

Schedule	Volume, liters	Volume/kg body weight, ml/kg
Commander		
Preflight	16.1	257
Recovery day	15.8	262
13 days postflight	16.0	263
Science pilot		
Preflight	15.5	197
Recovery day	15.2	204
13 days postflight	15.3	204
Pilot		
Preflight	15.5	190
Recovery day	15.7	206
13 days postflight	15.7	203

TABLE 1-XI.- TOTAL BODY EXCHANGEABLE POTASSIUM (meq K)

Schedule	Flight crewmen		
	CDR	SPT	PLT
Preflight mean ± SD	3266 ± 50	3911 ± 58	3846 ± 52
Recovery day	2998	3678	3528
13 days postflight	3186	3704	3777

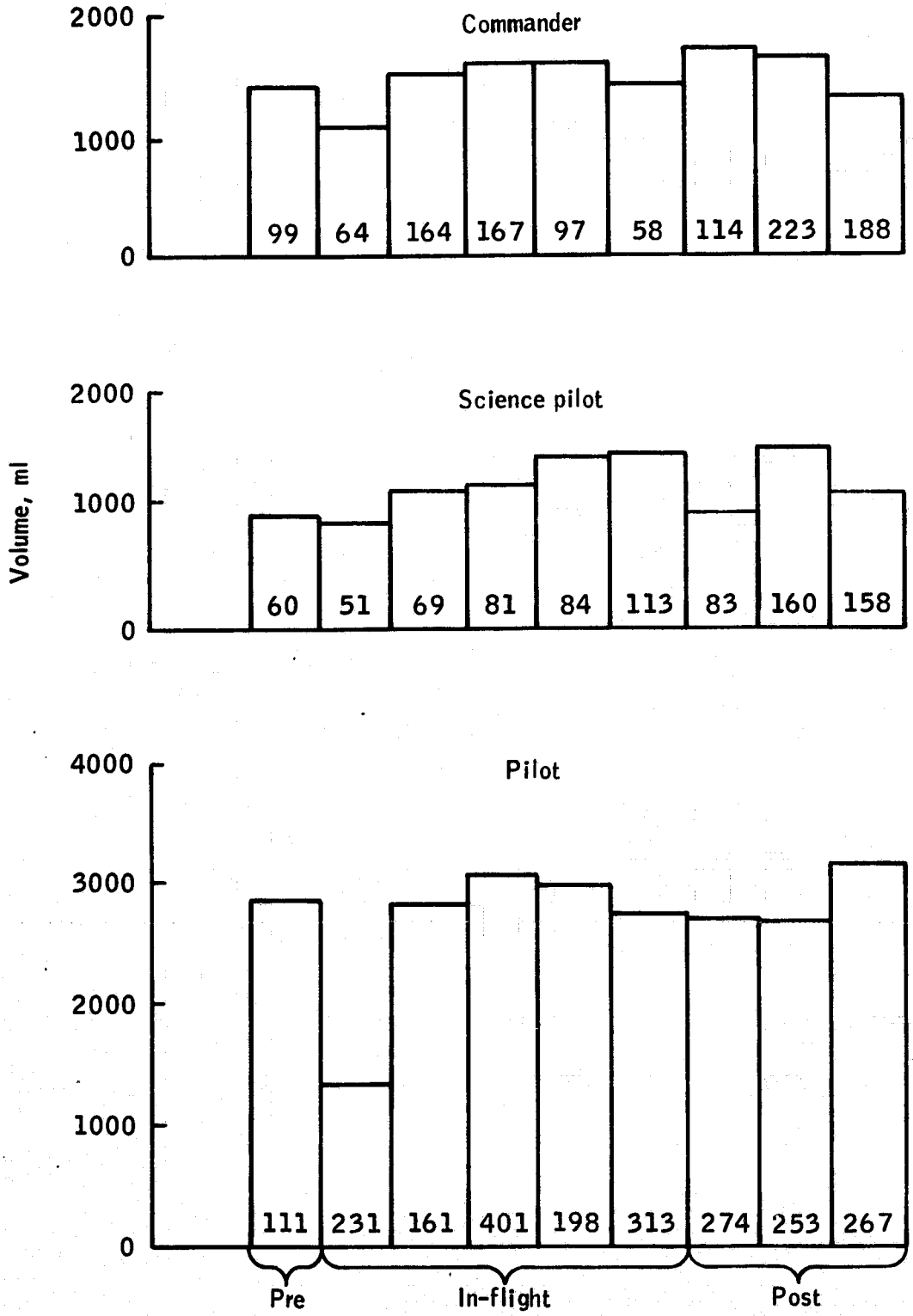

Figure 1-1.- Twenty-four-hour urine volume analyses. Numbers within bars are standard error of the mean.

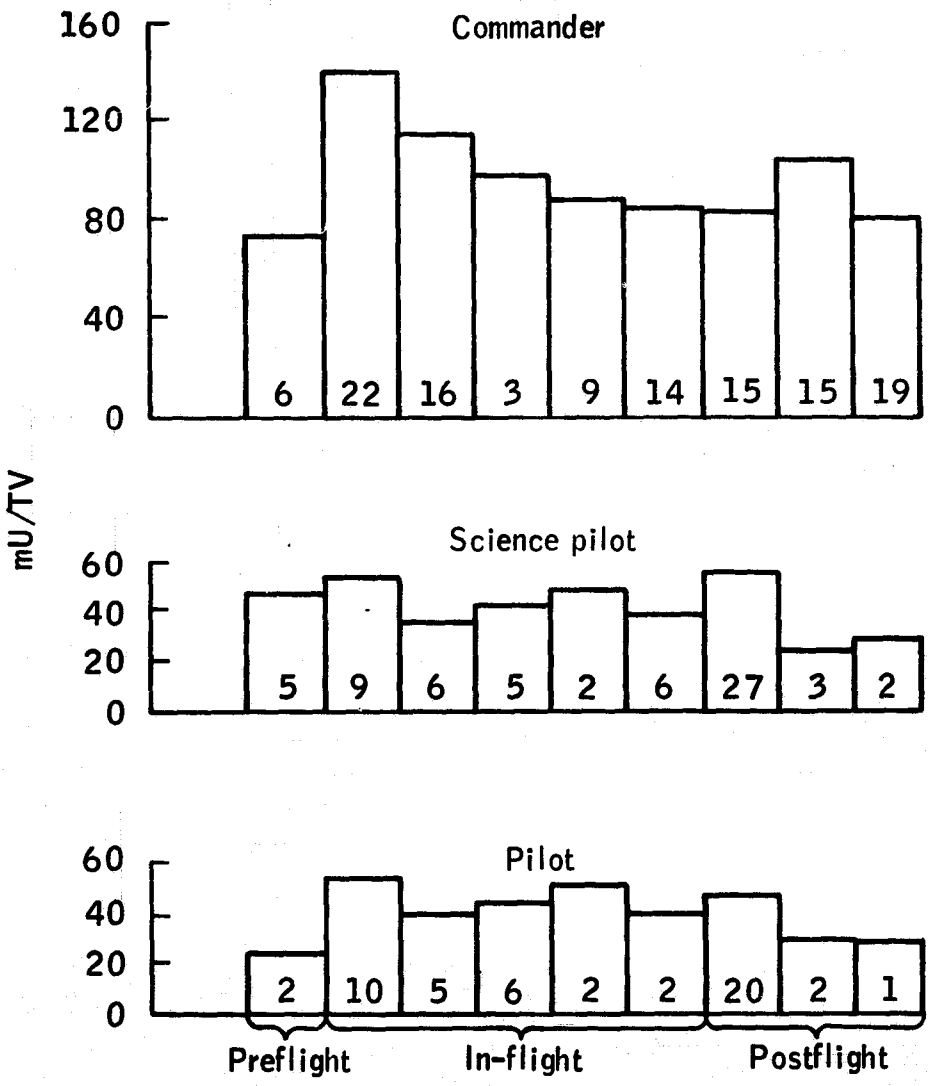

Figure 1-2.- Urinary ADH analyses (milliunits per total volume (mU/TV)). Numbers within bars are standard error of the mean.

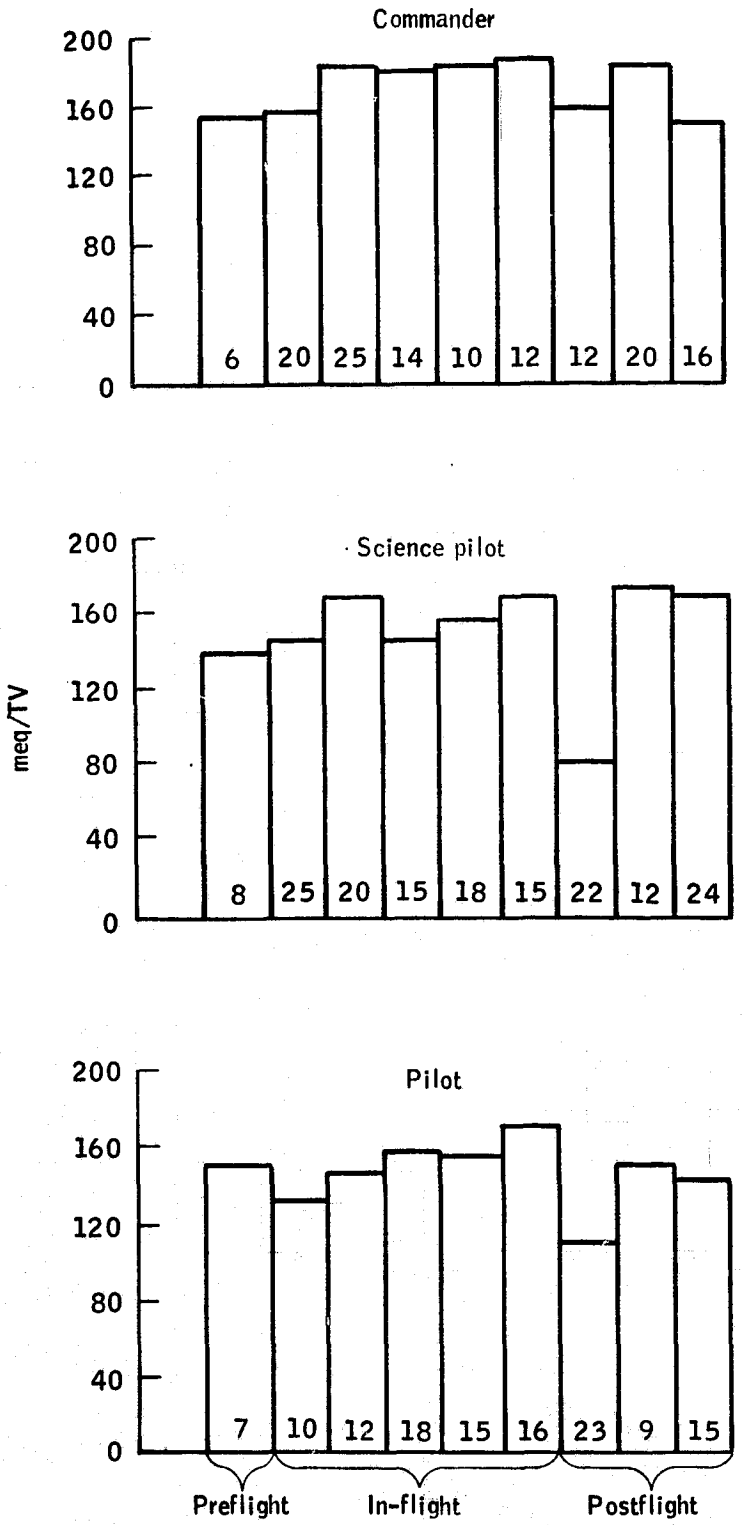

Figure 1-3.- Urinary sodium analyses. Numbers within bars are standard error of the mean.

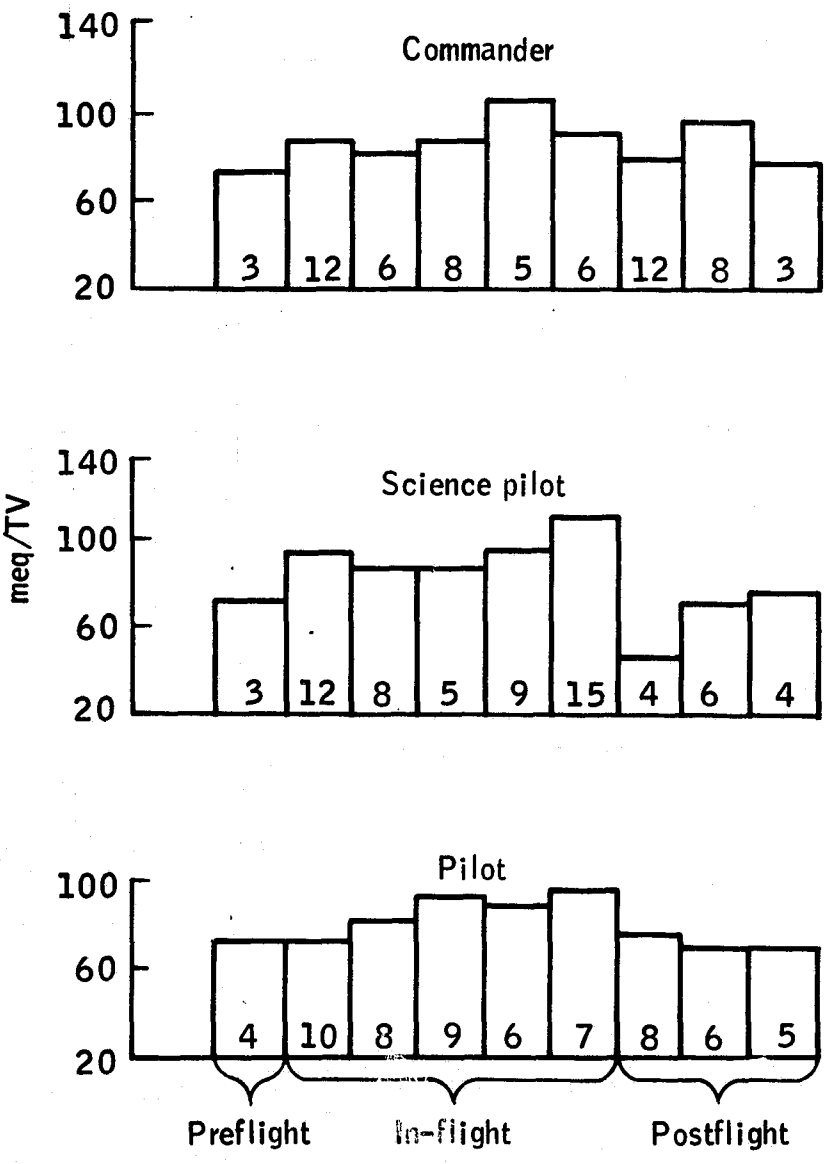

Figure 1-4.- Urinary potassium analyses. Numbers within bars are standard error of the mean.

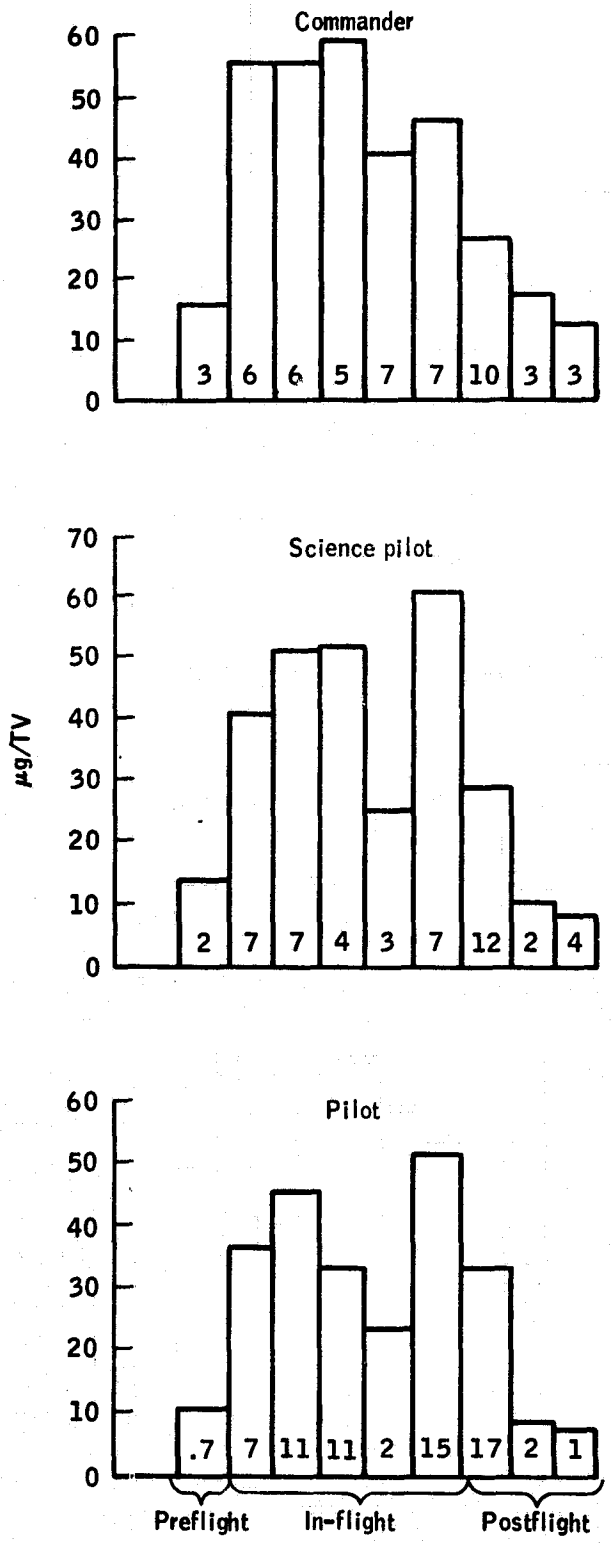

Figure 1-5.- Urinary aldosterone analyses. Numbers within bars are standard error of the mean.

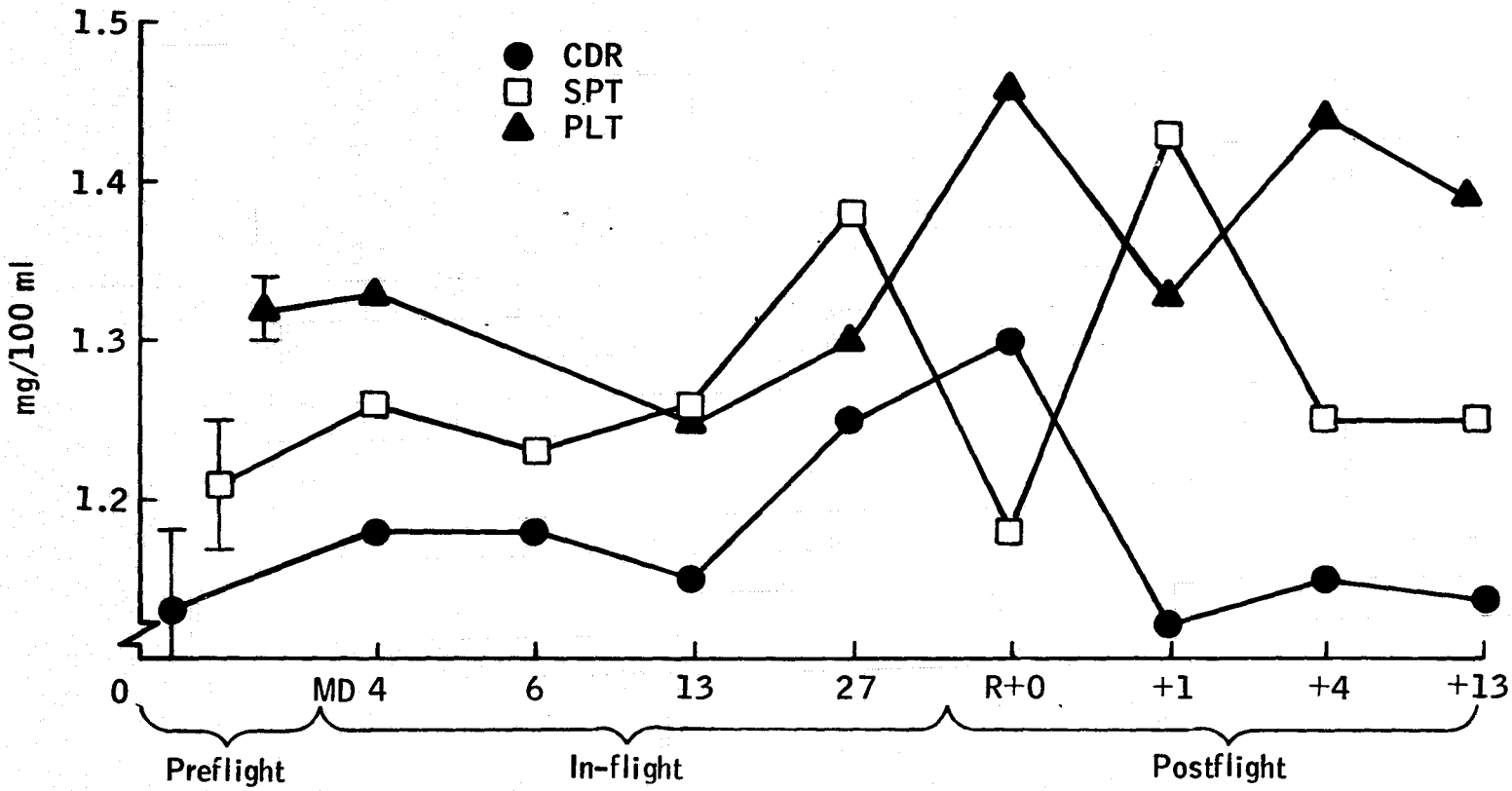

Figure 1-6.- Plasma creatinine analyses. Brackets represent mean ± standard error.

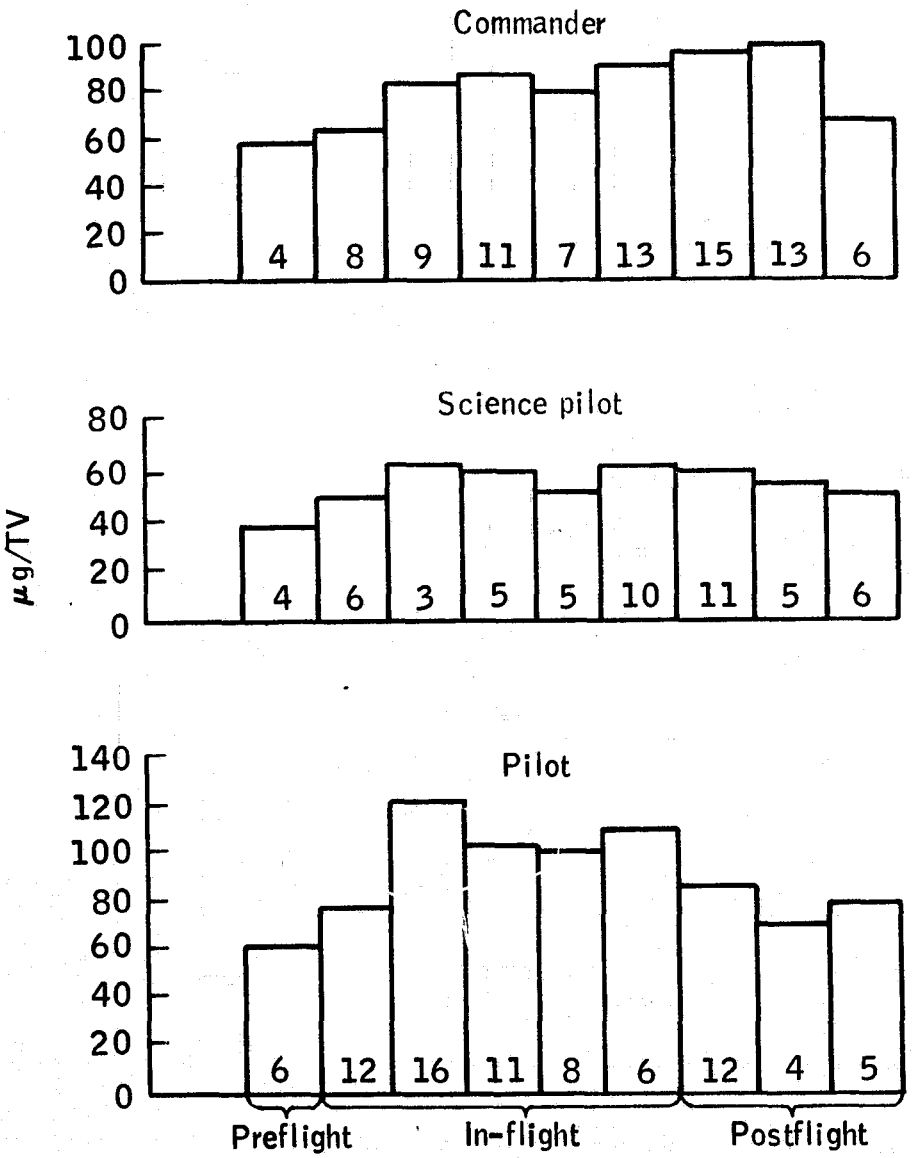

Figure 1-7.- Urinary cortisol analyses. Numbers within bars are standard error of the mean.

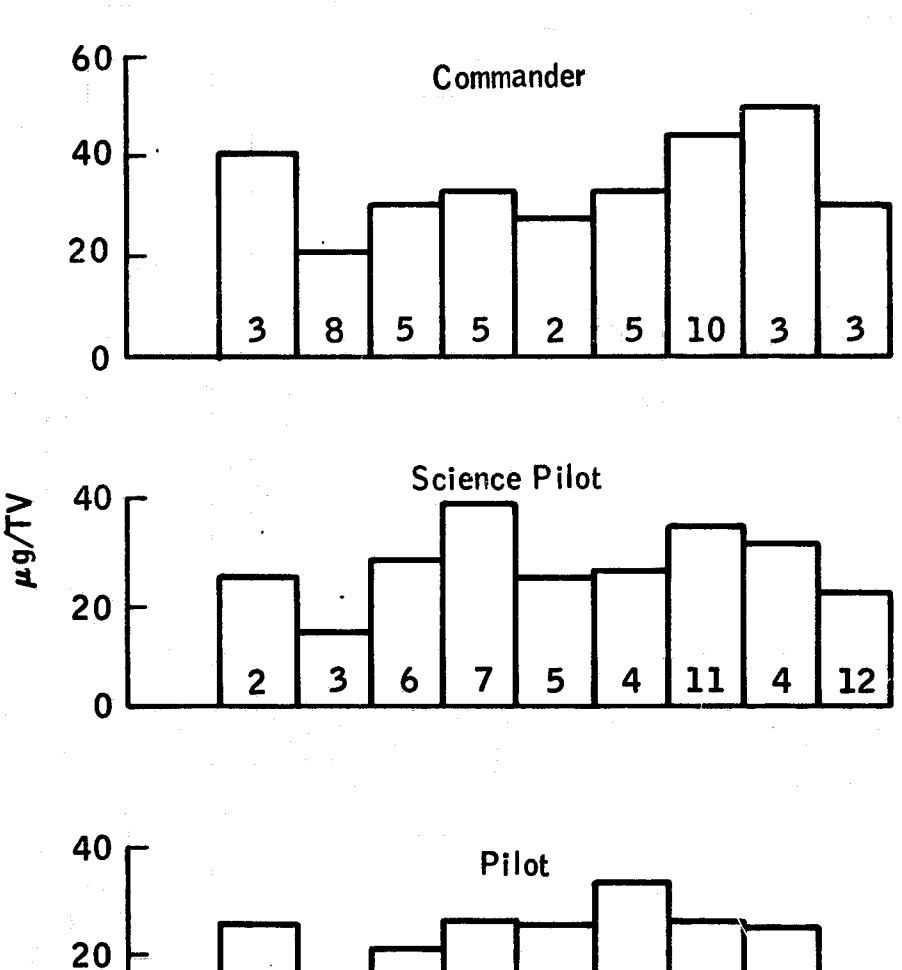

Figure 1-8.- Urinary epinephrine analyses. Numbers within bars are standard error of the mean.

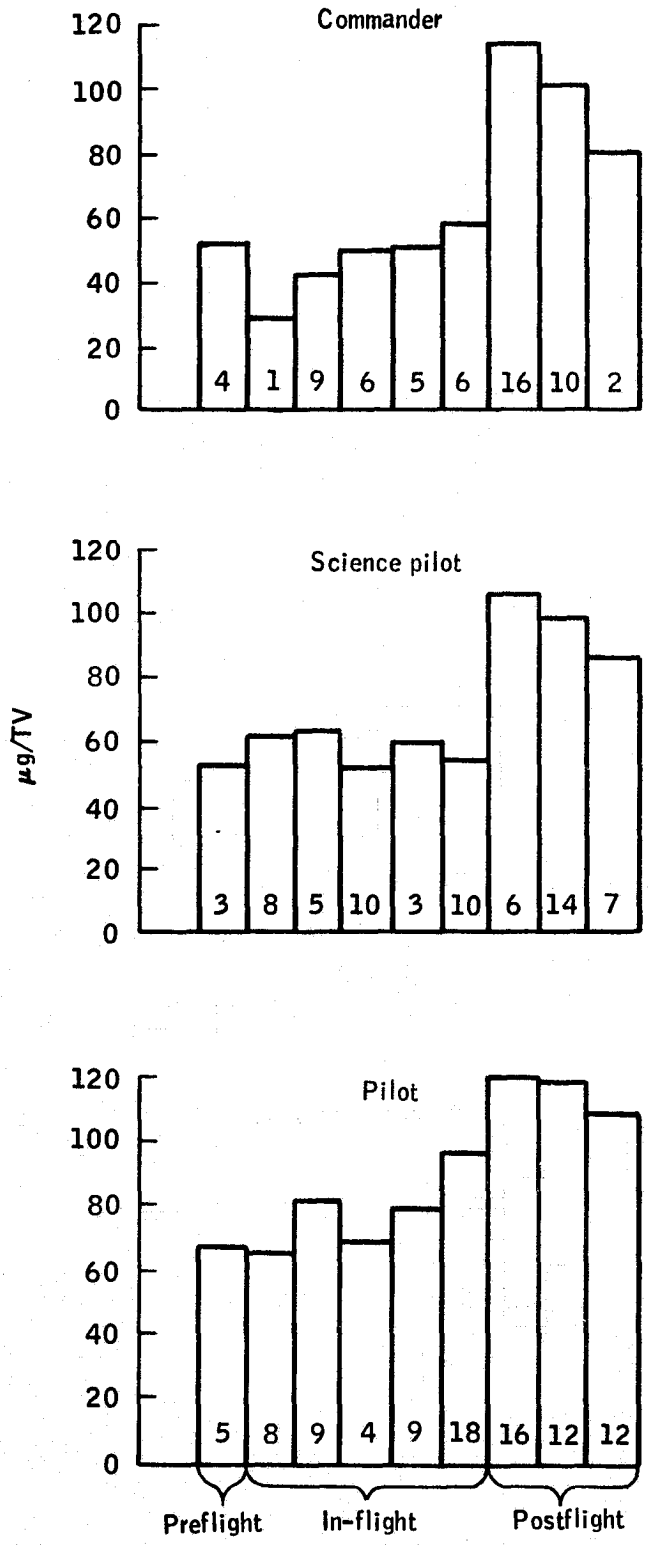

Figure 1-9.- Urinary norepinephrine analyses. Numbers within bars are standard error of the mean.

2. MODULATING THE PITUITARY-ADRENAL RESPONSE TO STRESS

By Joan Vernikos-Danellis, Ph. D.

INTRODUCTION

It is now generally accepted that the hypothalamic pituitary-adrenal response to stress is controlled by at least three mechanisms: corticosteroid negative feed back, central facilitatory, and central inhibitory, each of which can be demonstrated in the absence of steroids. All three mechanisms presumably exert their control by way of hypothalamic-hypophyseal neurohumoral pathways and are mediated by the adrenocorticotropic hormone (ACTH) releasing factor (CRF). Thus, it might be expected that the preexisting state of the individual, or "baseline," determines to a great extent the magnitude and the temporal characteristics of the response to a provocative stimulus or stress. This is true, for instance, of the hyperresponsiveness to reentry that has been observed in crewmembers following each space mission (ref. 2-1). It is true of animals following chronic exposure to cold (ref. 2-2), confinement (ref. 2-3), or water deprivation (ref. 2-4). It is also true of humans after 14 days of bed rest and possibly after relative confinement.[1] Therefore, a search has been made for a means of modulating the magnitude of this response to stress, a search based primarily on knowledge of the mechanisms regulating pituitary-adrenal activity.

Serotonin is believed to be a transmitter or regulator of neuronal function, and several reports have indicated a possible relationship between the pituitary-adrenal secretion of steroids and brain serotonin in the rat (refs. 2-5 and 2-6). This relationship was investigated further by evaluating the effects of altering brain 5-hydroxytryptamine (HT) levels on the daily fluctuation of plasma corticosterone and on the response of the pituitary-adrenal system to a stressful or noxious stimulus in the rat. The approach was either to inhibit brain 5-HT synthesis with para-chlorophenylalanine (PCPA) or to raise its level with precursors such as tryptophan or 5-hydroxytryptophan (5-HTP).

METHODS AND RESULTS

Male Sprague-Dawley rats (150 to 200 grams) were kept in an environment of 12 hours of light and 12 hours of dark (lights on at 0700) and given food and

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[1] J. Vernikos-Danellis and M. E. Dallman, unpublished observations, 1974.

and water ad libitum. The animals were injected intraperitoneally (I.P.) with pyrogen-free saline or PCPA in saline at a dose of 300 mg/kg/day.

Daily injections of PCPA for 2 or 4 days raised the morning low and prevented the evening rise in plasma corticosterone levels. This difference in levels was observed only in those animals killed within 24 hours after the last injection (fig. 2-1). Figure 2-2 shows that pretreatment with PCPA enhanced and sustained the response to the stress of ether for 1 minute or of electrical shock for 4.5 minutes. Pretreatment with PCPA also resulted in a 50-percent increase in anterior pituitary ACTH concentration (9.34 ± 1.89 ml/mg of pituitary tissue in PCPA-treated animals). Thus, inhibition of brain 5-HT synthesis enhanced the stress response in addition to altering the diurnal pattern of the pituitary-adrenal system.

The alternate approach in studying the role of brain 5-HT in the regulation of pituitary-adrenal function was to attempt to increase brain 5-HT by the use of precursors. L-tryptophan (408 mg/kg), or 5-HTP alone (212 mg/kg), or 5-HTP (50 mg/kg) with the peripheral dicarboxylase inhibitor, L-α-hydrazinomethyldihydroxyphenylalanine (MK 486, 20 mg/kg), were injected I.P. into groups of rats. The animals were then studied 2 hours later, when brain 5-HT had been reported to be at maximal levels after injection of precursors in a similar study (ref. 2-7) and when plasma corticosterone levels had usually returned to normal in control animals after the stress of an injection. The animals were decapitated and bled 2.5 or 15 minutes after the stress of ether. Figure 2-3 shows that, in adrenalectomized rats, tryptophan completely abolished the stress-induced secretion of ACTH. The stress response was reduced significantly ($P < 0.05$) by 5-HTP alone or in combination with MK 486. Hence, these precursors of 5-HT tended to decrease or inhibit the pituitary-adrenal response to stress. This inhibition was more evident in the absence of the adrenals.

Preliminary reports on human studies also appear to support this 5-HT/pituitary-adrenal relationship. The urinary 17-hydroxycorticosteroid (OHCS) response to oral ingestion of MK 486 and 5-HTP was determined in four normal male human volunteers. Figure 2-4 shows the results of this test in one of the four subjects where 24-hour urinary 17-OHCS excretion was measured daily for 12 days. After 2 days of control collections, the subjects were given placebo capsules containing lactose for 2 days. This was followed by 2 days of MK 486 (50 milligrams, four times a day) with placebo (for 5-HTP), to control for possible MK 486 effects on the steroid excretion; an additional 4 days of MK 486 (50 milligrams, four times a day) with increasing doses of 5-HTP (100, 200, 300, and 400 mg/day, respectively); and finally 2 days of placebo only. MK 486 raised 17-OHCS excretion and the combination of MK 486 and 5-HTP produced nausea and vomiting. However, when treatment was discontinued, a prompt drop in 17-OHCS excretion, and a return to baseline levels resulted. Although it is evident that the MK 486/5-HTP combination is not acceptable for human use, at least in the dosage used, the results provide encouraging evidence that 5-HT precursors could be used to suppress pituitary-adrenal secretion in humans.

It is now reasonably accepted that the pituitary-adrenal system functions as a closed-loop system. In this system, the positive vector, or "driving

force," corresponds to environmental stimuli activated by way of hypothalamic neurohumoral pathways (ref. 2-8), and the negative vector corresponds to the level of corticosteroids, which act by feedback inhibition on the pituitary-adrenal system (ref. 2-9). The author's findings of enhancement of the stress response by inhibition of serotonin synthesis and indications of a reduction of the stress response by serotonin precursors suggested that serotonin may mediate or modulate the negative feedback mechanism regulating pituitary-adrenal function (fig. 2-5). If this hypothesis were correct, then the inhibitory action of corticosteroids on pituitary ACTH secretion would be expected to be less effective in animals whose brain serotonin had been depleted. Figure 2-6 illustrates the results of an experiment to test this possibility and compares the corticosterone stress-response-inhibiting properties of two doses of prednisolone in control rats and in animals whose brain serotonin content was markedly reduced by pretreatment with PCPA. Both doses of the steroid effectively inhibited the response to the stress of ether and laparotomy in the control animals. In 5-HT-depleted animals, neither dose was capable of blocking the stress response, further indicating that serotonin is indeed involved in the negative feedback to the pituitary-adrenal system.

CONCLUDING REMARKS

These results have led to the suggestion that in some disease states such as Cushing's disease, in which there is a similar inability of corticosteroids to suppress the pituitary-adrenal system, there is a defect in serotonergic neuronal processes that impairs pituitary-adrenal feedback mechanisms. A similar but reversible serotonergic defect may be involved in the hyperresponsiveness to provocative stimuli during various chronic exposures wherein pretreatment with serotonin precursors or appropriate dietary manipulation may be effective in modulating the stress response.

REFERENCES

2-1. Leach, C.: The Endocrine Electrolyte and Fluid Volume Changes Associated With Apollo Missions. NASA SP-368, 1975.

2-2. Daniels-Severs, A.; Goodwin, A. L.; Keil, L. C.; and Vernikos-Danellis, J., et al.: Effect of Chronic Crowding and Cold on the Pituitary-Adrenal System: Responsiveness to an Acute Stimulus During Chronic Stress. Pharmacology, vol. 9, 1973, pp. 348-356.

2-3. Sakellaris, P. C.; and Vernikos-Danellis, J.: Increased Rate of Response of the Pituitary-Adrenal System Induced by Repeated Stress (abs.). Endocrin. Soc. Mtg., Chicago, Ill., vol. 92: A-80 (Abstract), 1973.

2-4. Sakellaris, P. C.; and Vernikos-Danellis, J.: Alteration of Pituitary-Adrenal Dynamics Induced by a Water Deprivation Schedule. Physiol. & Behav., vol. 12, June 1974, pp. 1067-1070.

2-5. Scapagnini, U.; Moberg, G. F.; Van Loon, G. R.; De Groot, J.; and Ganong, W. F.: Relation of Brain 5-hydroxytryptamine Content to the Diurnal Variation in Plasma Corticosterone in the Rat. Neuroendocrinology, vol. 7, 1971, pp. 90-96.

2-6. Krieger, Dorothy T.; and Rizzo, Frank: Serotonin Mediation of Circadian Periodicity of Plasma 17-hydroxycorticosteroids. Am. J. Physiol., vol. 217, no. 6, Dec. 1969, pp. 1703-1707.

2-7. Grahame-Smith, D. G.: Studies In Vivo on the Relationship Between Brain Tryptophan, Brain 5-HT Synthesis and Hyperactivity in Rats Treated with a Monoamine Oxidase Inhibitor and L-tryptophan. J. Neurochem., vol. 18, 1971, June 1971, pp. 1053-1066.

2-8. Harris, G.: Neural Control of the Pituitary Gland. Physiol. Rev., vol. 28, 1948, pp. 139-179.

2-9. Sayers, G.; and Sayers, M. A.: Regulation of Pituitary Adrenocorticotrophic Activity During Response of Rat to Acute Stress. Endocrinology, vol. 40, 1947, pp. 265-273.

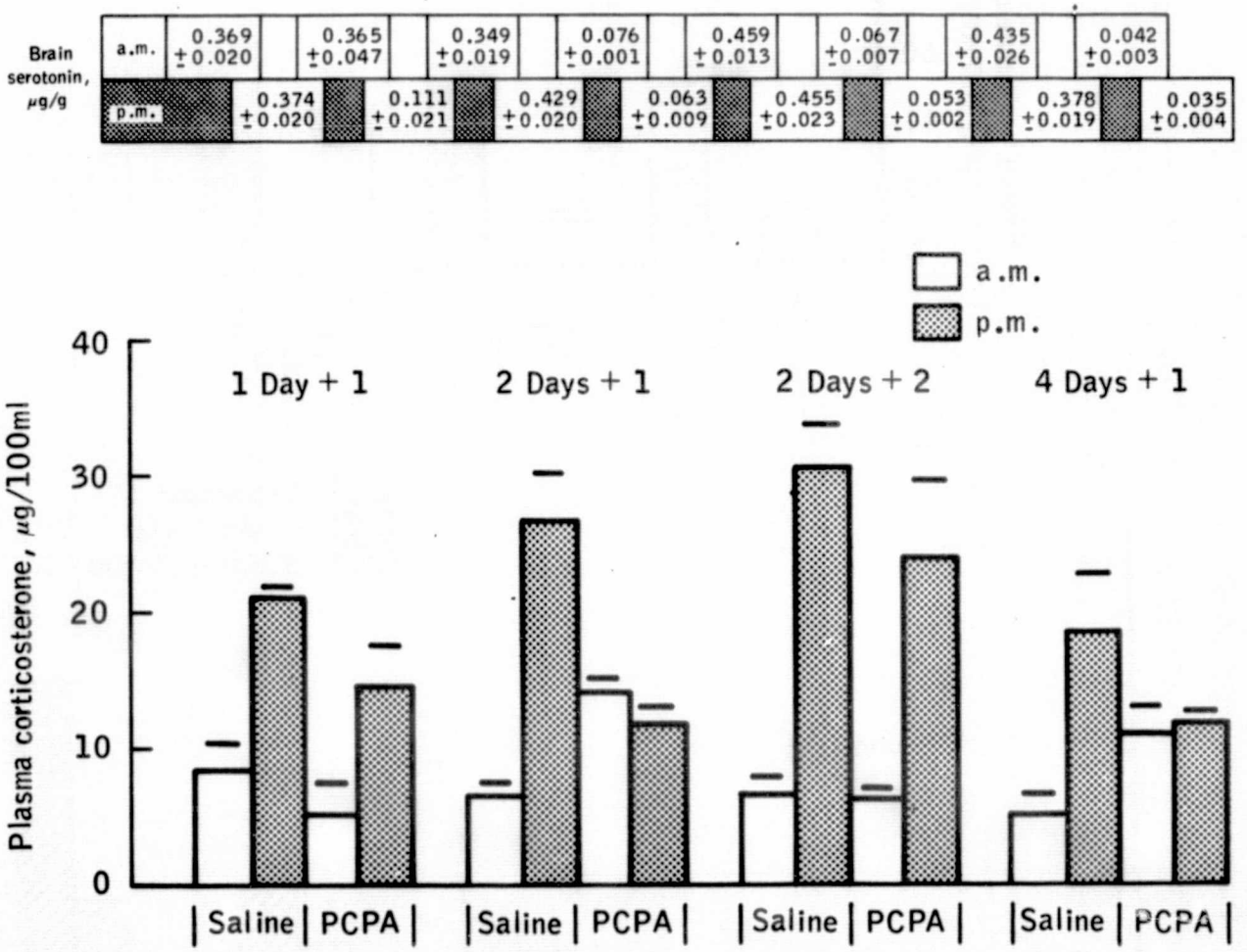
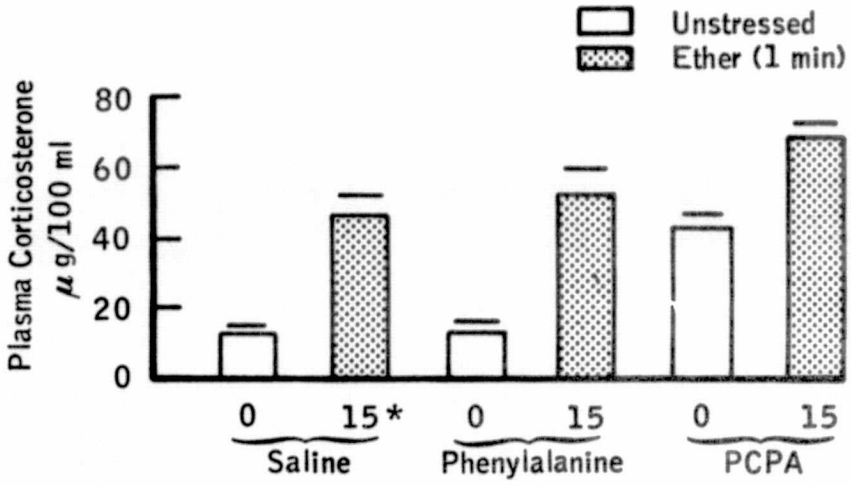


Figure 2-1.- Effect on PCPA pretreatment (300 mg/kg/day I.P.) on evening (2000) levels of plasma corticosterone in rats. Rats were killed 24 hours after one, two, or four daily injections; or 48 hours after two daily injections. Horizontal bars denote standard error of the mean (N = 7).



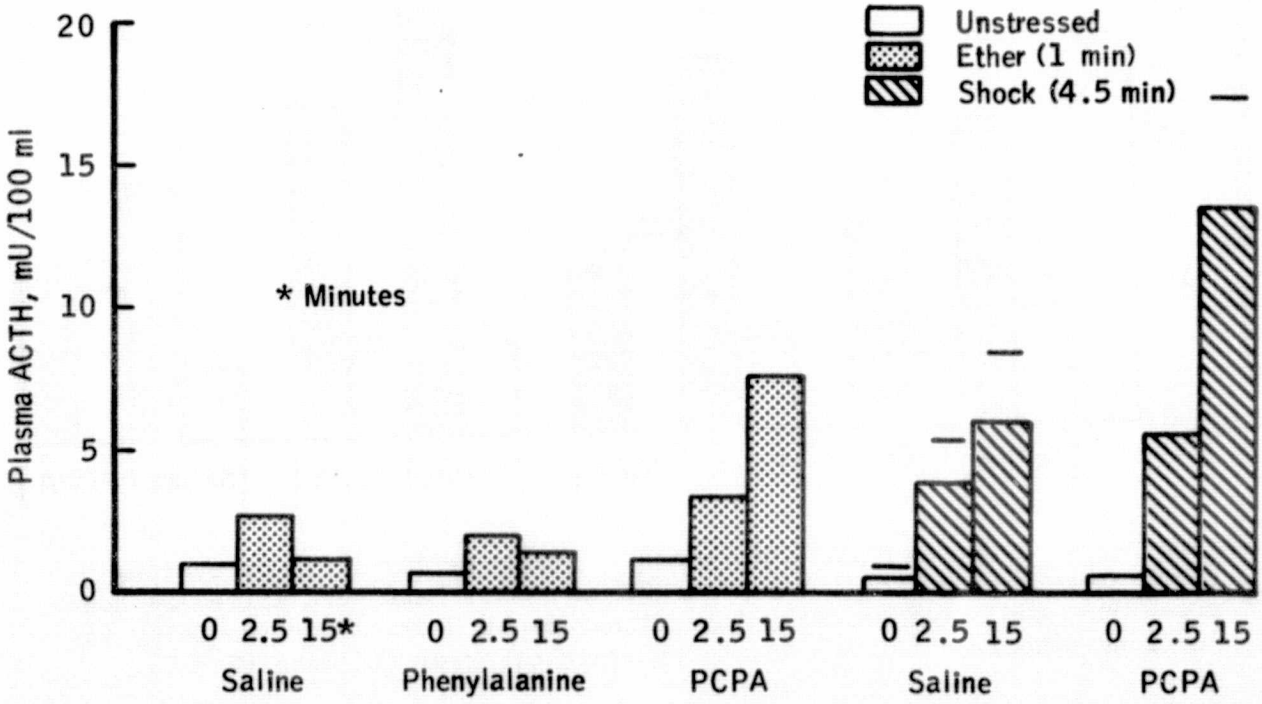


Figure 2-2.- Effect of PCPA on the corticosterone and ACTH concentrations in response to stress of ether (1 minute) or electrical shock (4.5 minutes). Horizontal bars denote standard error of the mean.

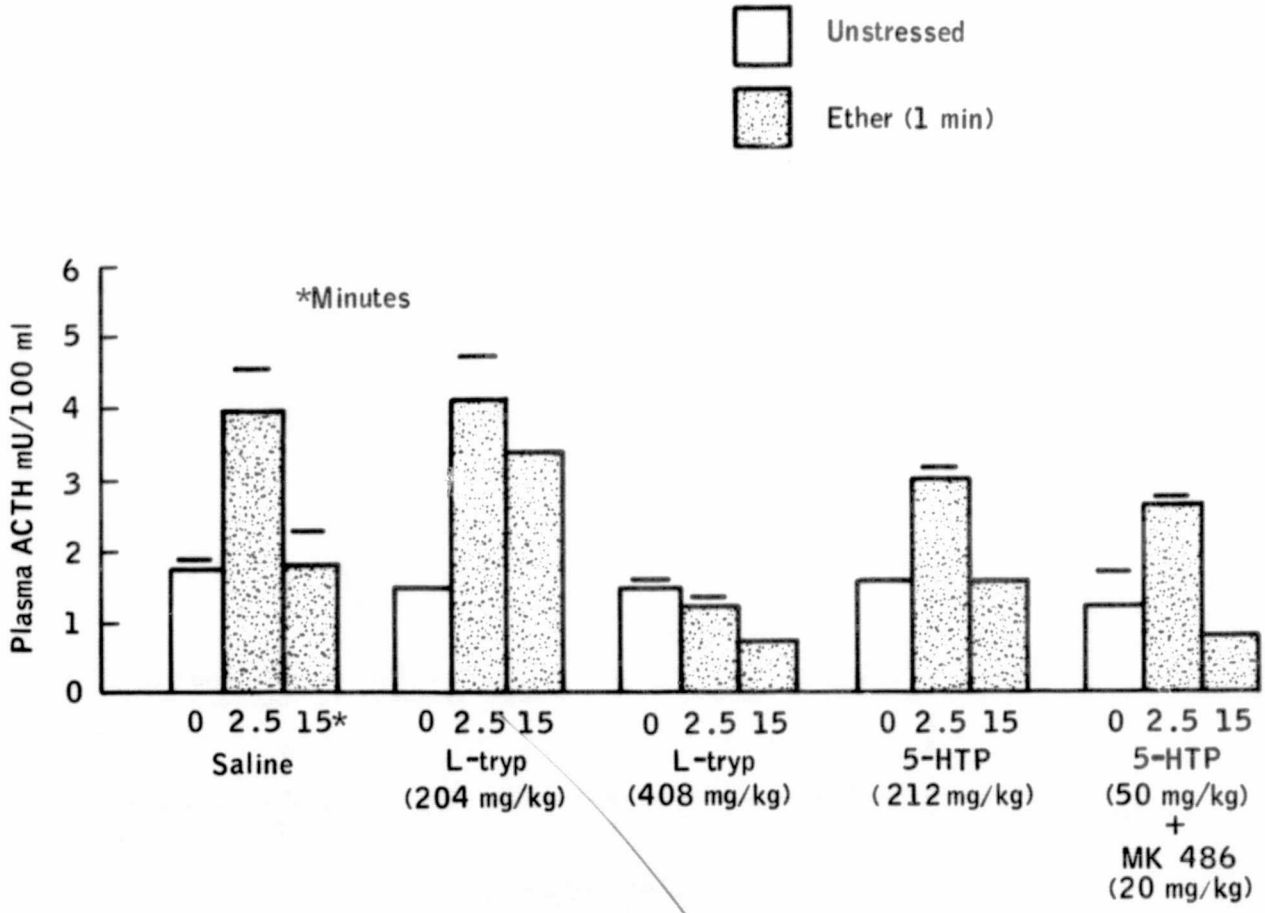


Figure 2-3.- Effect of L-tryptophan, of 5-HTP, or of 5-HTP with MK 486 on the pituitary-adrenal response to the stress of ether (for 1 minute) in rats adrenalectomized 24 hours previously. Horizontal bars denote standard error of the mean.

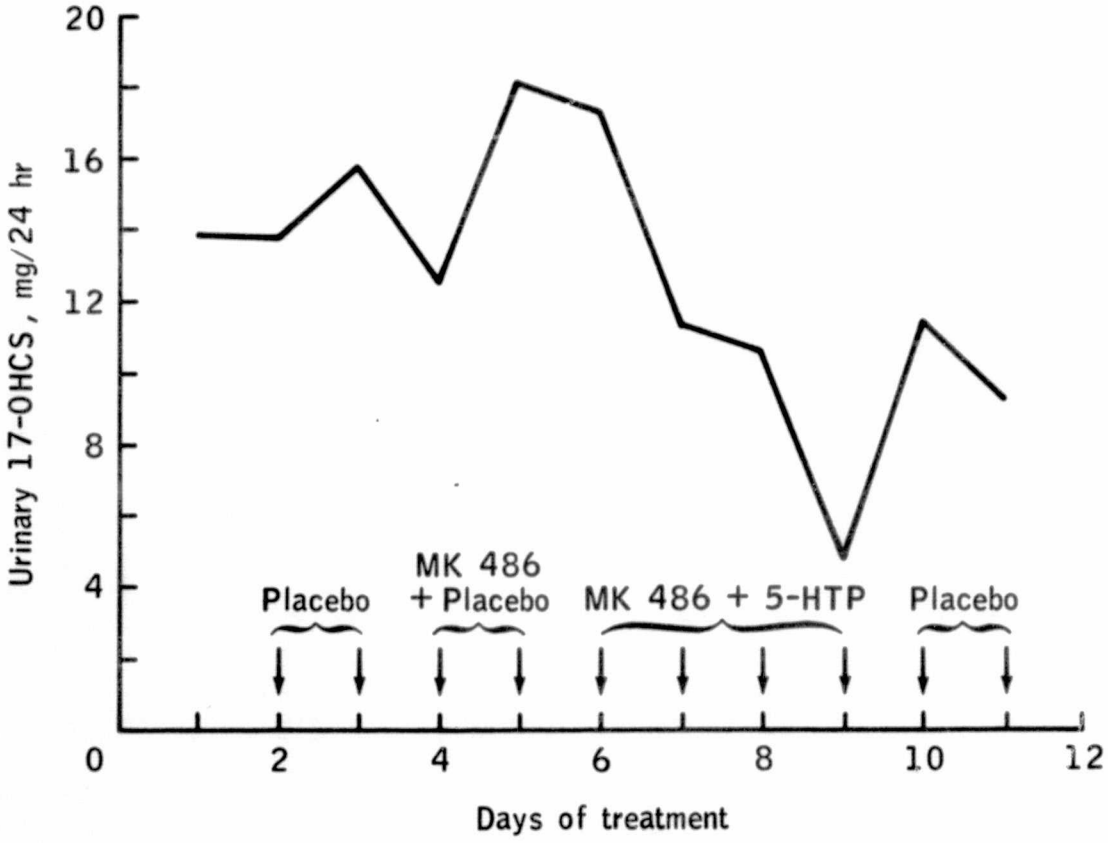


Figure 2-4.- Effect of 5-HTP and MK 486 on 24-hour urinary excretion of 17-OHCS in a normal human male.

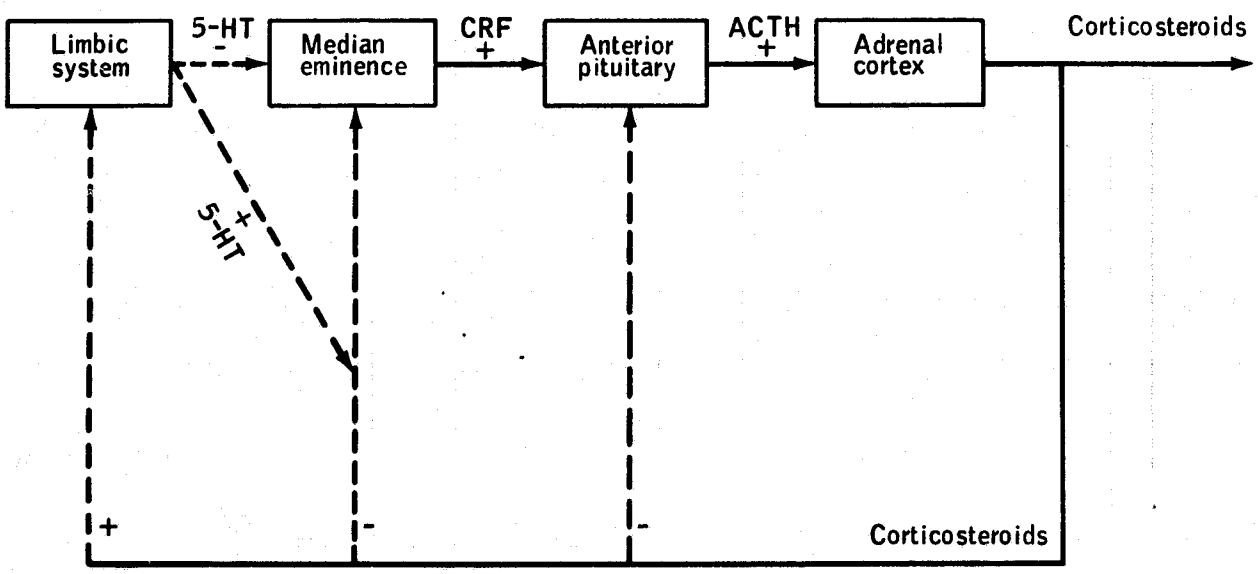


Figure 2-5.- Possible mechanisms of negative feedback regulation of ACTH secretion.

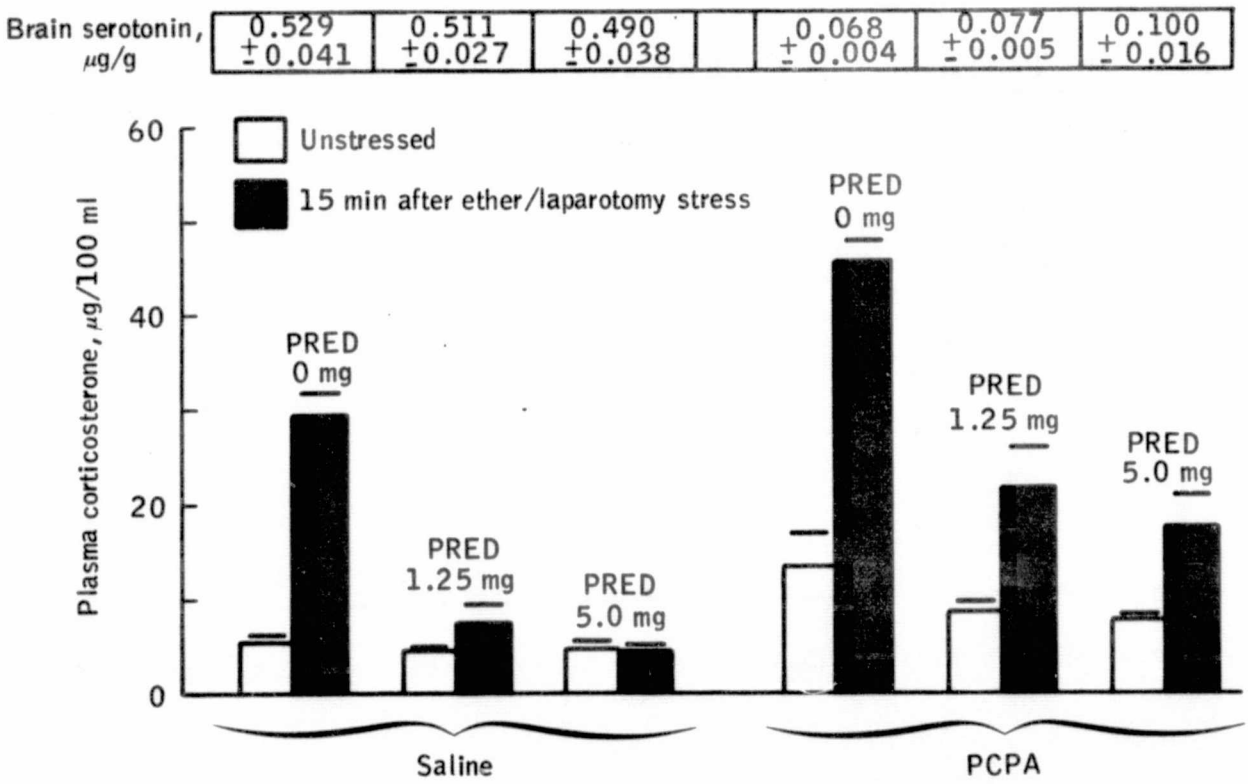


Figure 2-6.- Stress-inhibiting effects of prednisolone (PRED) in control or 5-HT-depleted rats. Rats were stressed 24 hours after two daily injections of PCPA (300 mg/kg/day I.P.) or saline, and 4 hours after injection of the steroid. Horizontal bars denote standard error of the mean.

3. SIGNIFICANCE OF BIORHYTHMS IN SPACE FLIGHT

Charles M. Winget, Ph. D.

INTRODUCTION

Every physiological system and every psychological function in the human body fluctuates temporally with a circadian period. No phase of medicine — from laboratory testing of new drugs and procedures, to clinical and public health programs, to the study of humans in the space environment or in industrial work-shift operations — is likely to remain untouched by the recent findings in the study of biologic rhythms. The object of this presentation is to provide evidence that the most important factor in the maintenance of optimal health and performance is the stability of the relationship of one body rhythm to another.

This evidence was obtained during a collaborative study conducted by investigators at the University of California at Davis and by those at the NASA Lyndon B. Johnson Space Center. The object of the study was to evaluate the effect of social interaction on performance, well-being, and physiological rhythm synchrony.

MATERIALS AND METHODS

Three groups of healthy males, ages 21 to 25, were confined in rooms (3.4 by 5.2 meters (11 by 17 feet)) for a total period of 105 days. Two of the groups were in rooms in which the environment could be regulated. The third group served as the control group and was exposed to ambient experimental conditions. The confined subjects were exposed for periods of several days either to 16 hours of light (L) and 8 hours of dark (D), also referred to as L:D, or to continuous light (24L:0D), also referred to as L:L, at a light intensity of 161 lm/m^2 (15 foot-candles). The confined subjects were deprived of all time cues, and meals were ad libitum. The subjects were observed throughout the study by a video camera and were scored for activity. Communications were limited to meal and sample-collection information, and meals and samples were passed through a two-way hatch. Rectal temperature and heart rate (HR) were sampled every 30 minutes by telemetry throughout the study.

The design of the experiment is shown in figure 3-1. The subjects in the north and south rooms were exposed to identical environmental conditions for the first 63 days of the experiment.

Figure 3-2 shows the summation dials of body temperature (BT) of the three subjects that served as controls in the east room. The occurrence of the peak in BT was remarkably stable, as indicated by the direction of the vector that points to 2000 hours. The same stability was true for the HR rhythms of the subjects, as shown in figure 3-3.

In contrast, the group in the south room (fig. 3-4) showed a phase shift and an increase in period length of about 12 minutes every 24 hours during their 21-day exposure to constant light at 161 lm/m^2 (15 foot-candles). After a return to the 16L:8D cycle, the HR rhythm promptly recovered.

A similar desynchronization of the HR rhythm, with the environment in the constant-light, 161-lm/m^2 (15 foot-candles) condition, was observed for the subjects in the north room, although their period length increased by 60 minutes every 24 hours (fig. 3-5). Thus, by the end of 21 days, this group had "lost" an entire day. Despite the identical conditions for the two rooms, the rate of desynchronization with the environment was strikingly different although the subjects within each group were remarkably synchronized. This difference suggests that social synchronizers may play a very important role in the regulation of human biologic rhythms. During this free-running section of the experiment, the investigators noted that internal synchrony was maintained. That is, although the individual was out of synchrony with sidereal time and the rhythms were free running, the relationship of one physiological rhythm to another remained stable. However, when the subjects were again exposed to the 16L:8D regime, the rate of recovery of each body rhythm differed from that of the others, resulting in marked internal desynchronization. For example, under "normal" conditions during the control period, cortisol leads HR by 8 to 10 hours and HR leads BT by 2 to 4 hours. During constant light, when the rhythms were free running, these lead times were maintained. However, during the recovery in 16L:8D, the BT peak returned to its original 2000 hours occurrence within 8 days, whereas it took the HR 4 days to fully recover and the plasma cortisol peak 30 days to recover. Thus, considerable internal desynchronization existed in these subjects for at least this 30-day recovery period.

The intricate relationship of several physiological rhythms under "normal" conditions is shown in figure 3-6. A delicate balance is somehow maintained so that the peak activity of each rhythm occurs at the appropriate time for its specific regulatory function in the body.

Figure 3-7 depicts the relationship of the synchrony of BT and HR to performance when either the synchrony with the environment (free running) or the internal synchrony is disturbed by an alteration of the light cycle. Performance did not appreciably change when the rhythms were free running. However, a severe increase in the number of errors made by the subjects on their performance tasks was noted when the subjects were returned to the controlled environment (16L:8D) and when maximum internal desynchronization was present.

CONCLUDING REMARKS

Evidence from studies involving experimental animals indirectly suggests that the period of internal desynchronization may be characterized not only by a decrease in performance, but also by a decrease in the ability to cope behaviorally in response to stressful conditions and by an increase in sensitivity to infection and in the reaction to the toxicity of various drugs.

Several additional means of producing internal rhythm desynchronization have been suggested over the years. These include bed rest of more than 20 days (refs. 3-1 and 3-2), weightlessness (ref. 3-3), the jet lag of east-west or west-east travel (refs. 3-4 and 3-5), and work-shift cycles such as those found in industry or hospitals (refs. 3-6 and 3-7). Such internal desynchronization could be produced by therapeutic procedures, surgery, infection, or other forms of stress, although this hypothesis has not been proved. The implications of the disruption of this internal temporal organization to human mental and physical health and the impact of such disruption on performance are self-evident. Through studies of biological rhythms, many aspects of human variability in symptoms of illness, in response to medical treatment, in learning, and in job performance are being quantitated so that peaks in strength and productivity can be anticipated and predicted.

As a result, timing will become an important factor in preventive health programs and medicine. For example, since the effects of drugs depend in part upon the time of administration, timing may be used as a critical aspect of treatment. Evidence now also suggests that the results of X-ray treatment, surgery, and even psychotherapy are influenced by timing. Research of biological rhythms will probably have an impact on problems of work performance, including accidents and absenteeism; in the future, a new concept of scheduling as a function of health may influence the determination of work shifts for transportation and communications personnel and members of various professions.

REFERENCES

3-1. Winget, C. M.; Vernikos-Danellis, J.; Cronin, S. E.; Leach, C. S.; et al.: Circadian Rhythm Asynchrony in Man During Hypokinesis. J. Appl. Physiol. vol. 33, no. 5, 1972, pp. 640-643.

3-2. Vernikos-Danellis, J.; Leach, C. S.; Winget, C. M.; Rambaut, P. C.; and Mack, P. B.: Thyroid and Adrenal Cortical Rhythmicity During Bed Rest. J. Appl. Physiol. vol. 33, no. 5, 1972, pp. 644-648.

3-3. Halberg, Franz; Vallbona, Carlos; Dietlein, Lawrence F.; Rummel, John A.; et al.: Human Circadian Circulatory Rhythms During Weightlessness in Extraterrestrial Flight or Bedrest With and Without Exercise. Space Life Sciences, vol. 2, no. 1, 1970, pp. 18-32.

3-4. Klein, K. E.; Wegmann, H. M.; and Brüner, H.: Circadian Rhythm in Indices of Human Performance, Physical Fitness Stress Resistance. Aerospace Medicine, vol. 39, no. 5, May 1968, pp. 512-518.

3-5. Klein, K. E.; Wegmann, H. M.; and Hunt, Bonnie, I.: Desynchronization of Body Temperature and Performance Circadian Rhythm as a Result of Outgoing and Homegoing Transmeridian Flights. Aerospace Medicine, vol. 43, no. 2, Feb. 1972, pp. 119-132.

3-6. Colquhoun, W. P.; Blake, M. J. F.; and Edwards, R. S.: Experimental Studies of Shift-Work II. Stabilized 8-Hour Shift Systems. Ergonomics, vol. 11, no. 6, 1968, pp. 527-546.

3-7. Colquhoun W. P.; Blake, M. J. F.; and Edwards, R. S.: Experimental Studies of Shift-Work I. A Comparison of 'Rotating' and 'Stabilized' 4-Hour Shift Systems. Ergonomics, vol. 11, no. 5, 1968, pp. 437-453.

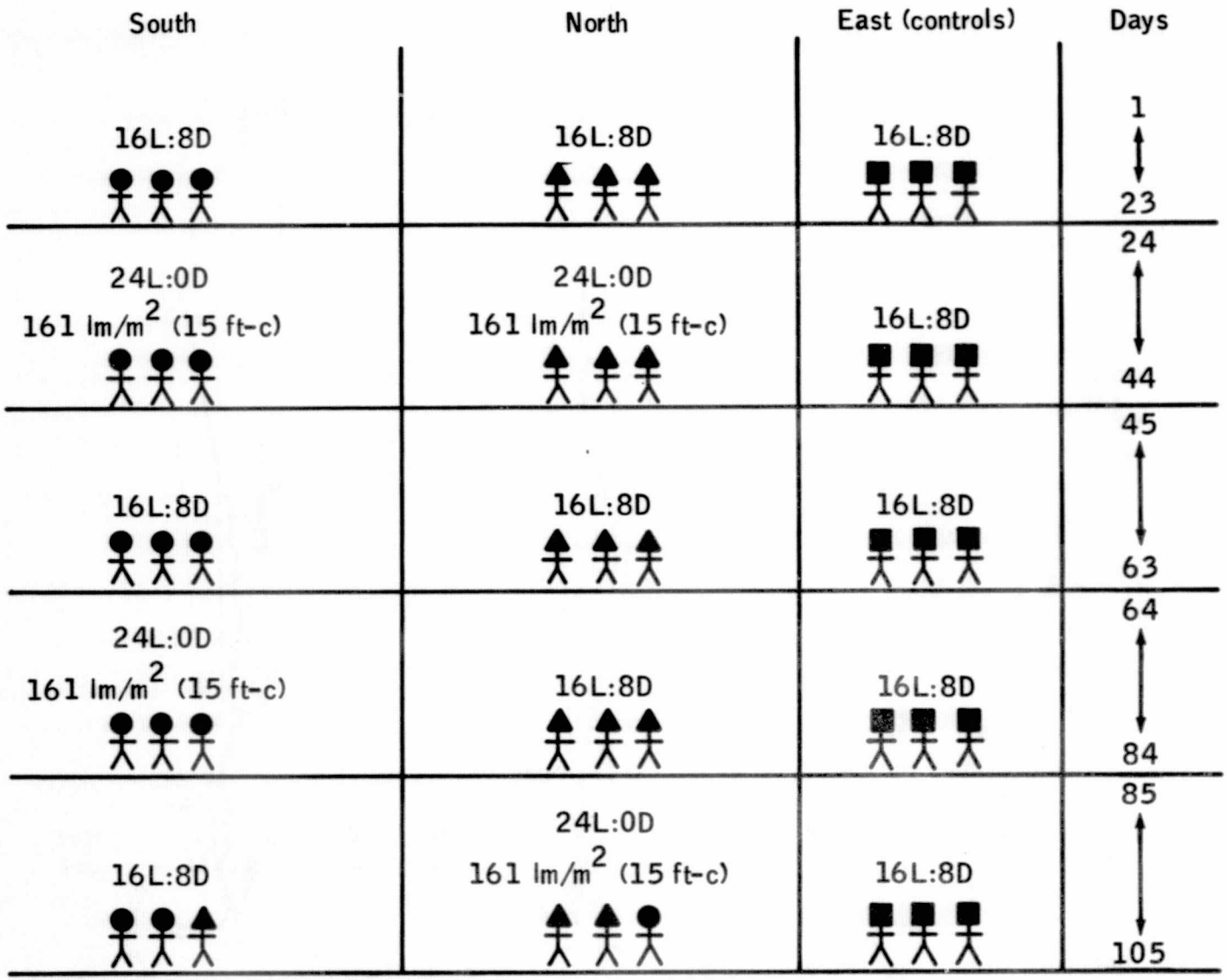


Figure 3-1.- The experimental design for the social interaction study. The subjects in the east room served as controls for the subjects in the north and south rooms and were exposed to ambient experimental conditions.

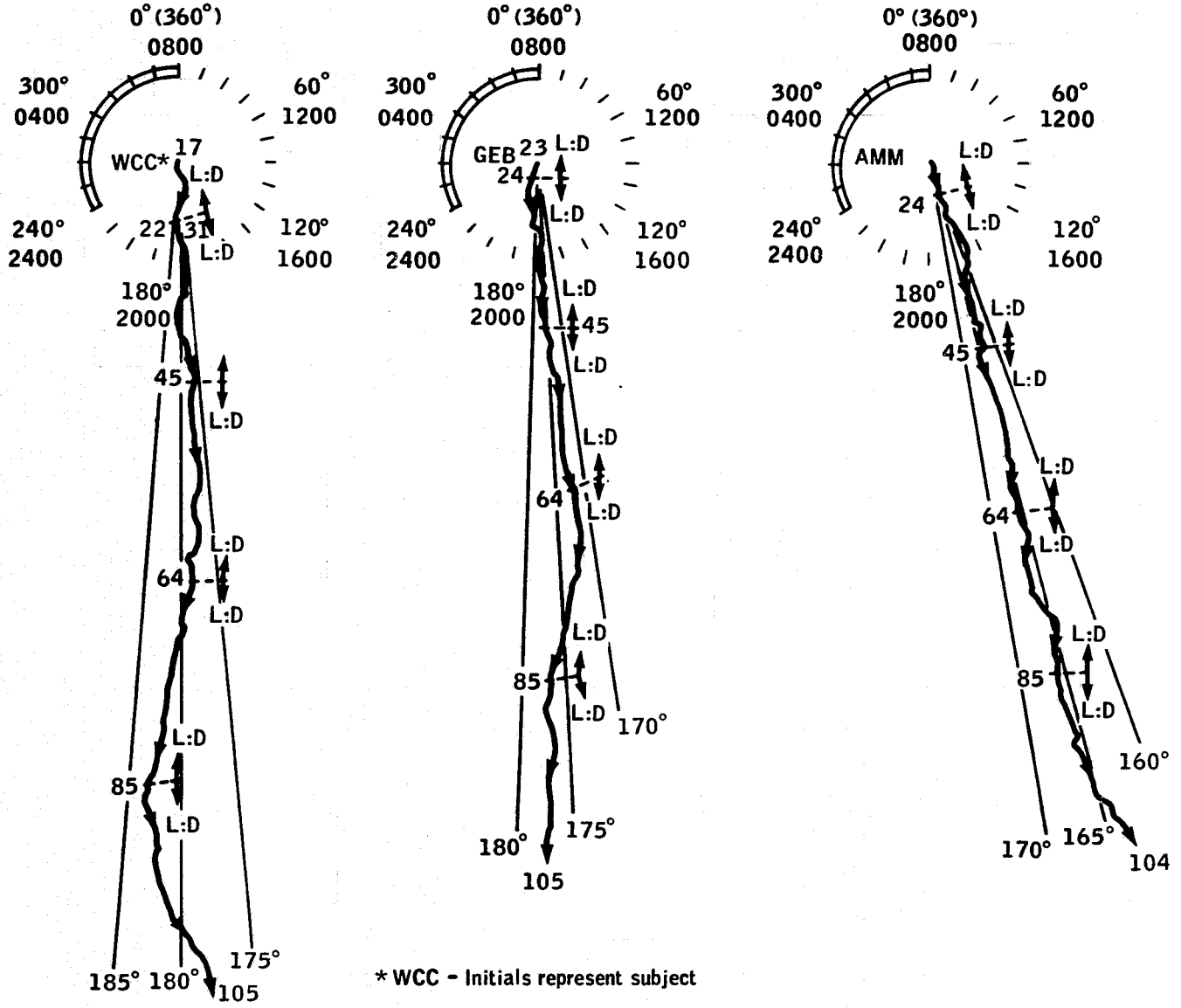


Figure 3-2.- Summation dials of body temperatures (rectal) of the three subjects that served as controls in the east room.

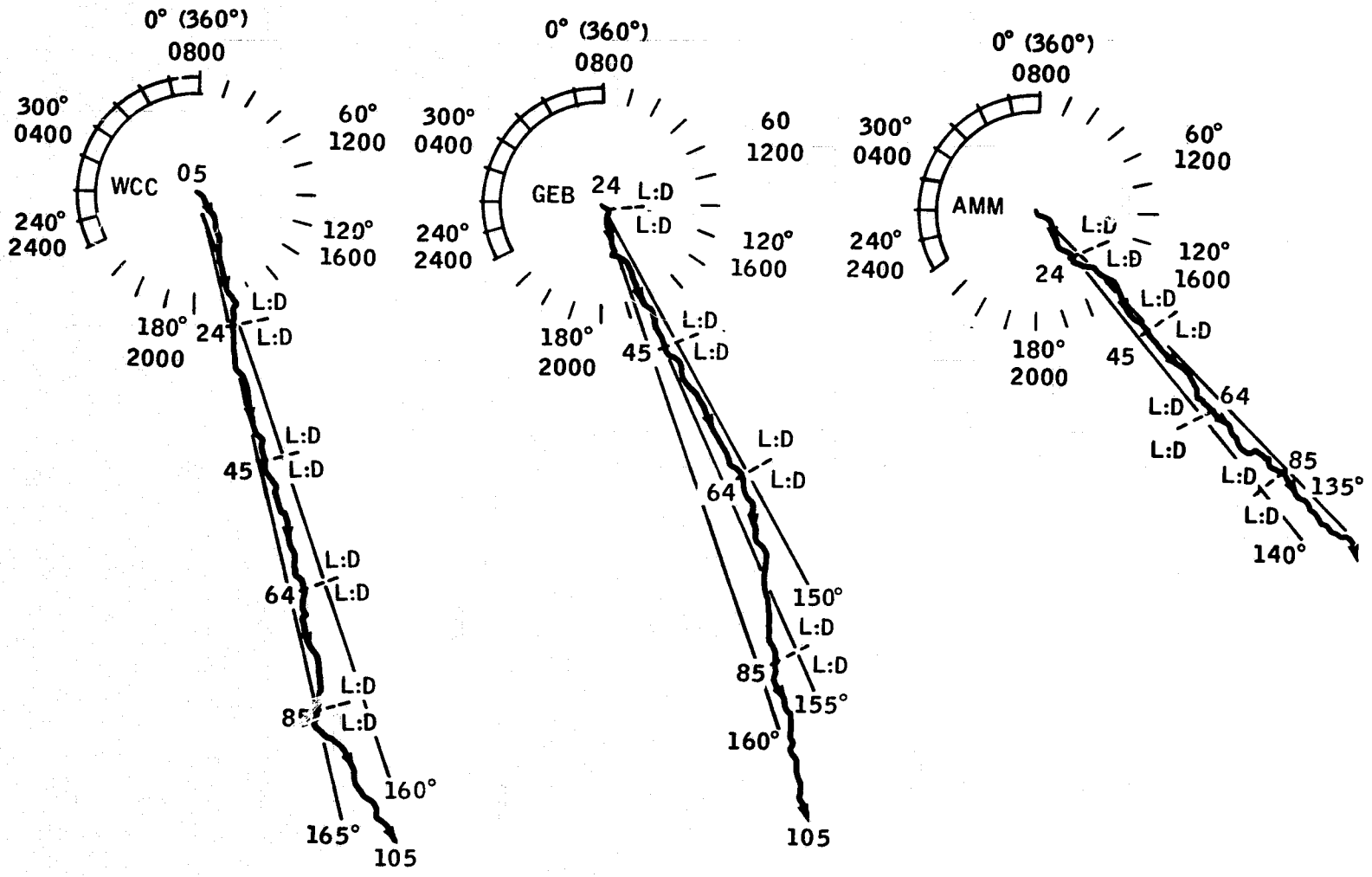


Figure 3-3.- Summation dials of the heart rate rhythms of the three subjects that served as controls in the east room.

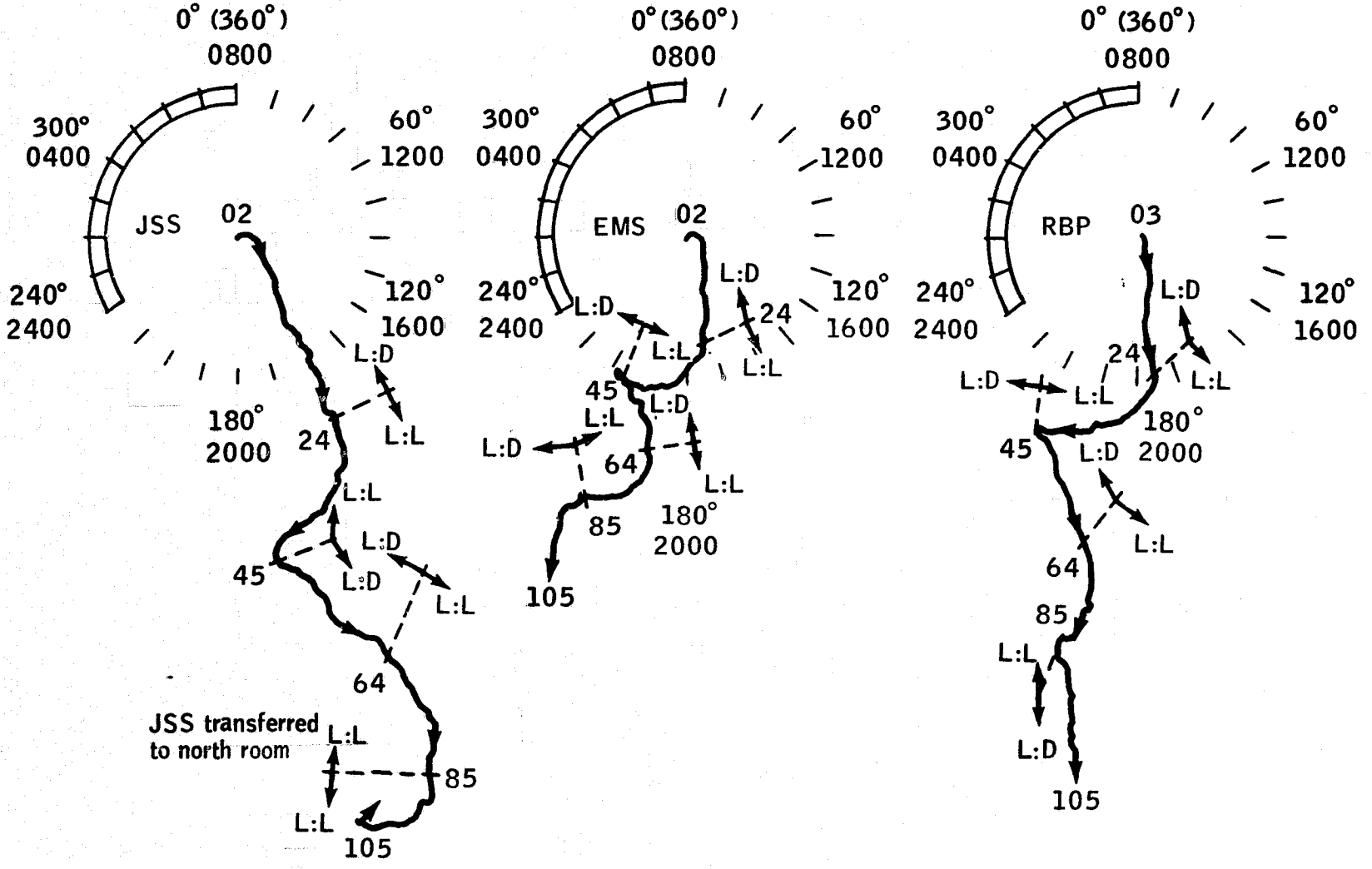


Figure 3-4.- Heart rate summation dials for the subjects of the south room. A phase shift and an increase in period length are clearly indicated during their 21-day exposure to constant light at 161 lm/m^2 (15 foot-candles).

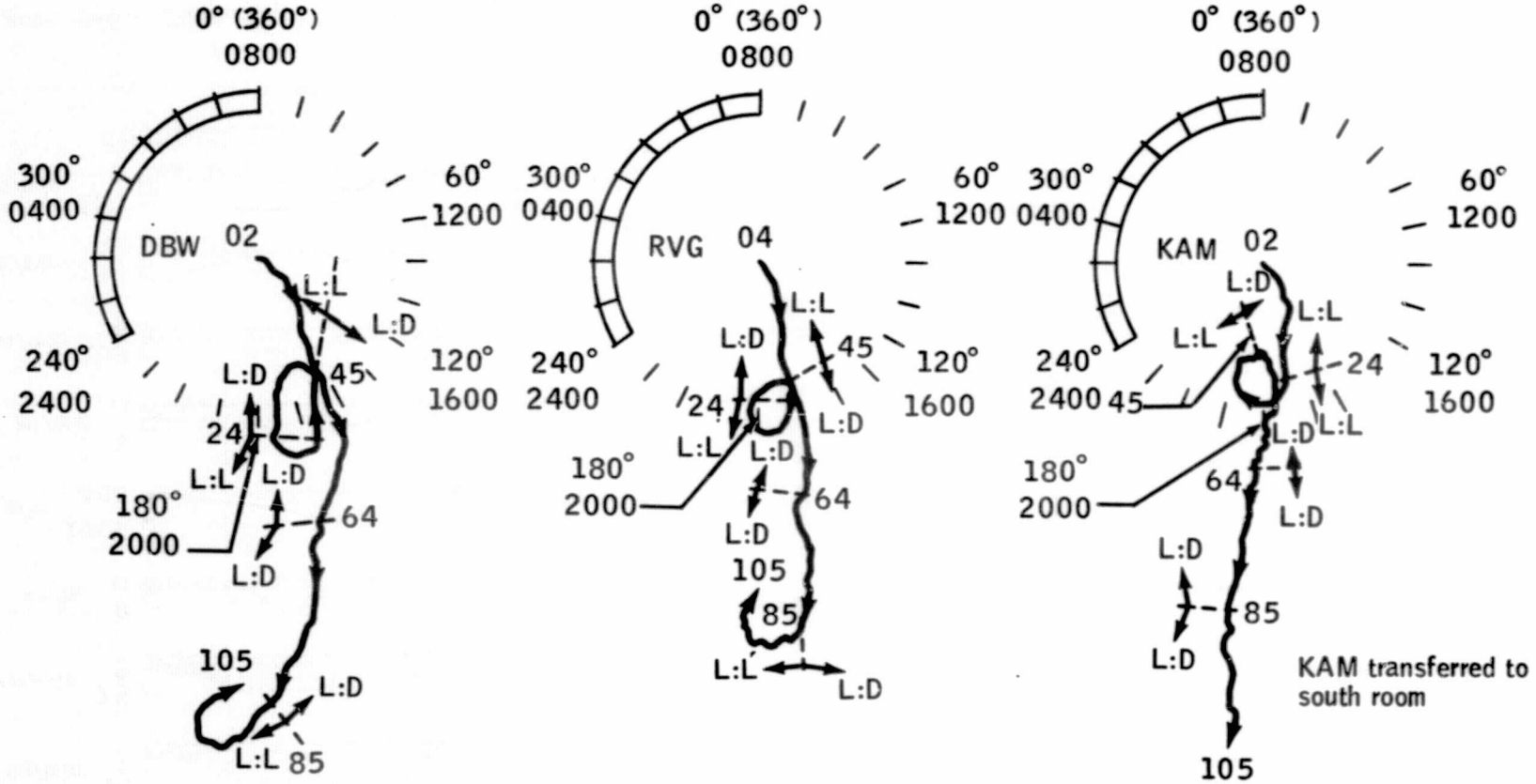


Figure 3-5.- Heart rate summation dials for the subjects of the north room. A desynchronization of the HR rhythm is clearly indicated with a period length increased by 60 minutes every 24 hours.

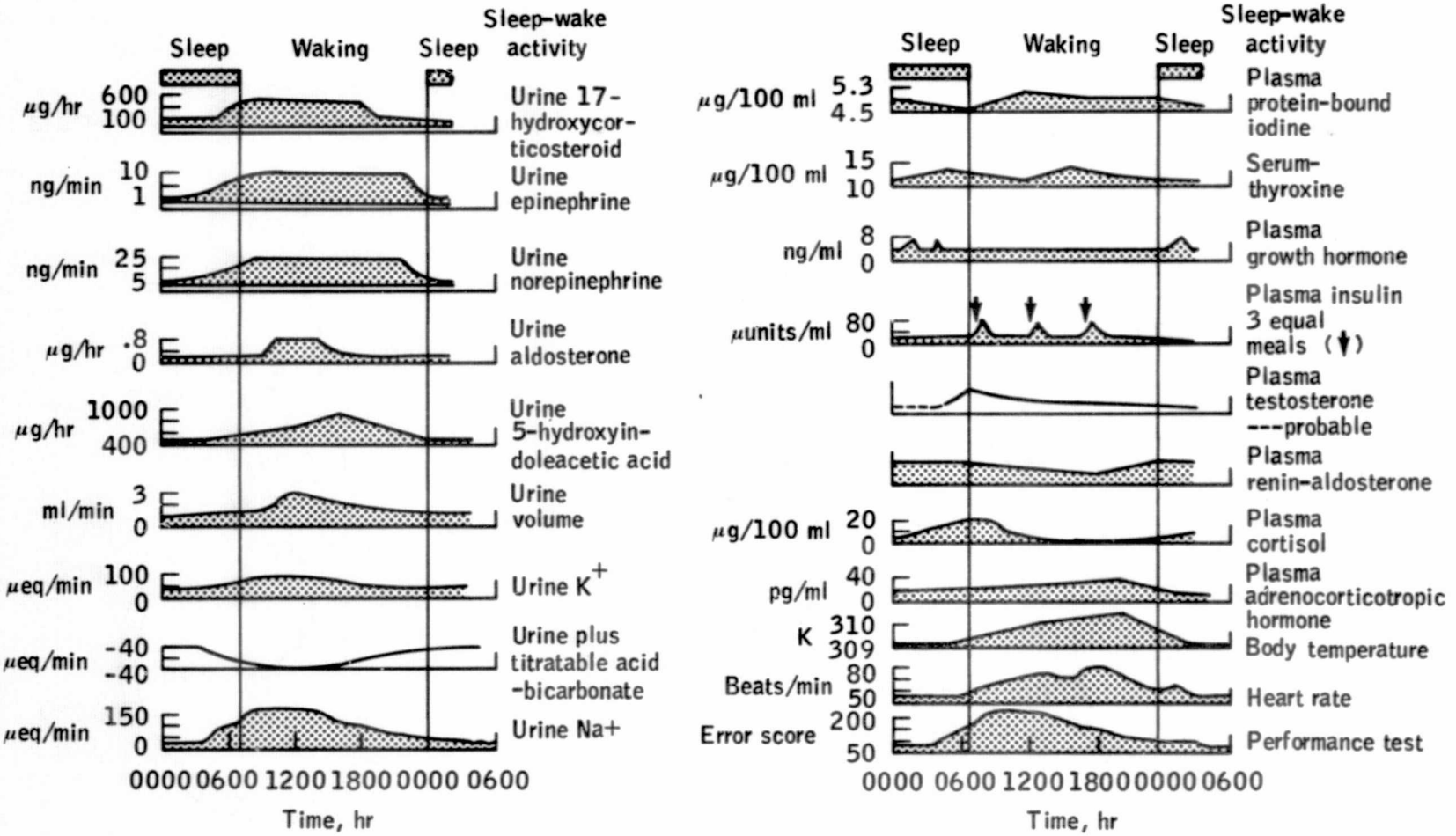


Figure 3-6.- Summary of material depicting approximate amplitudes, shapes, and phase relationships among certain physiological cycles. The curves portray idealized values for a hypothetical individual who retires at midnight, rises regularly at 0700, and consumes three meals per day at conventional times.

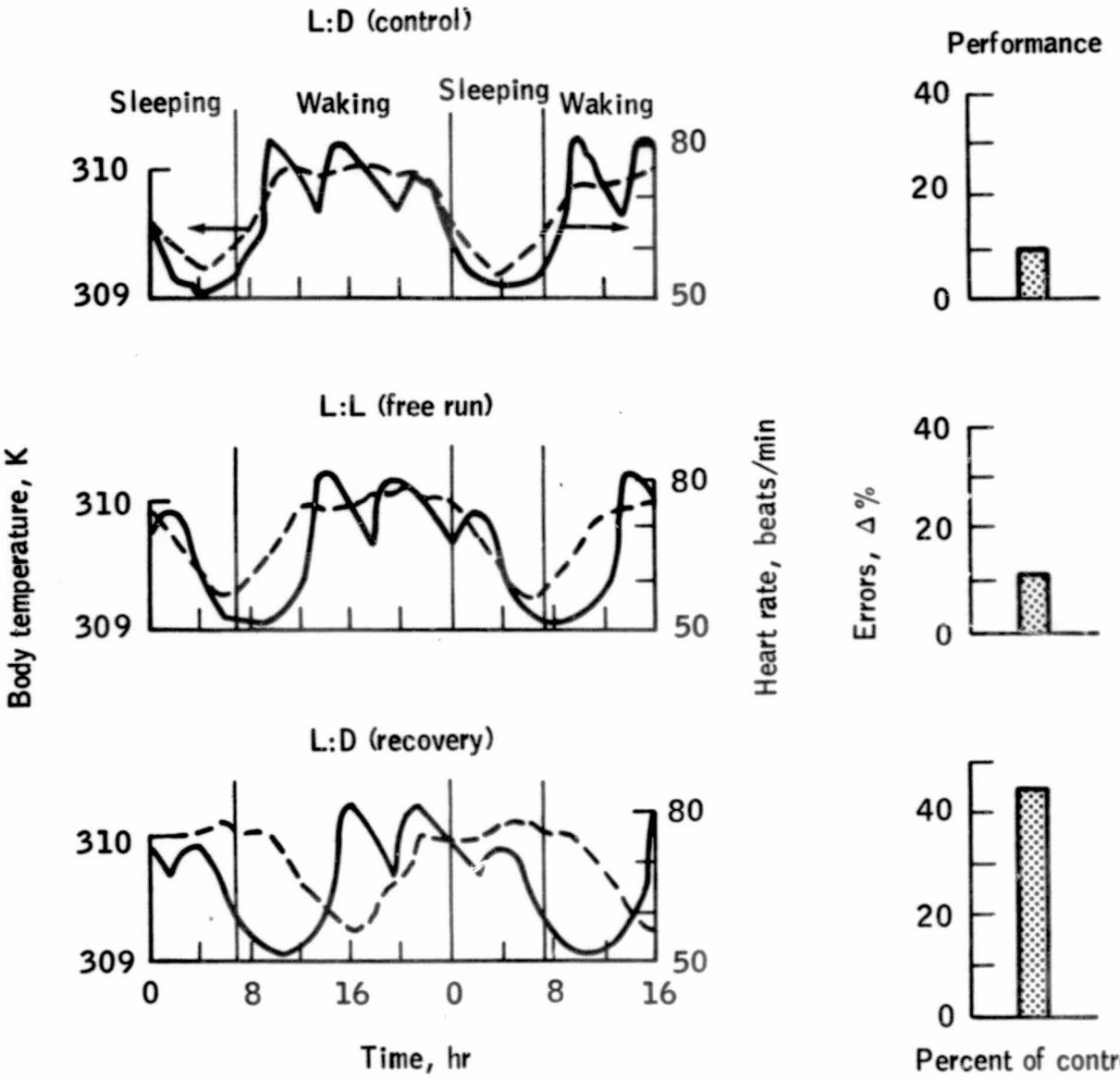


Figure 3-7.- The synchronous relationship of body temperature and heart rate to performance in various defined environmental conditions. Solid line represents heart rate; dashed line represents body temperature.

4. THE IMPORTANCE OF THE RENIN-ANGIOTENSIN SYSTEM IN NORMAL CARDIOVASCULAR HOMEOSTASIS

By Edgar Haber, M.D.*

INTRODUCTION

The role of the renin-angiotensin system in the maintenance of normal blood pressure has been a subject of considerable dispute and conflicting experimental data. In the normal, conscious animal on a normal salt intake, angiotensin II is not required for the maintenance of blood pressure. This concept has recently been supported through studies using two specific peptide inhibitors working at different points on the pathway from renin release to vasoconstriction. Several derivatives of angiotensin II are now available that compete directly with this hormone at its effector site in vascular smooth muscle (refs. 4-1 and 4-2). Several investigators have now shown that these compounds, which effectively block the vasoconstrictor action of angiotensin II, have no effect on blood pressure (ref. 4-3, 4-4, and 4-5).

A second series of peptide inhibitors competes effectively with angiotensin I for converting enzyme. The most potent of these peptides, which has the sequency Pyr-Trp-Pro-Arg-Pro-Gly-Ile-Pro-Pro, is well tolerated in large doses by normal animals. At doses of 0.25 mg/kg, the pressor response to angiotensin I is completely inhibited, whereas that to angiotensin II is unaffected (ref. 4-6). This compound has now been examined in the investigators' laboratory under a variety of physiologic circumstances to define the contributory role of angiotensin II to blood pressure maintenance.

METHODS

Studies were carried out on adult mongrel dogs (20 to 30 kilograms) which were housed in air-conditioned rooms. Each dog was fed a fixed diet that had been adjusted during the training period to maintain constant weight. Salt intake varied with the experimental circumstance, and water intake was carefully measured but permitted ad libitum. Before any study, each dog was trained to

*This report is based on collaborative investigations performed in the Departments of Medicine and Physiology of the Harvard Medical School by A. I. Samuels, E. D. Miller, C. S. Fray, E. Haber, and A. C. Barger.

lie motionless on its side on a padded table in a quiet air-conditioned laboratory. Only the investigators were permitted to enter the laboratory during the training and study sessions.

Blood pressure was measured by catheterization of the abdominal aorta with a polyvinyl chloride tubing by the method of Herd and Barger (ref. 4-7). The proximal end of each catheter was exteriorized through a hollow needle and tied in a plastic loop implanted in the skin. The catheters were protected by a cotton jacket. Systemic blood pressure was monitored through the output of a P23Db Statham pressure transducer and recorded on a Grass polygraph. Heart rate was determined from the blood pressure record or from the electrocardiogram. Blood pressure and heart rate were remarkably steady in these well-trained animals. Mean blood pressure in the control studies rarely fluctuated more than ±3 or 4 mmHg, although blood pressure did rise or fall beyond this limit momentarily when the animal occasionally yawned or stretched. Heart rate was also constant within ±2 to 3 beats per minute.

Renin activity was determined in duplicate by radioimmunossay (ref. 4-8). The values were expressed as nanograms per milliliter per hour of angiotensin I generated.

RESULTS

The intravenous administration of 5 milligrams of the converting enzyme inhibitor blocks the usual pressor response induced by the intravenous injection of 1 microgram of angiotensin I. The pressor response of angiotensin II was unchanged. In normal dogs on normal salt intake, the injection of 5 milligrams of converting enzyme inhibitor did not alter arterial pressure or systemic plasma renin activity. However, in dogs on a low salt diet with slight systemic hypotension and significant elevation of plasma renin activity, blood pressure fell approximately 10 mmHg when converting enzyme inhibitor was given (fig. 4-1), and the plasma renin activity increased severalfold. In adrenalectomized dogs adequately treated with cortisone acetate (25 mg/day), but maintained in a hypovolemic state on a low dosage of deoxycorticosterone acetate and with sodium restriction (10 meq/day), blood pressure fell strikingly within 3 minutes of the injection of converting enzyme inhibitor (fig. 4-2). Plasma renin activity rose more than fivefold basal levels. When a constant infusion of angiotensin II was given to maintain blood pressure after converting enzyme inhibitor administration, plasma renin activity did not rise (fig. 4-3). However, when blood pressure was maintained at the same levels with an alpha-adrenergic agent, phenylephrine, which by itself has no effect on renin activity, a considerable but somewhat lesser increase in renin activity following converting enzyme inhibitor administration was noted (fig. 4-4).

DISCUSSION

Earlier experiments indicating that the renin-angiotensin system has a small role in the maintenance of normal blood pressure have been confirmed by the observation that no hemodynamic effect in response to converting enzyme inhibitor is observed in the normal salt-replete animal. Conversely, when severe sodium depletion has occurred, striking hypotension is a consequence of converting enzyme inhibitor administration. Under these circumstances, it seems that angiotensin II is essential for the maintenance of blood pressure and that other compensatory mechanisms are insufficient. There is no evidence of sympathetic blockade in these animals, and, indeed, tachycardia occurs in association with hypotension. However, sufficient vasoconstriction cannot be effected in order to maintain blood pressure. A second observation of considerable interest concerns feedback control of renin secretion. Renal arterial pressure is known to be a major determinant of renin secretion (ref. 4-9). It is not surprising then that a marked increase in renin activity occurs in response to hypotension induced by converting enzyme inhibitor (fig. 4-2). However, the maintenance of normal blood pressure after converting enzyme inhibitor administration by phenylephrine results in far higher renin activities than when blood pressure is maintained at the same level by angiotensin II (fig. 4-3 and 4-4). This result suggests a direct feedback on renin secretion by angiotensin II that is independent of renal arterial pressure. When converting enzyme is inhibited and angiotensin II is absent, renin activity is much higher than under similar circumstances in which angiotensin II levels are maintained by exogenous infusion.

SUMMARY

1. The renin-angiotensin system plays a major role in the maintenance of circulatory homeostasis when extracellular fluid volume is depleted.

2. Angiotensin II concentration, in addition to renal perfusion pressure, is an important factor in the regulation of renin release.

REFERENCES

4-1. Pals, Donald T.; Masucci, Frederick D.; Sipos, Frank; and Denning, George S., Jr.: A Specific Competitive Antagonist of the Vascular Action of Angiotensin II. Circ. Res., vol. XXIX, Dec. 1971, pp. 664-672.

4-2. Türker, R. K.; Hall, M. M.; Yamamoto, M.; Sweet, C. S.; and Bumpus, F. M.: A New, Long-Lasting Competitive Inhibitor of Angiotensin. Science, vol. 177, no. 4055, Sept. 1972, pp. 1203-1205.

4-3. Bing, J.; and Nielsen, K.: Role of the Renin-System in Normo- and Hypertension. Acta Pathol. Microbiol. Scand., Sec. A, vol. 81, no. 3, May 1973, pp. 254-262.

4-4. Johnson, J. Alan; and Davis, James O.: Angiotensin II: Important Role in the Maintenance of Arterial Blood Pressure. Science, vol. 179, no. 4076, March 1973, pp. 906-907.

4-5. Gavras, Haralambos; Brunner, Hans R.; Vaughan, E. Darracott, Jr.; and Laragh, John: Angiotensin-Sodium Interaction in Blood Pressure Maintenance of Renal Hypertensive and Normotensive Rats. Science, vol. 180, no. 4093, June 1973, pp. 1369-1372.

4-6. Ondetti, Miguel A.; Williams, Nina J.; Sabo, Emily F.; Pluscec, Josip; et al.: Angiotensin-Converting Enzyme Inhibitors from the Venom of Bothrops jararaca. Isolation, Elucidation of Structure, and Synthesis. Biochemistry, vol. 10, no. 22, Oct. 1971, pp. 4033-4039.

4-7. Herd, J. A.; and Barger, A. C.: Simplified Technique for Chronic Catheterization of Blood Vessels. J. Appl. Physiol., vol. 19, 1964, pp. 791-792.

4-8. Haber, Edgar; Koerner, Theresa; Page, Lot B.; Kliman, Bernard; and Purnode, Andre: Application of a Radioimmunoassay for Angiotensin I to the Physiologic Measurements of Plasma Renin Activity in Normal Human Subjects. J. Clin. Endocrinol. Metab., vol. 29, no. 7, July 1969, pp. 1349-1355.

4-9. Gutmann, F. D.; Tagawa, H.; Haber, E.; and Barger, A. C.: Renal Arterial Pressure, Renin Secretion, and Blood Pressure Control in Trained Dogs. Am. J. Physiol., vol. 224, no. 1, Jan. 1973, pp. 66-72.

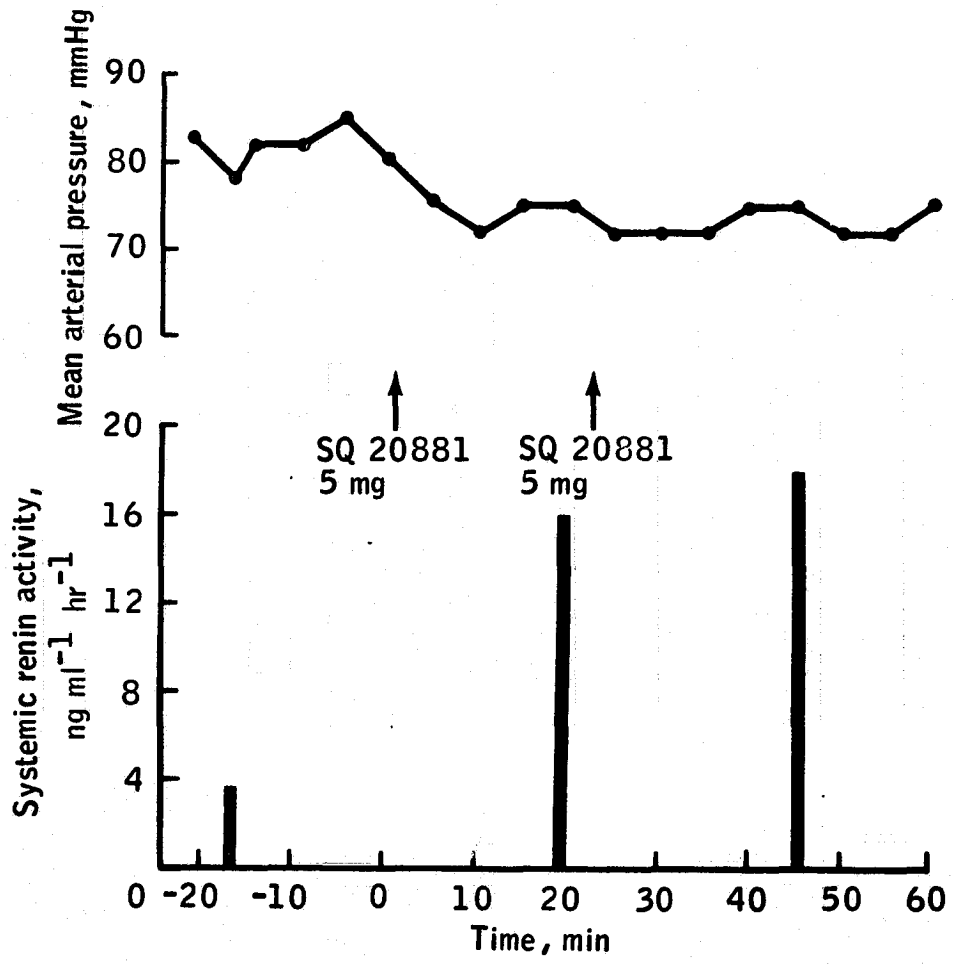


Figure 4-1.- Normal salt-depleted dog treated with converting enzyme inhibitor (SQ 20881, 5 mg).

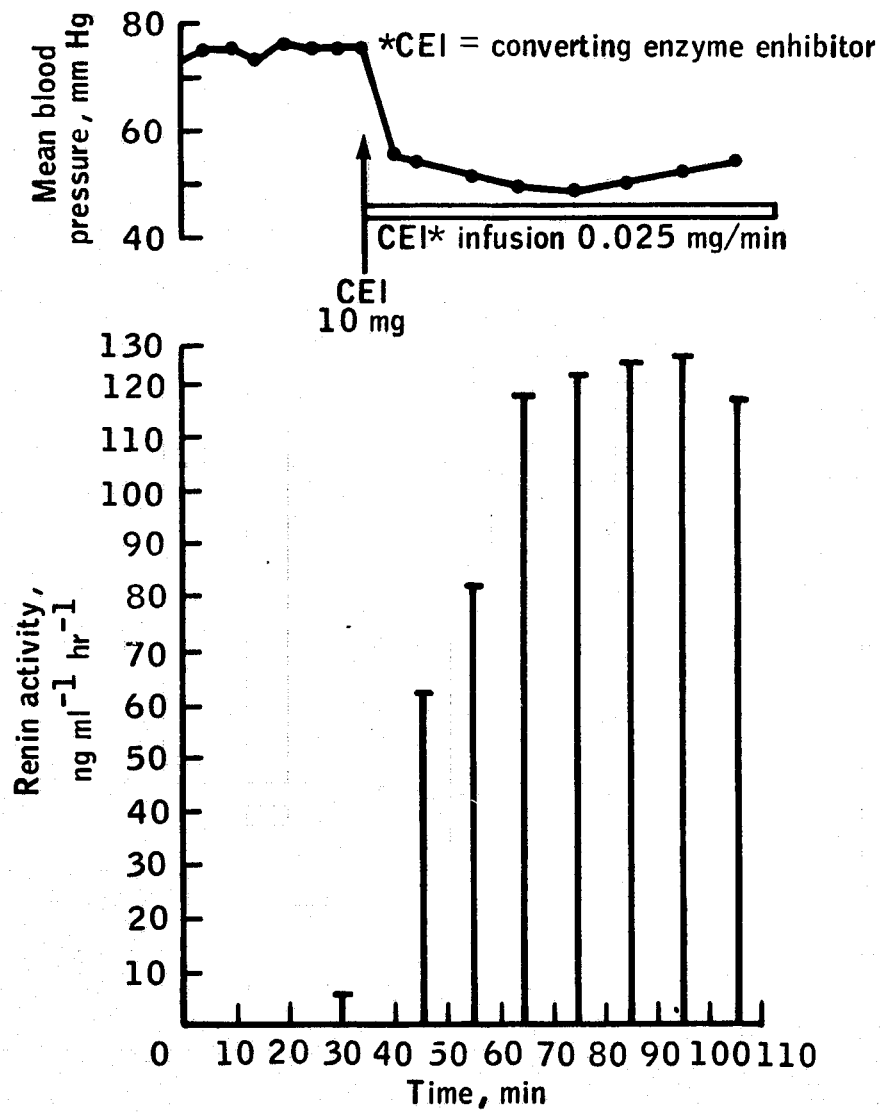


Figure 4-2.- Adrenalectomized sodium-depleted dog treated with converting enzyme inhibitor.

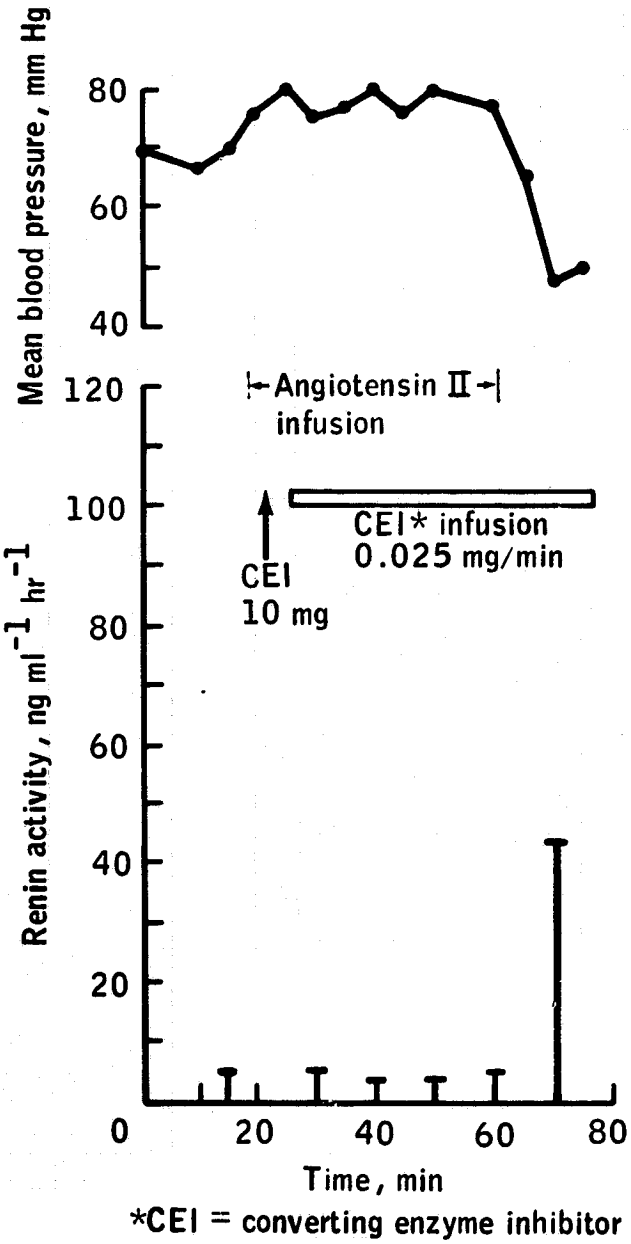


Figure 4-3.- Adrenalectomized sodium-depleted dog. Blood pressure maintained with angiotensin II infusion after converting enzyme inhibitor treatment.

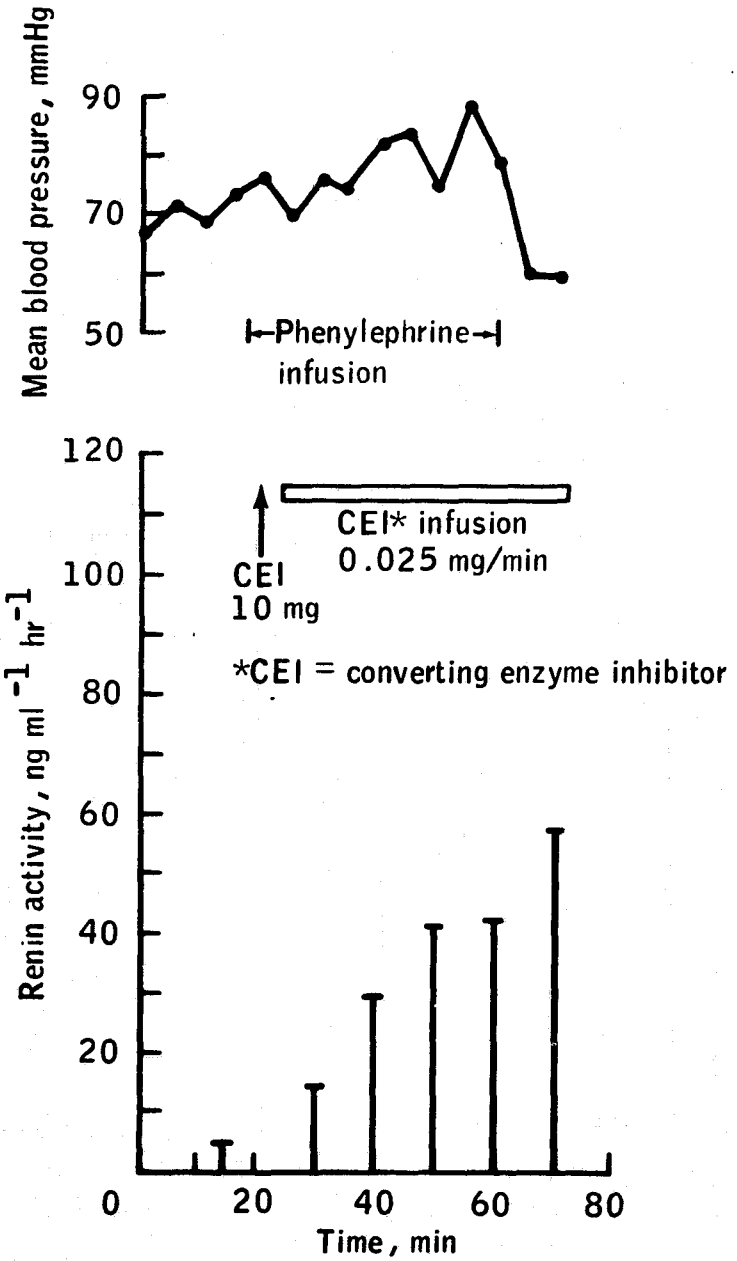


Figure 4-4.- Adrenalectomized sodium-depleted dog. Blood pressure maintained with phenylephrine infusion after converting enzyme inhibitor treatment.

5. STRESS-INDUCED CHANGES IN CORTICOSTEROID METABOLISM: A PROGRESS REPORT

By Martha M. Tacker, Ph. D.*

At the 1972 Endocrine Conference, the author presented a proposal to analyze in detail the effects of stress on corticosteroid metabolism (ref. 5-1). This proposal was prompted by the recurring difficulty in correlating and explaining corticosteroid concentrations measured in various NASA-supported studies. Because plasma and urine corticosteroid concentrations are influenced by several factors in addition to adrenal cortex secretion (fig. 5-1), the effect of stress on all of these factors must be determined before a meaningful interpretation of plasma and urine concentrations can be made. Furthermore, such evaluation must precede determination of the most appropriate and optimal index of the adrenocortical response to stress.

Funds to support this study were received in the summer of 1972 and the effort to date has been directed toward developing the methodology necessary for these studies. Following the suggestion of last year's conference members, methods for the determination of the plasma concentrations of free and transcortin-bound cortisol were established (ref. 5-2). Plasma is equilibrated with a tracer amount of ^{3}H-cortisol at 310 K (37° C) and an aliquot (1 or 2 milliliters) is placed inside a length (approximately 25 centimeters) of dialysis tubing that has been soaked in distilled water for 24 hours. The tubing is draped inside a glass centrifuge tube and the two ends are secured over the top of the centrifuge tube with a rubber band; the glass tube is then set in a covered water bath 310 K (37° C) for 5 minutes to saturate the air with water vapor and is then sealed with Parafilm. Centrifugation for 45 minutes produces an ultrafiltrate of plasma, the volume of which is approximately 10 percent of the original plasma volume. The volume of ultrafiltrate does not unacceptably alter the free-to-bound cortisol equilibrium (ref. 5-3).

A comparison of the concentration of radioactivity in the ultrafiltrate with that in the original plasma reveals the relative amount of unbound cortisol in the plasma at normal body temperature 310 K (37° C). Multiplication of the amount of total (free plus bound) plasma cortisol (μg/100 ml) by this percentage gives the concentrations of cortisol in both the free and bound fractions of the plasma. The addition of increasing amounts of cortisol to the plasma increases the amounts of free cortisol after a concentration of 20 μg/100 ml is reached (fig. 5-2).

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*The technical assistance of Douglas Robbins is gratefully acknowledged.

The other area of methodology being set up is the measurement of individual corticosteroids. Methods commonly used to measure corticosteroids have varying degrees of specificity, and all require a preliminary chromatographic separation to measure each corticosteroid specifically. The scheme considered optimal (fig. 5-3) consists of separation of plasma corticosteroids with Sephadex LH-20 column chromatography (ref. 5-4), followed by radioimmunoassay of the individual steroids (cortisol, corticosterone, cortisone, 11-deoxycortisol, and 11-deoxycorticosterone) (ref. 5-5).

The addition of tracer amounts of each steroid to the plasma sample permits correction for methodological losses. A methylene chloride or ethanol extract of the plasma is passed through a 20-centimeter column of Sephadex LH-20 using methylene chloride/methanol (99/1) as the eluting solvent. Separation of the five steroids of interest (fig. 5-4) is very reproducible; recoveries of cortisol and corticosterone are about 75 percent, and blank contribution to the radioimmunoassays is negligible.

The potential problem of the poor separation of corticosterone and 11-deoxycortisol can be approached in two ways. First, a longer column would separate the two steroids, but would also require custom glasswork. More importantly, a longer column would increase the volume of solvent necessary to elute the steroids, especially cortisol. The second approach, which is currently being used with success, is the analysis of aliquots of the appropriate fraction with specific antibodies. Recovery can be determined by using tritium for one steroid and carbon-14 for the other and by using dual counting technics.

Radioimmunoassay methods have been chosen for analysis of the amounts of each steroid. Standard curves for the radioimmunoassay of cortisol are quite reproducible (fig. 5-5), and the recovery of increasing amounts of cortisol added to plasma and assayed directly (without the chromatographic separation) is reproducible and virtually complete (fig. 5-6).

The antibody against cortisol also binds corticosterone almost as well as it does cortisol. Because these two corticosteroids are separated by the column chromatography, the cortisol antibody can then be used to measure corticosterone using ^{3}H-corticosterone in place of ^{3}H-cortisol. This antibody shows slightly less binding of 11-deoxycortisol (which is poorly separated from corticosterone); and, because the same solutions and procedures can be used to measure cortisol and corticosterone, the cortisol antibody is more convenient to use than the antibody directed against corticosterone itself. Recovery of standard corticosterone passed through the column and measured by radioimmunoassay in two studies was quite good, yielding averages of 0.42 nanogram and 0.46 nanogram compared to an expected average of 0.40 nanogram.

Deoxycortisol can be assayed with a specific antibody that does not cross-react with corticosterone. Antibodies are available to assay cortisone and deoxycorticosterone, but have not been received.

A preliminary exercise study was made in which blood was drawn from a subject before, during, and after an exercise test. This test consisted of four consecutive 4-minute periods of increasing workload on an exercise bicycle. Plasma corticosteroids measured with the corticosteroid binding globulin assay showed a decrease during exercise from relatively high preexercise levels followed by a transient increase immediately post exercise (fig. 5-7).

REFERENCES

5-1. Tacker, Martha M.: Stress-induced Changes in Corticosteroid Metabolism in Man. Proceedings of the 1972 NASA Lyndon B. Johnson Space Center Endocrine Program Conference, NASA TM X-58134, 1974.

5-2. Maickel, R. P.; Miller, F. P.; and Brodie, B. B.: Interaction of Nonsteroidal Antiinflammatory Agents With Corticosterone Binding to Plasma Proteins in the Rat. Arzneimittleforshung, vol. 19, Nov. 1969, pp. 1803-1805.

5-3. Sandberg, Avery A.; Rosenthal, H.; Schneider, S. L.; and Slaunwhite, W. Roy, Jr.: Protein-Steroid Interactions and Their Role in the Transport and Metabolism of Steroids. Steroid Dynamics, Gregory Pincus, Takeshi Nakao, and James F. Tait, eds., Academic Press, 1966, pp. 1-61.

5-4. Newsome, H. H., Jr.; Clements, A. S.; and Borum, E. H.: The Simultaneous Assay of Cortisol, Corticosterone, 11-Deoxycortisol, and Cortisone in Human Plasma. J. Clin. Endocrinol. Metab., vol. 34, no. 3, Mar. 1972, pp. 473-483.

5-5. Plasma Cortisol Radioimmunoassay Procedure. Technical Bulletin No. F21-53, Endocrine Sciences (Tarzana, Calif.), Nov. 1972.

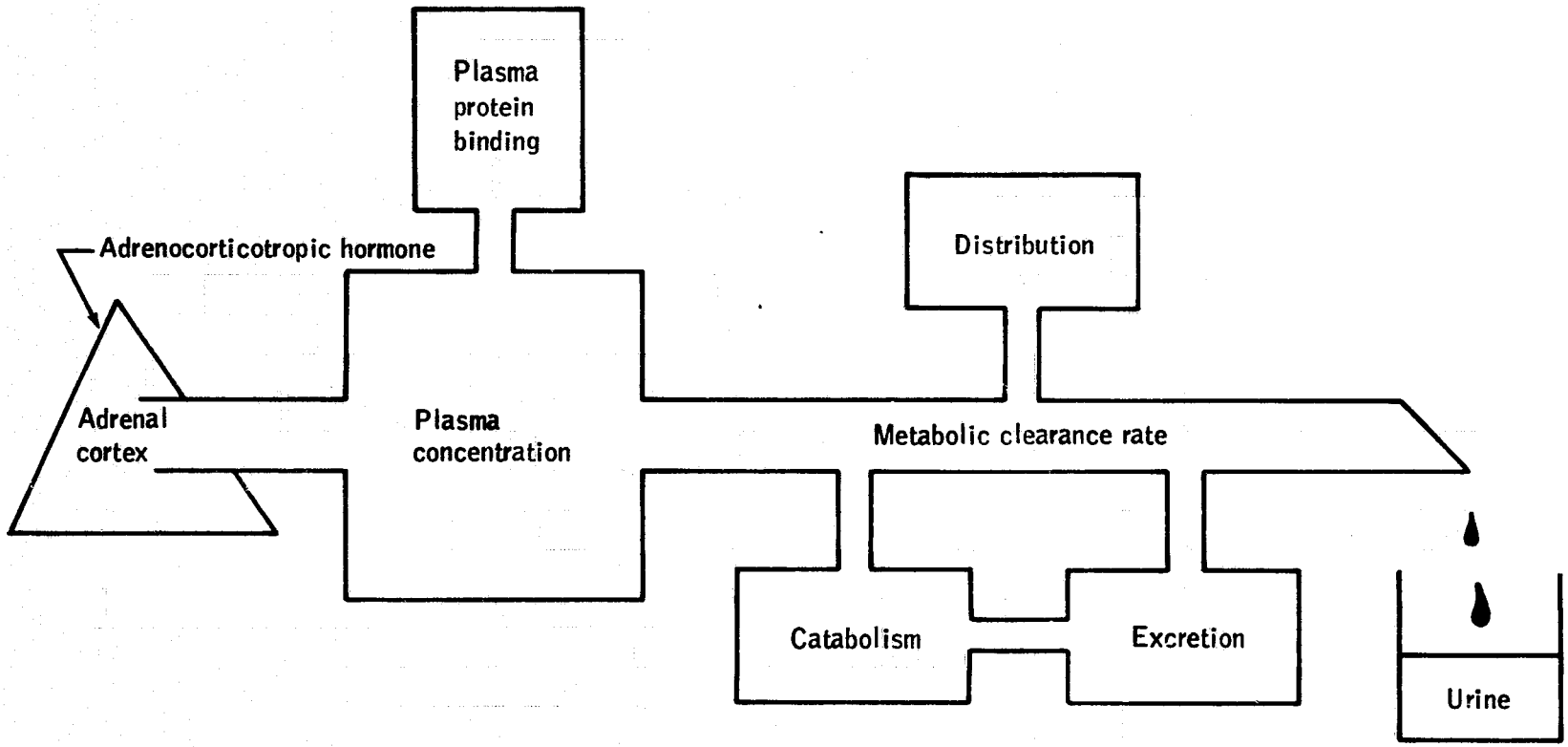

Figure 5-1.- Illustration of various factors that can influence plasma and urine concentrations of corticosteroids.

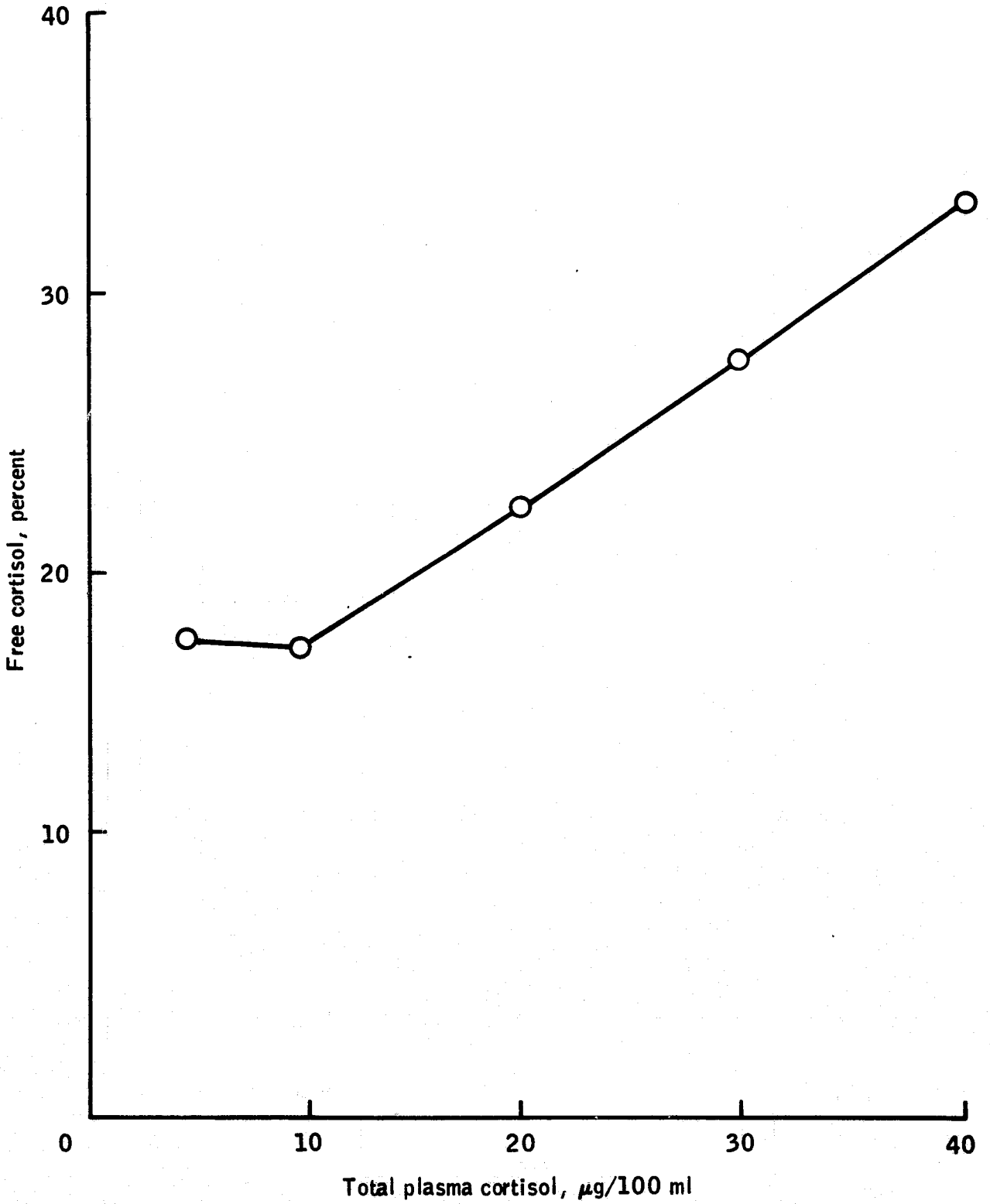

Figure 5-2.- Increase in unbound cortisol with addition of cortisol to plasma.

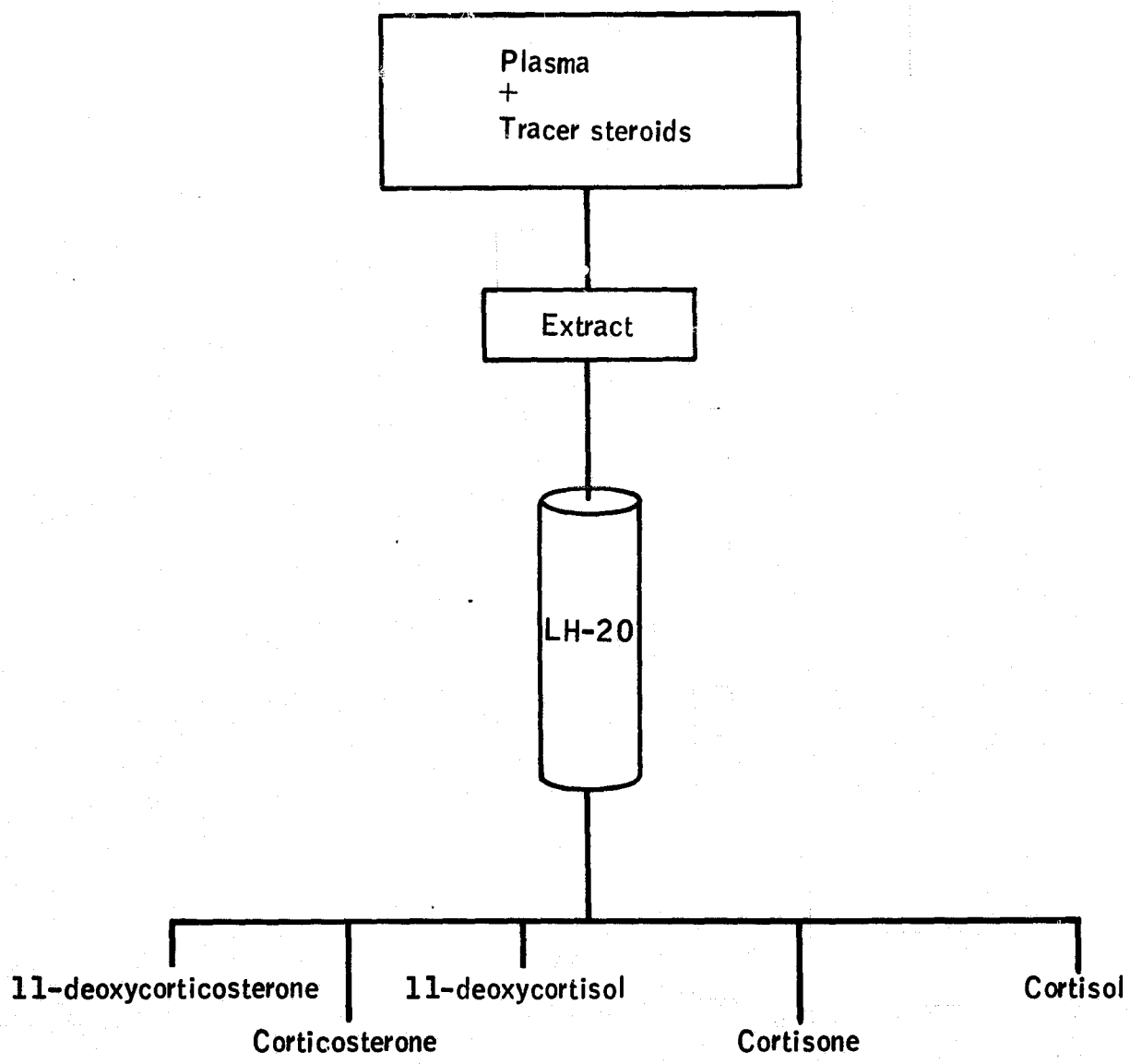

Figure 5-3.- Scheme for separation and quantitation of five plasma corticosteroids.

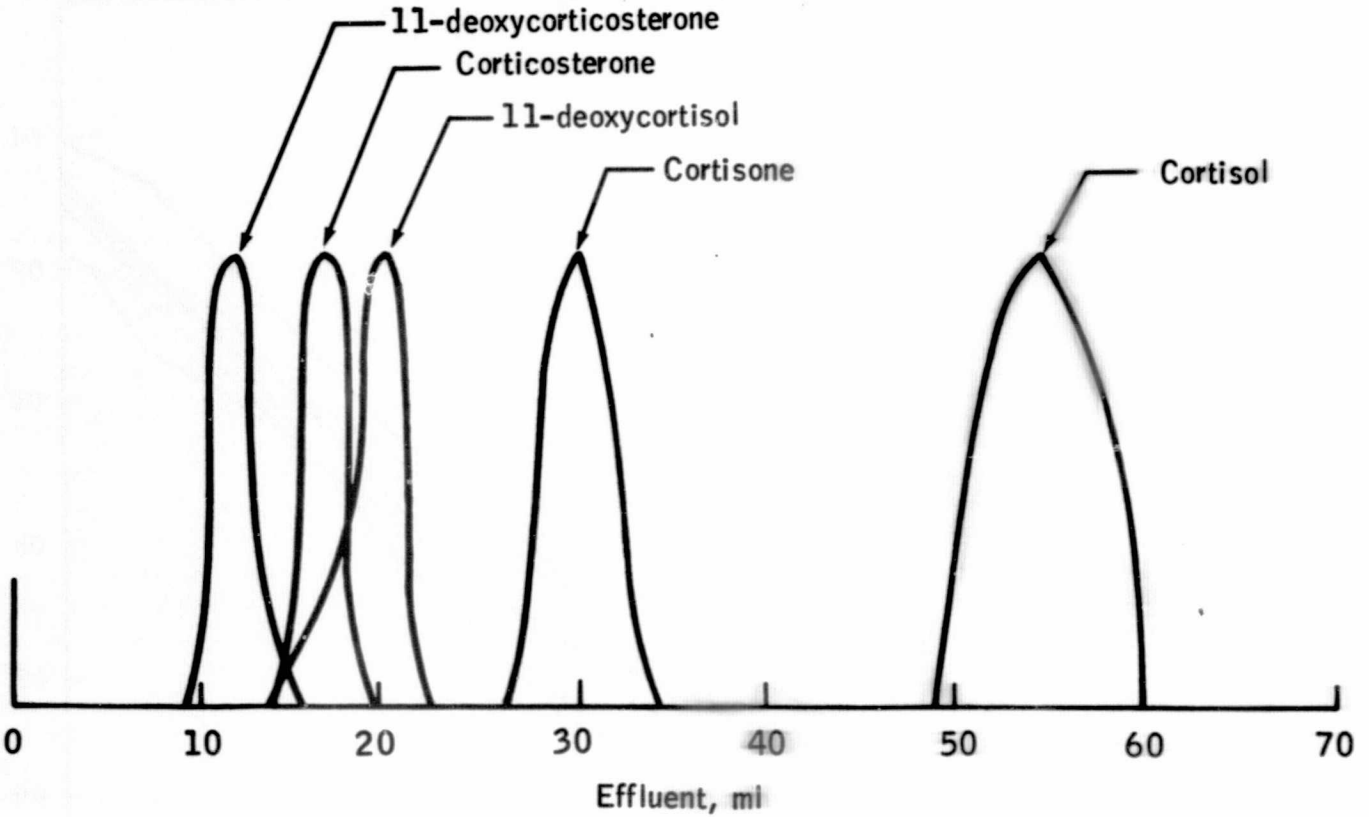

Figure 5-4.- Separation of radioactive corticosteroids by column chromatography (Sephadex LH-20, 1 by 20 cm column, eluted with methylene chloride/methanol, 99/1).

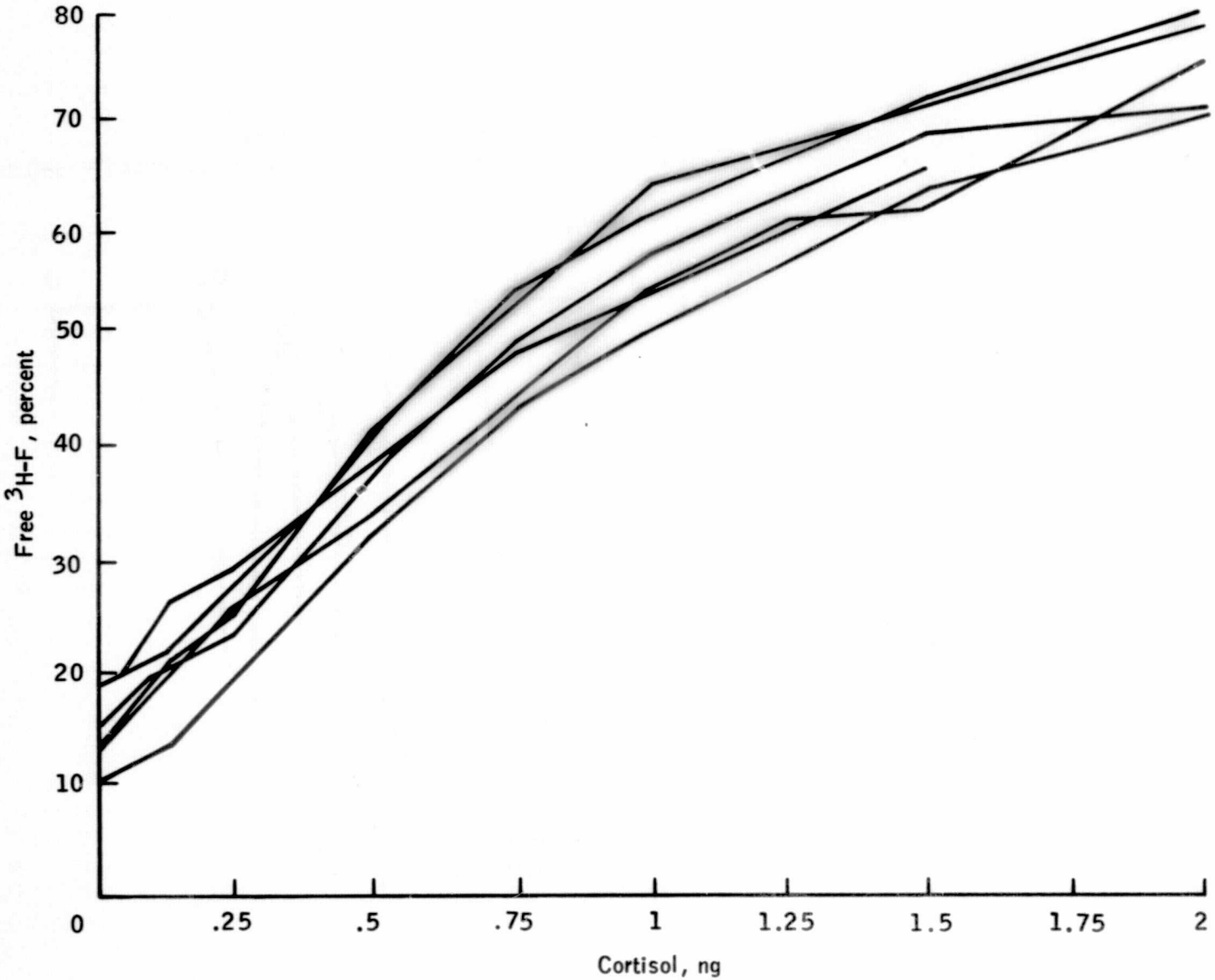

Figure 5-5.- Standard curves for radioimmunoassay of cortisol.

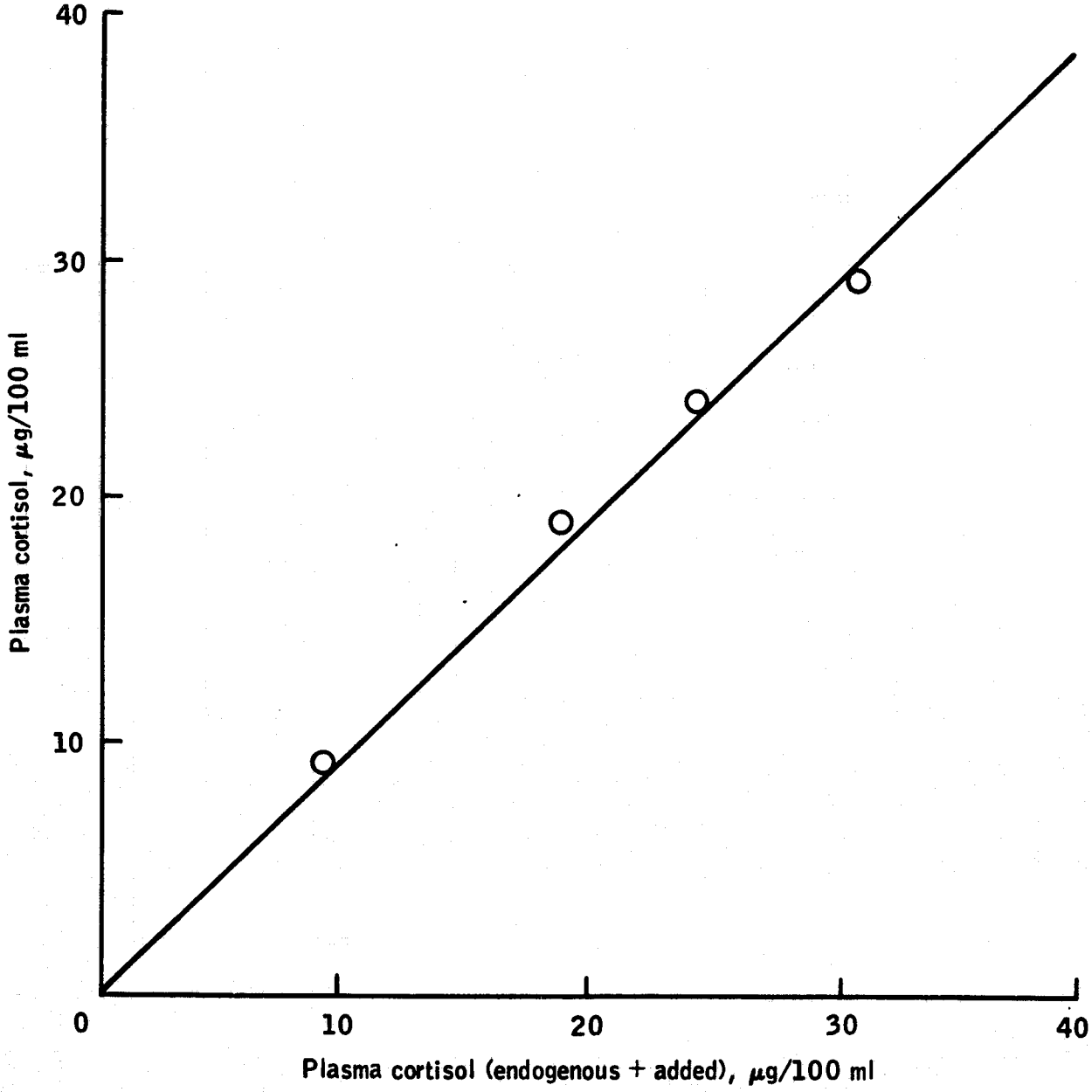

Figure 5-6.- Measurement of cortisol added to plasma by radioimmunoassay.

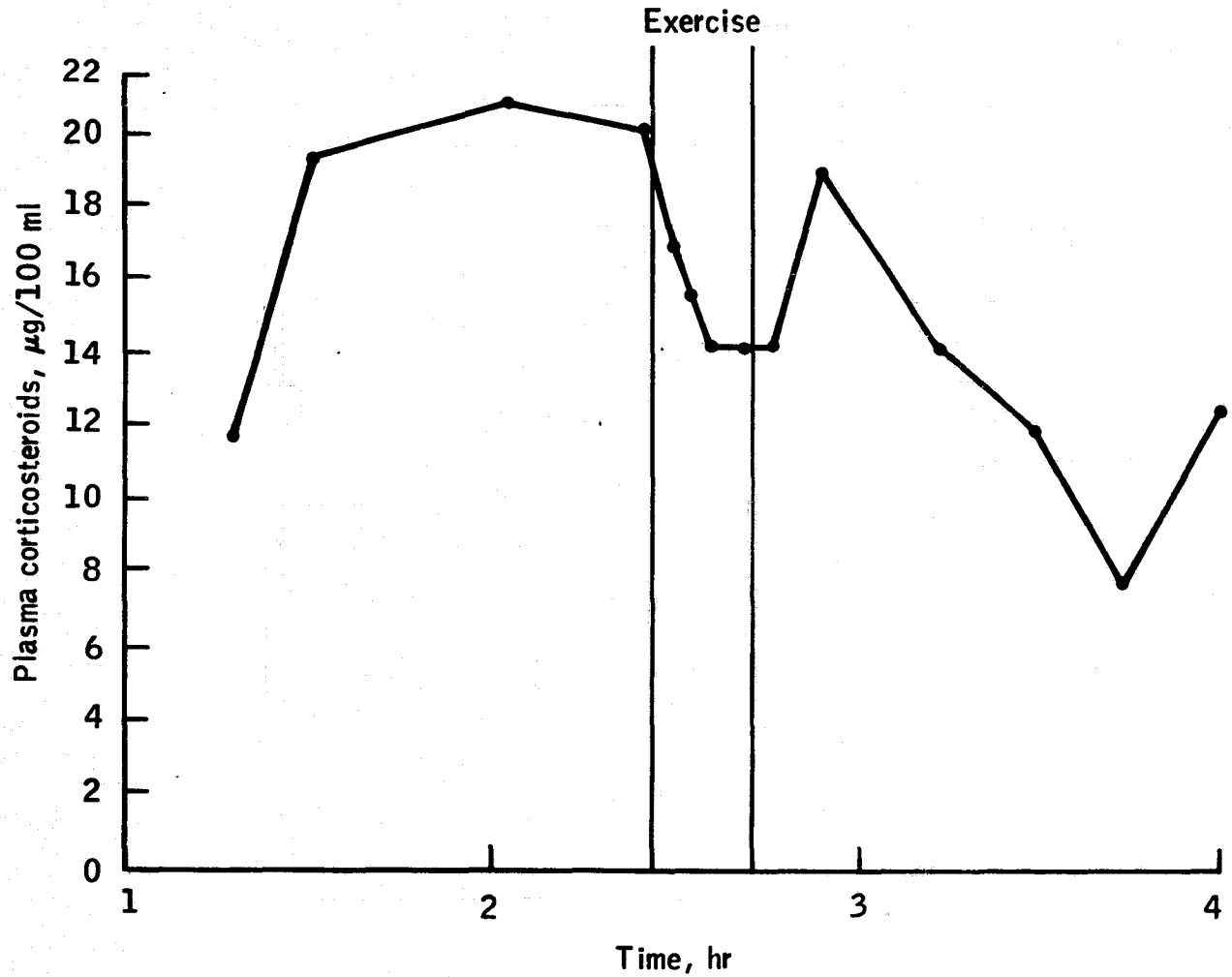

Figure 5-7.- Preliminary exercise study: changes in plasma corticosteroid concentration, single subject.

6. RECENT STUDIES OF PHYSIOLOGICAL FACTORS INVOLVED IN THE REGULATION OF SEROTONIN CONTENT AND TURNOVER IN THE BRAIN

By William W. Morgan, Ph. D.

DISCUSSION AND RESULTS

In recent years, considerable effort has been expended in an attempt to elucidate the physiological role(s) of serotonin (5-HT) in the brain. No clear functional role for the brain serotonergic system has yet been conclusively demonstrated. However, a considerable amount of evidence has been accumulated to indicate roles for brain 5-HT in a number of physiological processes. For example, 5-HT pathways in the hypothalamus may be involved in temperature regulation, particularly in the production of increased body heat in response to cold exposure (ref. 6-1). Serotonergic pathways may also play a regulatory role in neuroendocrine secretion. Intraventricularly administered 5-HT has been shown to decrease the blood level of luteinizing hormone (refs. 6-2 and 6-3). Serotonin containing neurons may also be involved in the regulation of adrenocorticotropic hormone (ACTH) release (ref. 6-4). The brain serotonergic system is known to be activated by stress (ref. 6-5); thus brain serotonergic pathways may play an important role in the adaptation to stress, probably in part by affecting the release of ACTH. Available evidence suggests that 5-HT containing neurons may function to decrease neural excitability. An inverse correlation between brain 5-HT content and the susceptibility to audiogenically induced seizures has been demonstrated (ref. 6-6). Also, low 5-HT in the brain has been correlated with an increased seizure susceptibility (ref. 6-7). One of the more documented roles of brain 5-HT is in the regulation of slow-wave sleep (refs. 6-8 and 6-9). Changes in brain 5-HT levels have also been correlated with some types of behavior. A decrease in brain 5-HT induced either by chemicals or by lesions results in an increase in response to electrical shock (ref. 6-10) and to an increase in approach behavior (ref. 6-11). An increase in the activity of brain serotonergic pathways is believed to result in a decrease in "fearfulness" and anxiety (ref. 6-12).

Because of the evidence linking brain serotonergic system to sleep regulation, to emotional behavior, and to the adaptation to stress (partially through actions of neuroendocrine regulation), it is likely that this putative neurotransmitter system will be markedly altered in humans during prolonged space flight and will play important roles in the adaptation of humans to stress associated with spaceflight. It is thus important to gain an understanding of the physiological mechanisms that regulate the activity of the brain serotonergic system and to further investigate the role of brain serotonergic pathways in the stress response and in neuroendocrine regulation.

Daily changes in 5-HT content have been demonstrated in the brains of many different organisms, and a considerable amount of evidence suggests that the circadian change in 5-HT content may be a reflection of a daily change in the activity of the serotonergic system. Indeed, there is some evidence in humans that the changes in the incidence of some types of epileptic seizures (ref. 6-13) and the severity of cases of mental depression (ref. 6-14) may be correlated with daily fluctuations in the activity of the brain serotonergic system. It is conceivable that changes in serotonergic nerve activity or in the levels of 5-HT in the brain may affect the ability of an organism to respond to stress. However, considerable disagreement has arisen in the last few years concerning the time of day when the serotonergic system is most active. One of the research goals of the Department of Anatomy Laboratory at the University of Texas at San Antonio has been to study this question. The importance of plasma tryptophan in regulating the activity of the serotonergic system has also been studied.

The presence of diurnal rhythmic changes in 5-HT in the brains of rodents has been well documented (refs. 6-15 to 6-27). In most of these studies, the highest level of 5-HT over a 24-hour period occurred during the light phase of a regulated lighting regime with 12 to 14 hours of light. Friedman and Walker (ref. 6-17) observed that 5-HT levels in the midbrain and caudate nucleus of the rat were elevated during the light phase when the rats were relatively inactive or asleep. They hypothesized that the elevation of 5-HT was functionally related to the cyclic inactive or sleep state observed in rodents. In support of this hypothesis, a considerable volume of evidence from other studies, particularly those of Jouvet (refs. 6-8 and 6-9), also suggests a functional role for 5-HT in sleep regulation. Asano (ref. 6-24) reported that the development of the adult pattern for the daily rhythms in brain 5-HT correlated closely in time with the development of the sleep-and-wakefulness pattern. Schreiber and Schlesinger (refs. 6-26 and 6-28) positively correlated the daily elevation of 5-HT content in areas of the mouse brain with a daily decrease in the seizure susceptibility of mice prone to audiogenic seizures. In their studies, Schreiber and Schlesinger found that 5-HT content was high during the light phase when the mice showed a decrease in seizure susceptibility. The assumption made by most of the previously mentioned investigators is that an increase in 5-HT content reflects an increase in the activity of the serotonergic system. However, as Neff and Tozer (ref. 6-29) have pointed out, the content of tentative transmitter substances such as 5-HT may provide a rather poor indicator of the activity of the neurons in which the transmitters are located. More information is needed to relate the brain serotonergic system not only to the phases of a regulated lighting cycle but also to other cyclic phenomena, such as motor activity and seizure susceptibility.

Another way of examining the activity of serotonergic neurons is to measure the brain content of the primary metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) (ref. 6-29). Changes in the brain content of 5-HIAA have been correlated with changes in the turnover of brain 5-HT (refs. 6-5 and 6-30). Thus, experiments were undertaken to determine if the content of brain 5-HIAA changed in a predictable fashion at different times of the day. If the levels of 5-HIAA in the brain were found to change with the time of day, the next step would be to determine the pattern of these changes in relation to the

lighting regime, and further to determine how this pattern of changes in 5-HIAA content compared to similar diurnal changes in 5-HT content.

In three separate studies, 36 adult male mice were housed in groups of 6 in an animal room maintained at a nearly constant temperature (295 K (22° C)). Balb/C mice were studied in the first experiment and AJAX mice were studied in the second and third experiments. The mice were exposed for at least 3 weeks to a daily cycle of 12 hours of light and 12 hours of dark (12:12 LD), with the lights on from 0900 to 2100 (central daylight time). The animals were provided with food (Wayne Lab Blox) and water ad libitum.

On the day of each experiment, the animals were sacrificed in groups of six at 4-hour intervals over a 24-hour period. The cerebral cortex was dissected and 5-HT was extracted and quantitated using the method of Maickel et al. (ref. 6-31); 5-HIAA was also extracted and quantitated from the same homogenate by the method of Curzon and Green (ref. 6-32).

In two of these experiments, circadian changes in 5-HT content were observed (fig. 6-1). In the third experiment, 5-HT content was not measured. The highest daily level of brain 5-HT was observed at different hours in the two experiments. Generally, however, 5-HT appeared to be high late in the dark phase and/or during the early hours of the light phase (fig. 6-1). The level of 5-HT declined as the light phase continued, and the lowest levels of 5-HT occurred during the early to middle dark phase. This pattern for the changes in 5-HT content in the mouse cerebral cortex with time of day agrees with that reported for 5-HT in the whole mouse brain (refs. 6-15 and 6-25) or in regions of mouse brain (ref. 6-26). The variability of the peak in serotonin content observed in this study is probably due partly because data were collected at intervals of 4 hours and because the rhythm in 5-HT content is not very precisely regulated by the lighting regime. Strain differences are probably of minor significance in producing the variability in the hour of peak 5-HT because the hour of peak 5-HT in the Balb/C strain (experiment 1) compares favorably with that reported for whole brain in AJAX mice in a previous publication (ref. 6-25).

Significant circadian changes in 5-HIAA content in the cerebral cortex were observed in each of the three experiments. Levels of 5-HIAA were high just before the onset of the dark phase (fig. 6-2, experiment 1) or during the early hours of the dark phase (fig. 6-2, experiments 2 and 3). The results of experiment 2 suggest that the daily 5-HIAA rhythm may be bimodal, with the second elevation occurring late in the dark phase. This second elevation of 5-HIAA during the late dark phase is probably of only short duration since it was not observed in a repeat study (experiment 3) of the rhythm in 5-HIAA content in the brains of AJAX mice.

A comparison of the rhythms in 5-HT content and 5-HIAA content in experiments 1 and 2 indicates that the patterns of the daily changes in the content of 5-HT do not correlate well with the pattern of changes in the content of its major metabolite, 5-HIAA. Other investigators have correlated changes in the brain content of 5-HIAA with the rate of turnover or metabolism of serotonin (refs. 6-12 and 6-30). It is thus likely that the daily changes in

5-HIAA content observed in the current studies reflect circadian changes in the rate of turnover or metabolism of 5-HT in the cerebral cortex of the mouse. If this is true, these data further suggest that the daily changes in the content of 5-HT do not correlate well with circadian changes in the turnover or metabolism of 5-HT. The data suggest that serotonin is being metabolized more rapidly just before the onset or during the early hours of the dark phase. These results support Hery et al. (ref. 6-33) who reported an increase in the rate of formation of radioactively labeled 5-HIAA from tritiated serotonin with the onset of the dark phase. They concluded that 5-HT was synthesized more rapidly during the light phase, accounting for the increase in 5-HT content, but was utilized more rapidly during the dark phase.

Sheard and Aghajanian (ref. 6-34) observed an elevation of brain 5-HIAA after electrical stimulation of the area of the raphe nuclei, the region of the brain containing the bulk of the cell bodies of the brain serotonergic system (ref. 6-35). These results suggest that an increase in the production of 5-HIAA or an increase in the turnover of serotonin may reflect an increase in impulse activity of serotonergic neurons. It is tempting to speculate that the elevation of 5-HIAA observed in these present studies also reflects an increase in the activity of serotonergic neurons before or during the early hours of the dark phase. However, it is yet unclear whether the turnover of serotonin reflects the activity of the functional pool of serotonin or the activity of both the functional and the metabolic pool of serotonin (ref. 6-11). Thus, it cannot be presently assumed that the elevations of 5-HIAA observed at certain time points in the current studies are a positive reflection of changes in the impulse activity of serotonergic neurons. However, the data indicate a need to evaluate more carefully the suggested functional correlations that other investigators have made between diurnal changes in serotonin content and similar diurnal changes in other parameters, such as sleep and motor activity or audiogenic seizure sensitivity.

Because only the content of 5-HIAA was measured, it is possible that the elevations of 5-HIAA content observed during the dark phase do not reflect an increase in the metabolism of serotonin but are the result of a decrease in the rate at which 5-HIAA is being actively transported out of the brain. Data available from a recent experiment suggest indirectly that the higher levels of 5-HIAA observed during the early dark phase may indeed be due to a decrease in 5-HIAA elimination from the brain during this time period. Figure 6-3 is a summary of the results of a preliminary experiment in which the turnover of 5-HT in the cerebral cortex of AJAX mice was determined during three separate time intervals over a 24-hour day. The turnover of 5-HT was determined by measuring the accumulation of 5-HIAA in the cerebral cortex after the active transport of this metabolite from the brain was inhibited by the injection of probenecid (200 mg/kg). The primary metabolite of 5-HT is 5-HIAA, and like 5-HT its passive diffusion from the brain is prevented by the blood-brain barrier (ref. 6-36). This acidic metabolite of 5-HT is normally eliminated from the brain by an acid transport system (ref. 6-37). Neff and Tozer (ref. 6-29) have shown that probenecid, a drug known to block acid transport in the kidney and liver, also blocks 5-HIAA transport in the brain. These investigators have also shown that the rate of accumulation of 5-HIAA in the brain after probenecid treatment is equal to the rate of turnover of 5-HT as calculated by other means (ref. 6-29). In the present experiment, the experimental animals

received probenecid intraperitoneally 2 hours before sacrifice. At the same time that the experimental animals received probenecid, a group of control animals was sacrificed. Since probenecid inhibits the transport of 5-HIAA out of the brain, the mean level of 5-HIAA in the brains of the control animals represents the content of 5-HIAA in the brains of the experimental animals at the time they were injected with probenecid. At 1000, 5-HIAA content had increased 75 percent over that observed in control mice sacrificed 2 hours earlier (fig. 6-3). At 1800, the probenecid-injected animals had accumulated 45 percent more 5-HIAA than the control animals. At 0400, the 5-HIAA content of the probenecid-injected animals was only 5 percent greater than that observed in the control animals sacrificed 2 hours earlier. In this particular experiment, the increase in 5-HIAA content in the control animals at 0400 was not statistically significant. The decrease in the total level of 5-HIAA accumulated in the cerebral cortex of the probenecid-treated animals at 0400 was statistically significant ($p < 0.01$) compared to the levels found in the probenecid-injected animals at 1000 and 1800. As was anticipated from earlier studies, the content of 5-HIAA in the cerebral cortex of the control animals sacrificed at 0200 was greater than that observed in the control animals at 0800 and 1600.

The data from this experiment suggest that 5-HT turnover is greater during the light phase than during the dark phase. If 5-HT turnover is correlated with the impulse activity of the serotonergic neurons, the results suggest that the serotonergic system is more active during the light phase when 5-HT levels are higher. This conclusion is contrary to that made on the basis of the data obtained from studies of diurnal changes in the content of 5-HIAA in the cerebral cortex. The results of the probenecid study suggest that the elevations of 5-HIAA observed during the dark phase are the result of a decrease in the rate of elimination of 5-HIAA from the brain rather than an increase in serotonergic activity.

However, several criticisms can be made of the pharmacological measurement of 5-HT turnover using probenecid. Probenecid not only inhibits the acid transport of 5-HIAA but also elevates the level of free tryptophan in the blood and the content of tryptophan in the brain (ref. 6-38). As will be discussed later in this report, there is evidence that the rate of turnover of 5-HT is influenced by the level of tryptophan in the brain (ref. 6-39), indicating that probenecid itself may affect 5-HT turnover by elevating the level of tryptophan in the brain. Also, any alteration of the steady state levels of 5-HT or its metabolites, such as that induced by probenecid may affect neuronal activity and thus may indirectly affect 5-HT turnover (ref. 6-40). It has been known for some time that there is a circadian variation in the rate of drug metabolism by the liver. Generally, drug metabolism is greater during the dark phase (ref. 6-41). Thus, the decrease in 5-HIAA accumulation observed in the probenecid-treated mouse brain during the dark phase may reflect a decrease in the amount of probenecid that reaches the brain because of the more rapid metabolism of this drug during the dark phase.

Probably the most direct and ultimately the most reliable method for determining the rate of 5-HT turnover, thus arriving at some indication of serotonergic neuronal activity, is the use of radioisotopic procedures. Bapna et al. (ref. 6-42) have recently described a radioisotopic method for

measuring 5-HT turnover in small regions of brain by measuring the rate of synthesis of radioactively labeled 5-HT from radioactive tryptophan injected into the lateral ventricle of the brain. This method combines the advantages of specificity (only serotonergic neurons that contain the specific enzyme tryptophan hydroxylase will synthesize radioactive 5-HT) with high sensitivity, because the labeled precursor is brought into immediate proximity to the brain. This method has been criticized because the concentration of tryptophan used in the procedure may itself alter 5-HT turnover in the brain (ref. 6-43); however, no direct evidence for such an effect of this procedure on 5-HT turnover has been experimentally shown. Because of the advantages of the Bapna method for measuring 5-HT turnover, experiments were undertaken to determine whether the intraventricular administration of tryptophan in quantities less than those used by Bapna would alter 5-HT turnover.

The right lateral ventricles of Sprague-Dawley rats were cannulated chronically using the procedure of Bapna et al. (ref. 6-42). One week later, the experimental animals received 5 micrograms of L-tryptophan dissolved in 20 microliters of Ringer's solution (pH7), and control animals received only Ringer's solution. Tryptophan, 5-HT, and 5-HIAA were extracted and quantitated as described by Hery et al. (ref. 6-33). After 15 minutes, 5-HIAA content was significantly elevated in both the cerebral cortex ($p < 0.01$) and brainstem ($p < 0.05$) of the tryptophan-treated animals (table 6-I). Levels of 5-HIAA in the cerebral cortex remained elevated throughout later time intervals and were significantly above control values after 30 minutes ($p < 0.05$) and 120 minutes ($p < 0.05$). The elevation of 5-HIAA in these brain areas probably reflects an increase in 5-HT turnover in the tryptophan-treated animals (refs. 6-12 and 6-30).

Tryptophan content was significantly elevated only in the cerebral cortex ($p < 0.05$) of the tryptophan-treated animals, and then for only 15 minutes after tryptophan administration (table 6-I). Apparently, most of the intraventricularly injected tryptophan either is not taken up or is rapidly cleared from the brain. Unlike 5-HIAA and tryptophan, 5-HT content was not significantly different in the brainstem or cerebral cortex of tryptophan-treated animals compared to control animals.

The experiment was repeated. However, in this study, 50 microcuries of tritiated (^3H) tryptophan (6 micrograms) was administered intraventricularly. After preliminary analysis, the results appeared to be inconsistent with those of the previous experiment. No significant elevations in 5-HT, tryptophan, or 5-HIAA were observed in either the right or left half of the cerebral cortex of tryptophan-treated rats compared to control rats after 15, 30, or 60 minutes (table 6-II). The level of tryptophan in the brainstem was significantly elevated 15 minutes after tryptophan administration (table 6-II). Evidence that the labeled tryptophan had been properly injected into the ventricular system was provided by the high specific activities of 5-HT, tryptophan, and 5-HIAA in both the cerebral cortex and brainstem when aliquots of these substances were counted in a liquid scintillation counter (table 6-III).

However, when regression and correlation analysis (ref. 6-44) were performed on the data from the two experiments, some striking and reproducible correlations between tryptophan and 5-HIAA content were observed, but only in tryptophan-treated animals. In both experiments, highly significant correlations between tryptophan and 5-HIAA content were observed in the cerebral cortex of animals given intraventricular injections of tryptophan ($p < 0.01$) (table 6-IV). No significant correlation between these two parameters was observed in the same brain area of control animals (table 6-IV).

Unlike the cerebral cortex, no correlation between tryptophan and 5-HIAA was observed in the brainstem of tryptophan-treated animals (table 6-IV). However, in the initial study, a small but significant correlation between tryptophan and 5-HIAA was observed in the brainstem of the control animals.

The data from these two studies indicate that the intraventricular administration of 5 to 6 micrograms of tryptophan is not sufficient to greatly increase 5-HT turnover. Therefore, brain 5-HIAA is not always significantly elevated when groups of tryptophan-treated animals are compared to control animals (table 6-II). However, when tryptophan and 5-HIAA are compared in individual tryptophan-treated animals by using correlation analysis, a significant effect of the administered tryptophan on the relationship between these two parameters is demonstrated. The significant correlation between tryptophan and 5-HIAA in the cerebral cortex of individual tryptophan-treated rats (table 6-IV) probably reflects a small increase in the size of the tryptophan pool in the cerebral cortex of these animals, with a subsequent increase in 5-HT turnover, reflected by 5-HIAA content. Because the turnover of brain 5-HT is normally regulated by a number of factors other than brain tryptophan, tryptophan and 5-HIAA are not closely correlated in the brains of control rats (table 6-IV). The potential influence of brain tryptophan on brain 5-HT turnover is suggested by the significant correlation observed between these parameters in the brainstem in the initial study. The lack of correlation of tryptophan and 5-HIAA in the brainstem of tryptophan-treated rats suggests that the intraventricular levels of tryptophan were not elevated in areas removed from the site of injection (the lateral ventricles) to produce a consistent and significant effect on 5-HT turnover. The cerebral cortex contains a predominance of serotonergic nerve endings, whereas almost all of the cell bodies for serotonergic neurons are located in the brainstem. Thus, the significant effect of intraventricularly injected tryptophan on the correlation between tryptophan and 5-HIAA in the cortex but not the brainstem may indicate that serotonergic nerve endings are more sensitive to changes in tryptophan than are cell bodies.

The results of these two experiments indicate that the intraventricular administration of tryptophan in quantities less than those used by Bapna et al. (ref. 6-42) is capable, in itself, of altering the turnover of 5-HT. Thus, this method should be used very carefully when studying changes in the rate of turnover of 5-HT during different times of the day and under different lighting conditions. A procedure similar to that of Bapna was used by Hery et al. (ref. 6-33). Thus, it may be necessary to reconsider their conclusions concerning the levels of activity of the serotonergic system during the light and the dark phase of a 12:12 LD 24-hour day. Work is continuing on a chronic cannula system for the external jugular vein by which high specific activity

^{3}H tryptophan could be injected into the blood vascular system. The ultimate use for this system will be to measure short-term changes in 5-HT turnover in the brain.

Considerable evidence has been accumulated in the last 2 years indicating that the circulating level of plasma tryptophan may have an important role in regulating brain 5-HT content. Fernstrom and Wurtman (ref. 6-39) found that the intraperitoneal injection of L-tryptophan was followed by a significant elevation in brain 5-HT content. The dosage of tryptophan given in their experiment caused a smaller increase in blood tryptophan than was normally seen when blood tryptophan, which shows a circadian variation in content, reached its peak content during the night. In a second study (ref. 6-45), the same investigators found that a carbohydrate diet caused an elevation in whole blood tryptophan content with an ensuing increase in brain 5-HT content. In this case, they suggested that the elevation of tryptophan content was the result of an increase in insulin release stimulated by the carbohydrate diet. These investigators have therefore hypothesized that plasma tryptophan content has an important physiological role in the regulation of brain 5-HT content. Furthermore, they felt that the daily rhythm in brain 5-HT content may be the result of similar daily changes in plasma tryptophan. Numerous studies have confirmed the presence of daily rhythmic changes in the concentration of plasma tryptophan in both rodents and humans (refs. 6-46 to 6-48). Perez-Cruet et al. (ref. 6-30) have proposed that the diurnal changes in brain 5-HT content may be secondary to diurnal cycles in feeding activity and to the influence of food intake on circulating levels of plasma tryptophan.

An experiment was undertaken to test the role of food intake and of plasma tryptophan content on the daily rhythms of 5-HT content in the whole brain of the AJAX mouse. In this experiment, 72 adult male AJAX mice were divided into two equal groups, and exposed to the 12:12 LD lighting regime. The control animals had free access to food (Wayne Lab Blox) and water. The experimental animals were given water ad libitum but were given food daily for only 4 hours, between 1000 and 1400. After 5 weeks under these experimental conditions, the mice were weighed and then sacrificed by decapitation in groups of 12 (6 controls and 6 experimentals) at 4-hour intervals over a 24-hour period. Blood was collected from the cervical vessels in heparinized 9-milliliter test tubes. The brain was removed and divided longitudinally into symmetrical right and left halves. The content of 5-HT was determined in one-half of the brain as described earlier. Tryptophan content in the other half of the brain was determined by the method of Denckla and Dewey (ref. 6-49). The blood was centrifuged and tryptophan content was determined in the plasma fraction. The statistical significance of the differences in plasma tryptophan, brain tryptophan, or brain 5-HT, with changes in the time of day in both control and experimental animals, was determined by the analysis of variance. Effects of the feeding regime on the phasing (clock hour of peaks and troughs) of the daily rhythms in each of the above parameters were determined by comparing graphs of the rhythm for each parameter in control versus experimental animals.

The results in table 6-V indicate that the adult male AJAX control mice consumed, on the average, more than 80 percent of their food during the dark

phase of a 12:12 LD regime. Despite the restricted feeding time for the experimental animals, the body weights of these animals were equal to those of the control animals at the time of sacrifice (table 6-VI).

The diurnal rhythm in plasma total tryptophan content was markedly altered by the feeding regime (fig. 6-4). In both control and feeding-regime animals, the highest level of plasma total tryptophan was observed when the animals were consuming most of their daily intake of food.

By contrast, the circadian rhythms in brain tryptophan content and in brain 5-HT content were not appreciably altered in the experimental or feeding-regime animals when compared to those of control animals (figs. 6-5 and 6-6). These data suggest that the daily rhythms in brain 5-HT and tryptophan exist independently of the level of tryptophan in the plasma. Thus, more evidence was provided that further supported the hypothesis that daily rhythms in 5-HT and 5-HIAA content are a reflection of daily changes in the activity of the serotonergic system and not merely a metabolic phenomenon caused by a circadian rhythm in blood tryptophan. The comparison of the circadian rhythms in brain 5-HT and brain tryptophan (figs. 6-5 and 6-6) suggests an important correlation between these two parameters and may indicate that changes in the level of brain tryptophan have an important role in the daily changes in brain serotonergic activity.

Recently, Fernstrom and Wurtman (ref. 6-50) have indicated that the ratio of plasma tryptophan to other neutral amino acids in the blood that share a common transport mechanism with tryptophan for uptake into the brain is of more importance in regulating the turnover of 5-HT than is the level of plasma tryptophan alone. Also, more than 80 percent of the tryptophan in the plasma is bound to albumin and is not immediately available for transport into the brain (ref. 6-51). Tagliamonte et al. (ref. 6-52) have shown that the levels of "free" tryptophan do not always parallel the levels of plasma total tryptophan. Thus, it is possible that either the ratio of plasma tryptophan to other neutral amino acids or the level of free tryptophan in the blood may have a central role in regulating circadian variations in the activity of the brain serotonergic system. To test this hypothesis, an experiment was performed in the laboratory.

In this experiment, 72 Sprague-Dawley rats were divided into two groups. One-half of the rats, designated as controls, were given food and water ad libitum, and the other half, designated as experimental rats, were given food only between 1000 and 1400 daily. All of the animals were exposed to a 14:10 LD lighting regime. The animals were maintained under these experimental conditions for 6 weeks. Groups of six control and six experimental rats were sacrificed at 4-hour intervals over a 24-hour period. Cerebral cortex and brainstem 5-HT, tryptophan, and 5-HIAA were extracted and analyzed as described by Hery et al. (ref. 6-33). Plasma total tryptophan was analyzed as described previously, and tyrosine and phenylalanine were analyzed by the methods of Waalkes and Udenfriend (ref. 6-53) and McCaman and Robins (ref. 6-54), respectively. One milliliter of plasma was placed in a cellulose dialysis sack and 0.08 milliliter of dialyzate containing free tryptophan was collected following the procedure of Tagliamonte et al. (ref. 6-52).

In this experiment, consistently significant circadian rhythms in 5-HT content were not observed. In the cerebral cortex, a significant daily rhythm in 5-HT was observed in the experimental, or feeding-regime, animals but not in the control animals (fig. 6-7). In the brainstem, however, the 5-HT rhythm was statistically significant in the control animals but not in the experimental animals (fig. 6-7). The inconsistency of these results probably reflects the daily disturbance of all the animals used in the study when food was given to and then withdrawn from the experimental animals. In previous studies by other investigators as well as in the author's studies in which consistent circadian rhythms in brain 5-HT content were demonstrated, the animals were left undisturbed for as long as a week at a time.

Significant circadian changes in 5-HIAA content were observed in the cerebral cortex of control and experimental animals and in the brainstem of experimental animals (fig. 6-8). A comparison of the patterns of the daily changes in 5-HIAA content in experimental and control rats suggests that, in these animals, the feeding regime did alter the 5-HIAA rhythm to some extent. In the cerebral cortex, a second peak in 5-HIAA content was observed in the experimental animals at 1200 during the 4-hour period in which food was available. In the brainstem, the rhythm 5-HIAA content was significant in the experimental animals but not in the control animals, primarily because of a marked decrease in 5-HIAA at 1600, 2 hours after the daily time at which the food was removed.

Rosecrans and Schechter (ref. 6-12) felt that the ratio of 5-HIAA to 5-HT provided a better indication of serotonergic neuron activity than did measures of 5-HT or 5-HIAA alone. When the present data were expressed in this way, the ratio of 5-HIAA to 5-HT in the brainstem was considerably higher during the dark phase than during the light phase (fig. 6-9). These data provide further evidence that the serotonergic system may be more active during the dark phase when the animals are active. Although not shown in this illustration, in the cerebral cortex of the experimental animals, the peak of 5-HIAA at 1200 was even more exaggerated when expressed as a ratio of 5-HIAA to 5-HT.

Significant circadian changes in tryptophan content were also observed in both brain areas of both control and experimental rats (fig. 6-10). In general, the magnitude of the change in brain tryptophan content, as well as the level of statistical significance, was somewhat reduced in the experimental, or feeding-regime, animals. In particular, brain tryptophan content was reduced in the experimental animals at 0800, just 2 hours before the start of the daily 4-hour feeding period.

Statistically significant circadian rhythyms in both plasma total tryptophan and plasma free tryptophan were observed in both control and experimental rats (fig. 6-11). These data represent the first experimental evidence for the existence of a circadian change in free plasma tryptophan. Generally, the circadian rhythm in plasma free tryptophan either parallels or leads by 4 hours the rhythm in plasma total tryptophan. As in the earlier study on mice, plasma trypotophan in the control rats was at its peak value during the dark phase when the animals consumed the majority of their daily food intake. In the

experimental rats, a peak in plasma tryptophan was also observed at 1200, during the time the animals were eating. Evidence that the circadian rhythm in plasma tryptophan is not merely secondary to the intake of food is provided by the presence of a second peak in plasma tryptophan in the experimental animals during the dark phase. This peak coincides with the peak in plasma tryptophan observed in the control animals.

To obtain a clearer evaluation of correlations between the various parameters studied in this experiment, a correlational analysis and a regression analysis were performed on the data (table 6-VII) (ref. 6-44). The null hypothesis that the correlation coefficient (r) or the slope of the regression line is equal to zero was tested by a "t" test (ref. 6-44). Highly significant as well as consistent correlations between brain tryptophan and 5-HIAA content were observed in the cerebral cortex ($p < 0.005$) and the brainstem ($p < 0.001$) of both control and experimental animals. However, no consistently significant correlations were observed between plasma free or total tryptophan and brain 5-HT, 5-HIAA, or tryptophan. Plasma free tryptophan and brain 5-HIAA were slightly but significantly ($p < 0.05$) correlated in the cerebral cortex of control rats (table 6-VII), and free tryptophan was also correlated with 5-HT in the brainstem of these same animals ($p < 0.01$). No correlations were observed between the ratio of plasma tryptophan to plasma tyrosine plus phenylalanine and brain 5-HT, 5-HIAA, or tryptophan (table 6-VII).

These data obtained from correlational analysis suggest that brain tryptophan content may be intimately related, perhaps in a causative manner, to the daily changes in the content of brain 5-HT and 5-HIAA. This interpretation is consistent with the coincidence in the phasing of the daily rhythms in brain tryptophan and 5-HT observed in the mouse feeding-regime experiment described earlier. The daily changes in 5-HIAA may reflect circadian fluctuations in the turnover of 5-HT, and thus circadian changes in brain tryptophan content may have a role in regulating a daily rhythm 5-HT turnover. A significant correlation between two parameters does not always reflect a cause and effect relationship; however, tryptophan and 5-HIAA are already known to be related as the precursor and principal metabolite of 5-HT.

The lack of significant correlations between plasma free or total tryptophan and brain 5-HT, 5-HIAA, or tryptophan indicates that these parameters are not linearly related to 5-HT rhythms in the brain and suggests that plasma tryptophan does not have an immediate or predominant role in the regulation of circadian rhythms of brain tryptophan or 5-HT or in the turnover of 5-HT. However, some role for plasma tryptophan in relation to the circadian rhythm in 5-HT turnover in the cerebral cortex is suggested by the consistent coincidence of the peaks of the circadian rhythms of plasma tryptophan (fig. 6-11) and cerebral cortex 5-HIAA (fig. 6-8). However, the peak of the circadian rhythm in brainstem 5-HIAA is not consistently coincident with the plasma tryptophan. These particular data may again reflect a greater sensitivity of nerve endings to changes in tryptophan content. The lack of significant correlation between plasma free tryptophan or the ratio of plasma tryptophan to plasma phenylalanine plus tyrosine and brain tryptophan, 5-HT, or 5-HIAA suggests that plasma tryptophan also does not have a major or predominant role in the production of diurnal changes in brain tryptophan content or the turnover

of 5-HT. These data do not rule out a delayed effect of changes in plasma tryptophan on brain tryptophan or 5-HT turnover.

During the past year, another research approach has also been used in the author's laboratory to investigate the relationship between changes in the turnover of brain 5-HT and changes in other parameters, such as brain tryptophan, plasma total and/or free tryptophan, or the ratio of plasma tryptophan to other neutral amino acids. Perez-Cruet et al. (ref. 6-30) have shown that 24 hours of starvation causes a significant increase in 5-HT turnover in rats. However, the causes for the increase in 5-HT turnover were not investigated by these researchers. They did suggest that these changes might be due to changes in the ratio of plasma tryptophan to other plasma neutral amino acids. Experiments were undertaken to determine whether brain 5-HT turnover in male golden hamsters was affected by 24 or 48 hours of starvation. Possible relationships between changes in the levels of tryptophan and other amino acids in the plasma and the effects of food deprivation on hamster brain tryptophan, 5-HT, and 5-HIAA content were also investigated.

Groups of six control, six starved, and six starved and then fed (refed) hamsters were sacrificed at 0, 1, 2, and 3 hours beginning 24 or 48 hours after food deprivation. Because no statistically significant differences in brain tryptophan, 5-HT, or 5-HIAA were observed at 0 versus 1, 2, or 3 hours within any of the three experimental groups, the data collected after different hours were combined to compare brain tryptophan, 5-HT, and 5-HIAA in control versus starved or refed animals.

Food deprivation of 48 to 51 hours resulted in a consistent and significant increase in 5-HT turnover as evidenced by an increase in 5-HIAA content (tables 6-VIII and 6-IX) and by an increase in the rate of accumulation of 5-HT after monoamine oxidase inhibition (fig. 6-12). The elevation of 5-HT turnover was not reduced by the feeding of the 48-hour starved hamsters for 3 hours (tables 6-VIII and 6-IX).

The increase in 5-HT turnover in 48- to 51-hour starved hamsters was not consistently correlated with the levels of brain tryptophan, and in separate experiments, brain tryptophan was found to be elevated (table 6-VIII) or unchanged (table 6-IX) after 48 to 51 hours of starvation. Moreover, brain tryptophan remained elevated (table 6-VIII) or decreased below control levels (table 6-IX) in 48-hour starved animals that had refed for up to 3 hours.

The elevation of 5-HT turnover in 48- to 51-hour starved hamsters was not related to an increase in plasma total tryptophan (ref. 6-39) or to an increase in the ratio of plasma tryptophan to tyrosine (ref. 6-50). The data show that plasma tryptophan (fig. 6-13) and the ratio of plasma total tryptophan to tyrosine (table 6-X) decreased rather than increased in these animals.

In 24-hour starved hamsters, the levels of 5-HIAA, which probably reflect 5-HT turnover, were not significantly elevated. However, in the initial 24-hour starvation study (table-XI), 5-HIAA content was significantly increased after 27 hours of food deprivation. Lack of food was probably not the only

cause for the increase in 5-HIAA at 27 hours, because the 24-hour-starved-then-3-hour-refed hamsters also showed a significant elevation in brain 5-HIAA (table 6-XI).

Brain tryptophan in the 24-hour-starved hamsters was already elevated at 24 hours and remained elevated at 27 hours. The increased level of brain tryptophan in these hamsters coincided with the elevation of 5-HIAA at 27 hours. After 3 hours of feeding, brain tryptophan in the starved animals had decreased below levels found in the control animals. This decrease in brain tryptophan was not positively correlated with the elevation of 5-HIAA observed in the brains of these same animals during the same time period.

As in the earlier studies summarized in this report, the elevations of 5-HIAA content in the brains of the 27-hour-starved hamsters and the refed hamsters (24 hours starved plus 3 hours refed) were not well correlated with the change in the ratio of plasma tryptophan to tyrosine (table 6-XII). If the increase in 5-HIAA content or 5-HT turnover were due to a change in the ratio of plasma tryptophan to tyrosine, the ratio would be expected to increase coincidently with the elevation of 5-HIAA. However, the value of this ratio in the 24- to 27-hour-starved hamsters was not significantly different from that found in control animals (table 6-XII), and the same ratio in refed hamsters was significantly lower than that observed in control hamsters (table 6-XII).

The 24-hour-starvation study was repeated. In this study also, 5-HIAA content was found to be elevated in the starved and the refed hamsters; however, in this experiment, the elevations were not statistically significant (table 6-XI). In this repeat study, the ratio of plasma tryptophan to tyrosine + phenylalanine was significantly lower in both starved and refed hamsters (table 6-XII).

The underlying mechanisms responsible for the elevation of 5-HT turnover in 48-hour-starved hamsters are not clear. However, the elevation of 5-HIAA and 5-HT turnover observed in these animals is not due to changes in brain tryptophan, plasma tryptophan, or the ratio of plasma tryptophan to other neutral amino acids. It could be argued that not enough of the neutral amino acids were measured in these studies to obtain a proper ratio. However, Fernstrom and Wurtman (ref. 6-55) have reported that only tryptophan and tyrosine must be measured to show a correlation of this ratio with brain 5-HT synthesis. The elevation of 5-HT turnover in 48-hour-starved hamsters may be due to an increase in the level of free tryptophan in the plasma (ref. 6-52). But after the refeeding of 24-hour-food-deprived hamsters, a very rapid increase in plasma free tryptophan was observed without any subsequent increase in 5-HIAA content (fig. 6-14). Admittedly, if the increase in 5-HT turnover was very short term, it might have been missed in this experiment.

The increase in 5-HT turnover observed in these starvation studies probably does not reflect a component of the brain serotonergic system that is involved in the regulation of food intake and metabolism (ref. 6-30). This does not imply that serotonergic pathways in certain discrete areas of the hamster brain are not involved in the regulation of food intake, but rather that the

present data do not seem to demonstrate these particular pathways. The elevation of 5-HT turnover observed in the hamster brain after 48 hours of starvation may be due to stress induced by this period of starvation. The elevation of 5-HIAA observed in one of two studies after 27 hours of food deprivation may indicate the lower limit of time required for starvation to induce an appreciable increase in 5-HT turnover, or may indicate that 24-to-27-hour-starved hamsters are more sensitive than control animals to the stress of noise or handling.

SUMMARY

The presence of high levels of 5-HIAA, the principal metabolite of 5-HT, during the dark phase of a 12:12 LD lighting regime, suggests that the turnover of 5-HT may be higher during the dark phase than during the light phase. A significant increase in the ratio of 5-HIAA/5-HT during the dark phase in both the brainstem and cerebral cortex of rats exposed to 14:10 LD lighting regime provides further evidence that 5-HT turnover in the brain is greater during the dark phase. The increase in 5-HT turnover observed during the dark phase may reflect an increase in serotonergic activity at this time. However, preliminary evidence suggests that the dark-phase elevation of 5-HIAA may be due to a decrease in the rate of elimination of 5-HIAA from the brain, rather than to a change in serotonergic activity.

Direct experimental evidence was obtained to show that the intraventricular administration of 5 to 6 micrograms of tryptophan, a quantity considerably less than that frequently administered as a radioactive isotope to measure 5-HT turnover, is capable itself of slightly increasing 5-HT turnover. In the mouse, the daily rhythms in plasma total tryptophan were not well correlated with daily rhythms in brain tryptophan and 5-HT. However, a coincidence of the peaks of the daily rhythms in brain tryptophan and 5-HT suggests a possible interrelationship.

Highly significant correlations between brain tryptophan and 5-HIAA content were observed in the cerebral cortex and the brainstem of rats sacrificed at periods over a 24-hour day. Similar correlations between brainstem 5-HT and tryptophan were also observed in the brainstem of these animals. However, no consistently significant correlations were observed between either brain 5-HIAA or 5-HT and plasma-free or total tryptophan, or between brain 5-HIAA or 5-HT and the ratio of tryptophan to neutral plasma amino acids. These data again indicate that brain tryptophan may have an important role in the regulation of circadian changes in 5-HT turnover, whereas plasma tryptophan seems to be of less importance. Some role for plasma tryptophan in the regulation of circadian variations in 5-HT turnover is suggested by the coincidence of peaks observed in the circadian rhythms of plasma tryptophan and cerebral cortex and brainstem 5-HIAA.

A consistently significant increase in 5-HT turnover was observed in the whole brain of 48-hour-starved hamsters. Elevation of 5-HT turnover or 5-HIAA content was noted in the hamster brain after 27 hours of starvation. However,

this elevation of 5-HT turnover was not always statistically significant. The increase in 5-HT turnover in 48-hour-starved hamsters was not consistently correlated with an increase in brain tryptophan, and was never found to be correlated with an increase in plasma total tryptophan or with the ratio of plasma tryptophan to plasma tyrosine. These data obtained from starved hamsters suggest that, at least in the hamster, the turnover of 5-HT can be increased independently of changes in brain or plasma tryptophan.

REFERENCES

6-1. Myers, R. D.; and Beleslin, D. B.: Changes in Serotonin Release in Hypothalamus During Cooling or Warming of the Monkey. Am. J. Physiol. vol. 220, no. 6, June 1971, pp. 1746-1754.

6-2. Kamberi, Ibrahim A.; Mical, Renon S.; and Porter, John C.: Effect of Anterior Pituitary Perfusion and Intraventricular Injection of Catecholamines and Indoleamines on LH Release. Endocrinology, vol. 87, no. 1, July 1970, pp. 1-12.

6-3. Schneider, H. P. G.; and McCann, S. M.: Possible Role of Dopamine as Transmitter to Promote Discharge of LH-releasing Factor. Endocrinology vol. 85, no. 1, July 1969, pp. 121-132.

6-4. Vernikos-Danellis, J.; and Berger, P. A.: Brain Serotonin and the Pituitary-adrenal System. In J. Barchas and E. Usdin (eds.) Serotonin and Behavior, Academic Press (New York), 1973, pp. 173-177.

6-5. Bliss, E. L.: Effects of Behavioral Manipulations Upon Brain Serotonin and Dopamine. In J. Barchas and E. Usdin (eds.) Serotonin and Behavior, Academic Press (New York), 1973, pp. 315-324.

6-6. Schlesinger, Kurt; Boggan, William; and Freedman, Daniel X.: Genetics of Audiogenic Seizures: I. Relation to Brain Serotonin and Norepinephrine in Mice. Life Sci., vol. 4, no. 24, Dec. 1965, pp. 2345-2351.

6-7. Boggan, W. O.; and Seiden, L. S.: 5-hydroxytryptophan Reversal of Reserpine Enhancement of Audiogenic Seizure Susceptibility in Mice. Physiol. Behav., vol. 10, Jan. 1973, pp. 9-12.

6-8. Jouvet, M.: Insomnia and Decrease of the Cerebral 5-hydroxytryptamine After Destruction of the Raphe System in the Cat. In S. Garattini and P. A. Shore, (eds.) Advances in Pharmacology, Academic Press (New York), 1968, pp. 265-279.

6-9. Jouvet, Michel: Biogenic Amines and the State of Sleep. Science, vol. 163, no. 3862, Jan. 1969, pp. 32-41.

6-10. Harvey, John A.; and Lints, Carlton E.: Lesions in the Medial Forebrain Bundle: Relationship Between Pain Sensitivity and Telencephalic Content of Serotonin. J. Comp. Physiol. Psychol. vol. 74, no. 1, 1971, pp. 28-36.

6-11. Aprison, M. H.; and Hingtgen, J. N.: Serotonin and Behavior: A Brief Summary. Fed. Proc., vol. 31, Jan.-Feb. 1972, pp. 121-129.

6-12. Rosecrans, J. A.; and Schechter, M. D.: Brain 5-hydroxytryptamine Correlates of Behavior in Rats: Strain and Sex Variability. Physiol. Behav., vol. 8, Mar. 1972, pp. 503-510.

6-13. Halberg, F.: Some Physiological and Clinical Aspects of 24-hour Periodicity. J. Lancet, vol. 73, 1953, pp. 20-32.

6-14. Curzon, G.: Tryptophan Pyrrolase — A Biochemical Factor in Depressive Illness? Brit. J. Psychiat., vol. 116, no. 571-2, May 1970, pp. 1367-1374.

6-15. Albrecht, P.; Visscher, M. B.; Bittner, J. J.; and Halberg, F.: Daily Changes in 5-hydroxytryptamine Concentration in Mouse Brain. Proc. Soc. Exp. Biol. Med., vol. 92, 1956, pp. 703-706.

6-16. Dixit, Balwant N.; and Buckley, Joseph P.: Circadian Changes in Brain 5-hydroxytryptamine and Plasma Corticosterone in the Rat. Life Sci., vol. 6, no. 7, 1967, pp. 755-758.

6-17. Friedman, Alexander H.; and Walker, Charles A.: Circadian Rhythms in Rat Mid-Brain and Caudate Nucleus Biogenic Amine Levels. J. Physiol., vol. 197, no. 1, July 1968, pp. 77-85.

6-18. Friedman, Alexander H.; and Walker, Charles A.: Rat Brain Amines, Blood Histamine and Glucose Levels in Relationship to Circadian Changes in Sleep Induced by Pentobarbitone Sodium. J. Physiol., vol. 202, no. 1, May 1969, pp. 133-146.

6-19. Scheving, L. E.; Harrison, W. H.; Gordon, P.; and Pauly, J. E.: Daily Fluctuation (Circadian and Ultradian) in Biogenic Amines of the Rat Brain. Am. J. Physiol, vol. 214, no. 1, Jan. 1968, pp. 166-173.

6-20. Quay, W. B.: Regional and Circadian Differences in Cerebral Cortical Serotonin Concentrations. Life Sci., vol. 4, no. 3, Feb. 1965, pp. 379-384.

6-21. Quay, W. B.: Differences in Circadian Rhythms in 5-hydroxytryptamine According to Brain Region. Am. J. Physiol., vol. 215, no. 6, Dec. 1968, pp. 1448-1452.

6-22. Okada, Fumihiko: The Maturation of the Circadian Rhythm of Brain Serotonin in the Rat. Life Sci., vol. 10, pt. I, no. 2, Jan. 1971, pp. 77-86.

6-23. Scapagnini, U.; Moberg, G. P.; Van Loon, G. R.; De Groot, J.; and Ganong, W. F.: Relation of Brain 5-hydroxytryptamine Content to the Diurnal Variation in Plasma Corticosterone in the Rat. Neuroendocrinology, vol. 7, 1971, pp. 90-96.

6-24. Asano, Yutaka: The Maturation of the Circadian Rhythm of Brain Norepinephrine and Serotonin in the Rat. Life Sci., vol. 10, pt. I, no. 15, Aug. 1971, pp. 883-894.

6-25. Morgan, W. W.; and Yndo, C. A.: Daily Rhythms in Tryptophan and Serotonin Content in Mouse Brain: The Apparent Independence of These Parameters From Daily Changes in Food Intake and From Plasma Tryptophan Content. Life Sci., vol. 12, Mar. 1973, pp. 395-408.

6-26. Schreiber, Robert A.; and Schlesinger, Kurt: Circadian Rhythms and Seizure Susceptibility: Relation to 5-hydroxytryptamine and Norepinephrine in Brain. Physiol. Behav., vol. 6, no. 6, June 1971, pp. 635-640.

6-27. Morgan, W. W.; Yndo, C. A.; and McFadin, L. S.: Daily Rhythmic Changes in the Content of Serotonin and 5-hydroxyindoleacetic Acid in the Cerebral Cortex of Mice. Life Sci., vol. 14, Jan. 1974, pp. 329-338.

6-28. Schreiber, R. A.; and Schlesinger, K.: Circadian Rhythms and Seizure Susceptibility: Effects of Manipulations of Light Cycles on Susceptibility to Audiogenic Seizures and on Levels of 5-hydroxytryptamine and Norepinephrine in Brain. Physiol. Behav., vol. 8, Apr. 1972, pp. 699-703.

6-29. Neff, Norton H.; and Tozer, T. N.: In Vivo Measurement of Brain Serotonin Turnover. Advanc. Pharma., vol. 6, pt. A, 1968, pp. 97-109.

6-30. Perez-Cruet, J.; Tagliamonte, A.; Tagliamonte, P.; and Gessa, G. L.: Changes in Brain Serotonin Metabolism Associated with Fasting and Satiation. Life Sci., vol. 11, 1972, pp. 31-39.

6-31. Maickel, Roger P.; Cox, Raymond H., Jr.; Saillant, Jean; and Miller, Francis P.: A Method for the Determination of Serotonin and Norepinephrine in Discrete Areas of Rat Brain. Int. J. Neuropharmac., vol. 7, no. 3, May-June 1968, pp. 275-281.

6-32. Curzon, G.; and Green, A. R.: Rapid Method for the Determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic Acid in Small Regions of Rat Brain. Brit. J. Pharma., vol. 39, July 1970, pp. 653-655.

6-33. Hery, Francis; Rouer, E.; and Glowinsky, J.: Daily Variations of Serotonin Metabolism in the Rat Brain. Brain Res., vol. 43, 1972, pp. 445-465.

6-34. Sheard, Michael H.; and Aghajanian, George K.: Stimulation of the Midbrain Raphe: Effect on Serotonin Metabolism. J. Pharmacol. Exp. Ther., vol. 163, no. 2, 1968, pp. 425-430.

6-35. Fuxe, Kjell: Evidence for the Existence of Monoamine Neurons in the Central Nervous System. IV. The Distribution of Monoamine Terminals in the Central Nervous System. Acta Physiol. Scand. 64, suppl. vol. 247, 1965, pp. 39-85.

6-36. Roos, Björn-Erik: On the Occurrence and Distribution of 5-hydroxyindoleacetic Acid in Brain. Life Sci., vol. 1, no. 1, Jan. 1962, pp. 25-27.

6-37. Neff, N. H.; Tozer, T. N.; and Brodie, B. B.: Indole Metabolism. Part 2. Specialized Transport System to Transfer 5-HIAA Directly from Brain to Blood. Pharmacologist, vol. 62, 1964, p. 194.

6-38. Tagliamonte, A.; Biggio, G.; and Gessa, G. L.: Possible Role of "Free" Plasma Tryptophan in Controlling Brain Tryptophan Concentrations. Rivista di Farmacologia et Terapia, vol. 11, 1971, pp. 251-255.

6-39. Fernstrom, John D.; and Wurtman, R. J.: Brain Serotonin Content: Physiological Dependence on Plasma Tryptophan Levels. Science, vol. 173, no. 3992, July 1971, pp. 149-152.

6-40. Wurtman, R. J.; Anton-Tay, F.; and Anton, S.: On the Use of Synthesis Inhibitors to Estimate Brain Norepinephrine Synthesis in Gonadectomized Rats. Life Sci., vol. 8, pt. I, no. 17, 1969, pp. 1015-1022.

6-41. Heikkinen, E.; Karppanen, H.; Vapaatalo, H.; and Pelkonen, O.: Lack of Effect of Pinealectomy on the Diurnal Rhythm in Drug Metabolism. Acta Pharmacol. Toxicol., vol. 32, 1973, pp. 157-160.

6-42. Bapna, J.; Neff, N. H.; and Costa, E.: A Method for Studying Norepinephrine and Serotonin Metabolism in Small Regions of Rat Brain: Effect of Ovariectomy on Amine Metabolism in Anterior and Posterior Hypothalamus. Endocrinology, vol. 89, no. 6, Dec. 1971, pp. 1345-1349.

6-43. Hyyppä, M. T.; Cardinali, D. P.; Baumgarten, H. G.; and Wurtman, R. J.: Rapid Accumulation of H^3-serotonin in Brains of Rats Receiving Intraperitoneal H^3-tryptophan: Effects of 5, 6-dihydroxytryptamine or Female Sex Hormones. J. Neural Transm., vol. 34, 1973, pp. 111-124.

6-44. Sokal, Robert R.; and Rohlf, F. James: Biometry; The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, San Francisco, 1969.

6-45. Fernstrom, John D.; and Wurtman, Richard J.: Brain Serotonin Content: Increase Following Ingestion of Carbohydrate Diet. Science, vol. 174, no. 4013, 1971, pp. 1023-1025.

6-46. Rapoport, Morton I.; Feigin, Ralph D.; Bruton, Joseph; and Beisel, William R.: Circadian Rhythm for Tryptophan Pyrrolase Activity and its Circulating Substrate. Science, vol. 153, no. 3744, Sept. 1966, pp. 1642-1644.

6-47. Wurtman, Richard J.; Rose, Christopher M.; Chou, Chuan; and Larin, Frances F.: Daily Rhythms in the Concentrations of Various Amino Acids in Human Plasma. New Engl. J. Med., vol. 279, no. 4, July 1968, pp. 171-175.

6-48. Fernstrom, John D.; Larin, Frances; and Wurtman, Richard J.: Daily Variations in the Concentrations of Individual Amino Acids in Rat Plasma. Life Sci., vol. 10, pt. I, no. 14, July 1971, pp. 813-819.

6-49. Denckla, W. D.; and Dewey, Henry K.: The Determination of Tryptophan in Plasma, Liver, and Urine. J. Lab. Clin. Med., vol. 69, no. 1, Jan. 1967, pp. 160-169.

6-50. Fernstrom, John D.; and Wurtman, Richard J.: Brain Serotonin Content: Physiological Regulation by Plasma Neutral Amino Acids. Science, vol. 178, no. 4059, Oct. 1972, pp. 414-416.

6-51. McMenamy, Rapier H.; and Oncley, J. L.: The Specific Binding of L-tryptophan to Serum Albumin. J. Biol. Chem., vol. 233, no. 6, Dec. 1958, pp. 1436-1447.

6-52. Tagliamonte, A.; Biggio, G.; Vargiu, L.; and Gessa, G. L.: Free Tryptophan in Serum Controls Brain Tryptophan Level and Serotonin Synthesis. Life Sci., vol. 12, Mar. 1973, pp. 277-287.

6-53. Waalkes, T. P.; and Udenfriend, S. A.: Fluorometric Method for Estimation of Tyrosine in Plasma and Tissue. J. Lab. & Clin. Med., vol. 50, 1957, pp. 733-736.

6-54. McCaman, M. W.; and Robins, E.: Fluorometric Method for Determination of Phenylalanine in Serum. J. Lab. Clin. Med., vol. 59, May 1962, pp. 885-890.

6-55. Fernstrom, J. D.; Larin, F.; and Wurtman, R. J.: Correlations Between Brain Tryptophan and Plasma Neutral Amino Acid Levels Following Food Consumption in Rats. Life Sci., vol. 13, 1973, pp. 517-524.

TABLE 6-I.- COMPARISON OF 5-HT, TRYPTOPHAN, AND 5-HIAA CONTENT IN EXPERIMENTAL ANIMALS INJECTED INTRAVENTRICULARLY WITH 5 μg OF TRYPTOPHAN VERSUS CONTROL ANIMALS INJECTED WITH SALINE

Time after injection, min	Treatment group (a)	5-HT, μg/g ± SE[b] (c)	Tryptophan, μg/g ± SE (c)	5-HIAA, μg/g ± SE (c)
Cerebral cortex				
15	C	0.186 ± 0.030 (5)	2.70 ± 0.25 (6)	0.342 ± 0.009 (6)
	E	.230 ± .029 (7)	[d]3.62 ± .31 (6)	[e].398 ± .012 (6)
30	C	0.246 ± 0.034 (7)	3.30 ± 0.21 (7)	0.334 ± 0.017 (7)
	E	.263 ± .022 (7)	3.45 ± .21 (5)	[d].386 ± .015 (6)
60	C	0.233 ± 0.024 (7)	3.56 ± 0.19 (6)	0.367 ± 0.016 (7)
	E	.265 ± .035 (7)	3.54 ± .09 (5)	.379 ± .013 (7)
120	C	0.276 ± 0.028 (6)	2.98 ± 0.20 (4)	0.319 ± 0.016 (6)
	E	.220 ± .029 (6)	2.91 ± .24 (6)	[d].382 ± .020 (5)
180	C	0.277 ± 0.023 (6)	2.92 ± 0.25 (5)	0.341 ± 0.012 (6)
	E	.232 ± .019 (6)	3.00 ± .06 (6)	.334 ± .007 (6)
Brainstem				
15	C	0.717 ± 0.023 (7)	4.08 ± 0.23 (7)	0.766 ± 0.032 (6)
	E	.766 ± .062 (6)	4.00 ± .18 (6)	[d].923 ± .033 (5)
30	C	0.719 ± 0.080 (7)	3.97 ± 0.21 (7)	0.849 ± 0.057 (6)
	E	.729 ± .064 (7)	4.26 ± .27 (7)	.945 ± .045 (7)
60	C	0.654 ± 0.039 (7)	4.23 ± 0.23 (7)	0.880 ± 0.031 (6)
	E	.706 ± .074 (7)	4.37 ± .20 (7)	.918 ± .054 (7)
120	C	0.602 ± 0.077 (5)	3.78 ± 0.25 (5)	0.894 ± 0.033 (5)
	E	.714 ± .036 (6)	3.73 ± .27 (6)	.922 ± .065 (6)
180	C	0.680 ± 0.079 (6)	3.89 ± 0.20 (6)	0.929 ± 0.064 (6)
	E	.707 ± .030 (6)	3.65 ± .13 (6)	.822 ± .038 (6)

[a]C = control; E = experimental.

[b]SE = standard error.

[c]Numbers in parentheses indicates number of animals in group.

[d]$p < 0.05$; statistical significance was determined by analysis of variance.

[e]$p < 0.01$; statistical significance was determined by analysis of variance.

TABLE 6-II.- COMPARISON OF 5-HT, TRYPTOPHAN, AND 5-HIAA CONTENT IN EXPERIMENTAL ANIMALS INJECTED INTRAVENTRICULARLY WITH 6 μg (50 MICROCURIES) OF ^{3}H TRYPTOPHAN VERSUS CONTROL ANIMALS INJECTED WITH SALINE

Time after injection, min	Treatment group	5-HT, μg/g ± SE (a)	Tryptophan, μg/g ± SE (a)	5-HIAA, μg/g ± SE (a)
Right cerebral cortex				
15	C	0.363 ± 0.021 (8)	4.41 ± 0.21 (7)	0.332 ± 0.030 (8)
	E	.392 ± .016 (11)	4.46 ± .16 (9)	.292 ± .017 (11)
30	C	0.392 ± 0.027 (8)	4.55 ± 0.20 (7)	0.324 ± 0.009 (8)
	E	.386 ± .016 (8)	4.55 ± .06 (8)	.332 ± .016 (8)
60	C	0.404 ± 0.012 (8)	4.19 ± 0.13 (8)	0.309 ± 0.019 (8)
	E	.409 ± .005 (8)	4.41 ± .22 (8)	.309 ± .019 (8)
Left cerebral cortex				
15	C	0.323 ± 0.031 (8)	4.43 ± 0.12 (8)	0.379 ± 0.016 (8)
	E	.328 ± .023 (11)	4.31 ± .09 (9)	.342 ± .022 (10)
30	C	0.307 ± 0.040 (7)	4.44 ± 0.17 (8)	0.357 ± 0.014 (6)
	E	.336 ± .033 (8)	4.69 ± .19 (7)	.378 ± .024 (8)
60	C	0.313 ± 0.041 (8)	4.48 ± 0.10 (7)	0.353 ± 0.019 (8)
	E	.297 ± .037 (7)	4.67 ± .25 (8)	.356 ± .020 (7)
Brainstem				
15	C	0.706 ± 0.122 (5)	3.96 ± 0.13 (8)	0.619 ± 0.020 (8)
	E	.958 ± .113 (8)	[b]4.62 ± .16 (11)	.669 ± .021 (10)
30	C	0.786 ± 0.087 (6)	4.48 ± 0.21 (8)	0.704 ± 0.019 (8)
	E	.785 ± .082 (7)	4.42 ± .09 (8)	.674 ± .024 (7)
60	C	0.836 ± 0.063 (8)	4.32 ± 0.14 (8)	0.641 ± 0.017 (8)
	E	.877 ± .074 (7)	4.38 ± .17 (8)	.683 ± .035 (8)

[a]Number in parentheses indicates number of animals in group.

[b]$p < 0.01$; statistical significance determined by Student's "t" test.

TABLE 6-III.- THE SPECIFIC ACTIVITY OF BRAIN 5-HT, TRYPTOPHAN, 5-HIAA, AND TOTAL NONPROTEIN-BOUND RADIOACTIVITY IN BLOOD SERUM AT VARIOUS TIME INTERVALS AFTER THE INTRAVENTRICULAR INJECTION OF 50 MICROCURIES OF ^{3}H TRYPTOPHAN

Time after injection, min	Blood serum, [a]DPM/ml ± SE	5-HT, DPM/nmole ± SE	Tryptophan, DPM/nmole ± SE	5-HIAA DPM/nmole ± SE
		Right cerebral cortex		
15	$1291.8 \pm 118.2 \times 10^3$	$412.8 \pm 52.1 \times 10^3$	$48.6 \pm 9.8 \times 10^3$	$14.0 \pm 1.7 \times 10^3$
30	821.4 ± 60.0	296.6 ± 40.2	11.4 ± 2.7	9.9 ± 1.8
60	411.8 ± 69.4	230.6 ± 34.0	$5.7 \pm .8$	10.1 ± 1.6
		Left cerebral cortex		
15	$1291.8 \pm 118.2 \times 10^3$	$290.0 \pm 27.0 \times 10^3$	$35.1 \pm 15.8 \times 10^3$	$8.1 \pm 1.3 \times 10^3$
30	821.4 ± 60.0	265.9 ± 31.9	7.0 ± 1.7	7.0 ± 1.5
60	411.8 ± 69.4	345.1 ± 55.6	$3.3 \pm .5$	8.1 ± 1.5
		Brainstem		
15	$1291.8 \pm 118.2 \times 10^3$	$224.8 \pm 47.7 \times 10^3$	$30.6 \pm 5.3 \times 10^3$	$30.2 \pm 5.4 \times 10^3$
30	821.4 ± 60.0	294.9 ± 37.8	$14.9 \pm .9$	33.4 ± 2.6
60	230.4 ± 29.1	230.4 ± 29.1	$8.3 \pm .8$	24.5 ± 1.8

[a]DPM = Disintegrations per minute.

TABLE 6-IV.- CORRELATIONS BETWEEN BRAIN TRYPTOPHAN AND BRAIN 5-HIAA CONTENTS IN ANIMALS INJECTED INTRAVENTRICULARLY WITH TRYPTOPHAN OR SALINE

Brain area	Injection	Correlation coefficient (r) (a)	Percentage of 5-HIAA due to regression (b)	Statistical significance[c] of r	Statistical significance[c] of regression
Experiment 1					
Cerebral cortex	Tryptophan	0.488 (29)	24.0	$p < 0.05$	$p < 0.01$
	Saline	.145 (30)	2.0	NS[d]	NS
Brainstem	Tryptophan	0.282 (31)	8.0	NS	NS
	Saline	.381 (32)	15.0	$p < .05$	$p < .02$
Experiment 2					
Right cerebral cortex	Tryptophan	0.670 (24)	45.0	$p < 0.001$	$p < 0.001$
	Saline	.412 (22)	17.0	NS	NS
Left cerebral cortex	Tryptophan	0.686 (22)	47.0	$p < 0.001$	$p < 0.001$
	Saline	.091 (22)	1.0	NS	NS
Brainstem	Tryptophan	0.263 (26)	7.0	NS	NS
	Saline	.052 (24)	.3	NS	NS

[a]Number in parentheses indicates number of pairs of animals.

[b]The percentage of change in 5-HIAA that was due to a change in tryptophan was determined by squaring the correlation coefficient (ref. 6-14).

[c]The null hyphothesis (regression = 0 and correlation coefficient = 0) was tested by a "t" test (refs. 6-14 and 6-44).

[d]NS = not significant.

TABLE 6-V.- A COMPARISON OF THE PERCENTAGE OF FOOD CONSUMED BY ADULT MALE AJAX MICE DURING THE LIGHT AND THE DARK PHASES OF A 12:12 LD REGIME

Phase of lighting regime	Total number of determinations	Percentage of total consumption, mean ± SE	Statistical significance (a)
Light	42	12 ± 2	$p < 0.001$
Dark	42	88 ± 2	$p < 0.001$

[a]Determined by the Student's "t" test.

TABLE 6-VI.- THE EFFECT OF THE RESTRICTION OF FEEDING TO A DAILY FOUR-HOUR PERIOD ON BODY WEIGHT OF AJAX MICE

Animal group	Number of animals	Weight, g ± SE	Statistical significance[a]
Control	30	21.7 ± 0.44	NS
Experimental	30	22.5 ± .40	NS

[a]The statistical significance of the difference in body weights in the control and experimental groups was determined by the Student's "t" test.

TABLE 6-VII.- CORRELATIONS BETWEEN PARAMETERS IN THE BLOOD AND BRAIN OF CONTROL RATS FED AD LIBITUM OR EXPERIMENTAL RATS FED ONLY DURING A DAILY FOUR-HOUR PERIOD[a]

Parameter	Brain area	Treatment group	Correlation coefficient (r)	Percent of change Y due to X	Significance of r	Significance of regression
Correlations between parameters in brain						
Brain tryptophan compared with 5-HIAA	Cerebral cortex	C E	0.496 .474	24.6 22.5	$p < 0.005$ $p < .005$	$p < 0.005$ $p < .005$
	Brainstem	C E	0.532 .564	28.3 31.8	$p < 0.005$ $p < .005$	$p < 0.005$ $p < .005$
Brain tryptophan compared with 5-HT	Cerebral cortex	C E	0.056 .014	0.3 0.0	NS NS	NS NS
	Brainstem	C E	0.557 .494	31.0 24.4	$p < 0.01$ $p < .01$	$p < 0.01$ $p < .01$
Correlations between parameters in plasma and in brain						
Plasma free tryptophan compared with 5-HIAA	Cerebral cortex	C E	0.367 .044	13.5 .2	$p < 0.05$ NS	$p < 0.05$ NS
	Brainstem	C E	0.119 .033	1.4 .1	NS NS	NS NS
Plasma free tryptophan compared with 5-HT	Cerebral cortex	C E	0.068 .200	0.5 4.0	NS NS	NS NS
	Brainstem	C E	0.562 .252	31.5 6.3	$p < 0.01$ NS	$p < 0.01$ NS

[a]The null hypothesis that "regression = 0" and the "correlation coefficient = 0" was tested by "t" test (ref. 6-44). The percent change in Y due to X was determined by squaring the correlation coefficient (ref. 6-44).

TABLE 6-VII.- Concluded

Parameter	Brain area	Treatment group	Correlation coefficient (r)	Percent of change Y due to X	Significance of r	Significance of regression
Plasma tryptophan/tyrosine + phenylalanine compared with 5-HIAA	Cerebral cortex	C	0.223	5.0	NS	NS
		E	.090	.8	NS	NS
	Brainstem	C	0.139	1.9	NS	NS
		E	.166	2.8	NS	NS
Plasma tryptophan/tyrosine + phenylalanine compared with 5-HT	Cerebral cortex	C	0.086	0.7	NS	NS
		E	.139	1.9	NS	NS
	Brainstem	C	0.193	3.7	NS	NS
		E	.123	1.5	NS	NS
Free tryptophan compared with brain tryptophan	Cerebral cortex	C	0.008	0.0	NS	NS
		E	.029	.1	NS	NS
	Brainstem	C	0.330	10.9	NS	NS
		E	.157	2.5	NS	NS
Plasma tryptophan/tyrosine + phenylalanine compared with brain tryptophan	Cerebral cortex	C	0.009	0.0	NS	NS
		E	.118	1.4	NS	NS
	Brainstem	C	0.192	3.7	NS	NS

[a]The null hypothesis that "regression = 0" and the "correlation coefficient = 0" was tested by "t" test (ref. 6-44). The percent change in Y due to X was determined by squaring the correlation coefficient (ref. 6-44).

TABLE 6-VIII.- COMPARISON OF BRAIN TRYPTOPHAN, 5-HT, AND 5-HIAA IN THE BRAINS OF CONTROL, STARVED, AND REFED HAMSTERS[a]

Treatment group	Brain tryptophan, μg/g ± SE (b)	Brain 5-HT, μg/g ± SE (b)	Brain 5-HIAA, μg/g ± SE (b)
Control	3.18 ± 0.42 (24)	0.318 ± 0.01 (24)	0.226 ± 0.02 (24)
Starved	[c]3.96 ± .54 (23)	.309 ± .02 (22)	[c].363 ± .02 (23)
Refed	[c]4.07 ± .15 (24)	.283 ± .01 (23)	[c].342 ± .02 (24)

[a]No statistically significant differences were observed in the data obtained for any of these parameters within control, starved, or refed hamsters after 0, 1, 2, or 3 hours; therefore, all the data for a parameter were combined to compare control versus starved or refed.

[b]Number in parentheses indicates number of observations.

[c]$p < 0.001$.

TABLE 6-IX.- A REPEAT COMPARISON OF BRAIN TRYPTOPHAN, 5-HT, AND 5-HIAA IN THE BRAINS OF CONTROL, STARVED, AND REFED HAMSTERS[a]

Treatment group	Body weight, g ± SE (b)	Brain tryptophan, µg/g ± SE (b)	Brain 5-HT, µg/g ± SE (b)	Brain 5-HIAA µg/g ± SE (b)
Control	116 ± 1 (24)	2.95 ± 0.05 (24)	0.526 ± 0.02 (24)	0.242 ± 0.01 (24)
Starved	[c]103 ± 2 (24)	2.96 ± .10 (24)	.474 ± .02 (24)	[d].292 ± .01 (23)
Refed	[c]103 ± 1 (24)	[c]2.61 ± .09 (24)	.514 ± .02 (24)	[d].305 ± .01 (24)

[a]No statistically significant differences were observed in the data obtained for any of the parameters within the control, starved, or refed groups after 0, 1, 2, or 3 hours; therefore, all the data for a parameter were combined in order to compare control versus starved or refed animals.

[b]Number in parentheses indicates number of observations.

[c]$p < 0.001$.

[d]$p < 0.05$.

TABLE 6-X.- THE RATIO OF PLASMA TRYPTOPHAN TO TYROSINE IN CONTROL, STARVED, AND REFED HAMSTERS

Treatment group	Ratio of plasma tryptophan to tyrosine, after -			
	0 hr, μg/g ± SE	1 hr, μg/g ± SE	2 hr, μg/g ± SE	3 hr, μg/g ± SE
Control	2.06 ± 0.17	[a]2.44 ± 0.10	1.70 ± 0.09	1.84 ± 0.09
Refed	1.83 ± .18	[b,a]1.11 ± .07	[c,a]1.22 ± .09	1.74 ± .17
Starved	[d]1.57 ± .11	[b].99 ± .12	[b].98 ± .03	[b,e].76 ± .12

[a]Level of significance of that time period value from the values of other time periods in the same treatment group; $p < 0.005$.

[b]Level of significance from control group; $p < 0.001$.

[c]Level of significance from control group; $p < 0.005$.

[d]Level of significance of that time period value from the values of other time periods in the same treatment group; $p < 0.001$.

[e]Level of significance of the starved value from the refed value; $p < 0.001$.

TABLE 6-XI.- COMPARISON OF BRAIN TRYPTOPHAN, 5-HT, AND 5-HIAA IN CONTROL, STARVED, AND REFED HAMSTERS

Treatment group	Time after refeeding, hr	Brain tryptophan, µg/g ± SE (a)	Brain 5-HT, µg/g ± SE (a)	Brain 5-HIAA, µg/g ± SE (a)
Initial study				
Control	0	4.34 ± 0.14 (8)	0.640 ± 0.032 (8)	0.284 ± 0.013 (7)
	3	4.18 ± .06 (8)	.635 ± .029 (7)	.257 ± .013 (7)
Starved	0	[b,c]5.34 ± 0.22 (8)	0.551 ± 0.023 (8)	0.274 ± 0.007 (8)
	3	[d,e]4.78 ± .37 (8)	.589 ± .016 (7)	[f,c].368 ± .028 (8)
Refed	0	[b,c]5.18 ± 0.28 (6)	0.640 ± 0.024 (7)	0.295 ± 0.037 (8)
	3	[d,c]3.90 ± .37 (7)	.601 ± .030 (8)	[f,c].353 ± .031 (8)
Repeat study				
Control	0	3.43 ± 0.15 (8)	0.466 ± 0.015 (8)	0.239 ± 0.007 (8)
	3	3.46 ± .07 (7)	.532 ± .033 (7)	.234 ± .020 (7)
Starved	0	3.67 ± 0.20 (8)	0.498 ± 0.030 (8)	0.265 ± 0.017 (8)
	3	3.68 ± .25 (8)	.518 ± .040 (8)	.248 ± .011 (8)
Refed	0	3.69 ± 0.30 (8)	0.513 ± 0.030 (8)	0.270 ± 0.011 (8)
	3	3.49 ± .13 (8)	.530 ± .024 (8)	.253 ± .013 (7)

[a]Number in parentheses indicates number of animals in group.

[b]$p < 0.005$.

[c]Level of significance from control.

[d]$p < 0.05$.

[e]Level of significance of the starved value from the refed value.

[f]$p < 0.01$.

TABLE 6-XII.- COMPARISON OF THE RATIO OF PLASMA TRYPTOPHAN TO PLASMA TYROSINE PLUS PHENYLALANINE IN CONTROL, STARVED, AND REFED HAMSTERS

Treatment group	Ratio of plasma tryptophan to tyrosine plus phenylalanine, after -	
	0 hr, μg/g ± SE	3 hr, μg/g ± SE
Initial study		
Control	0.811 ± 0.042	0.886 ± 0.037
Starved	.832 ± .093	.944 ± .046
Refed	[a].419 ± .035	[a].609 ± .026
Repeat study		
Control	0.646 ± 0.010	0.672 ± 0.014
Starved	[a,b].529 ± .024	.780 ± .065
Refed	[a].332 ± .007	[a].453 ± .008

[a]Level of significance of the value from controls; $p < 0.001$.

[b]Level of significance of the starved value from the refed value; $p < 0.001$.

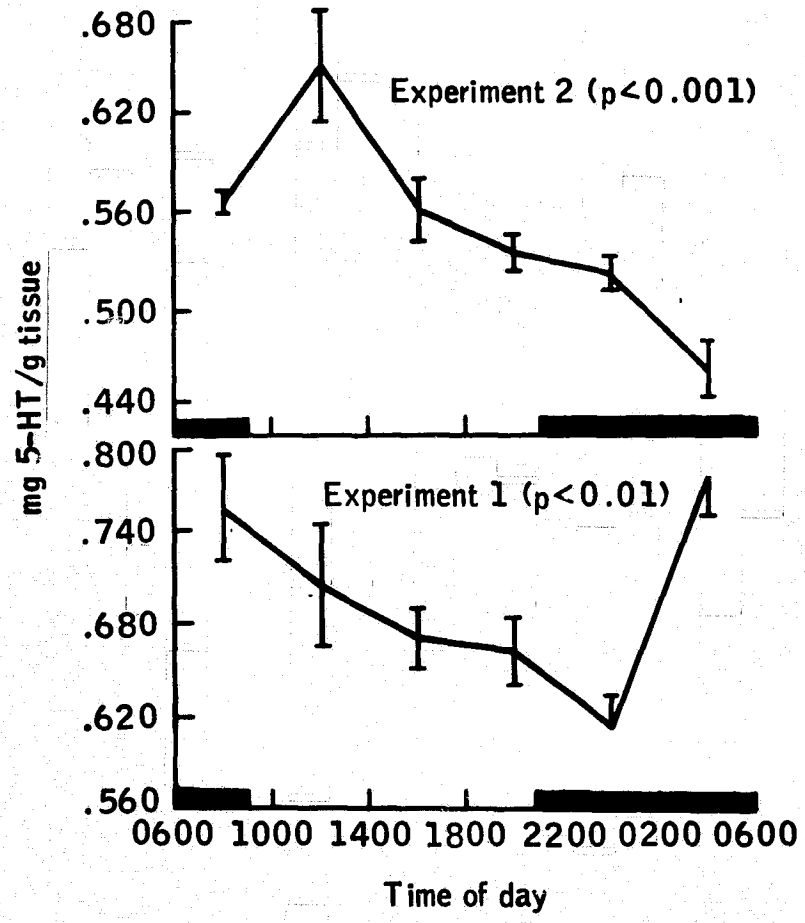

Figure 6-1.- Comparison of daily rhythmic changes in the content of serotonin in the cerebral cortex of Balb/C mice (experiment 1) and AJAX mice (experiment 2). Brackets represent mean ± standard error (SE); black bars represent dark phase of lighting regime.

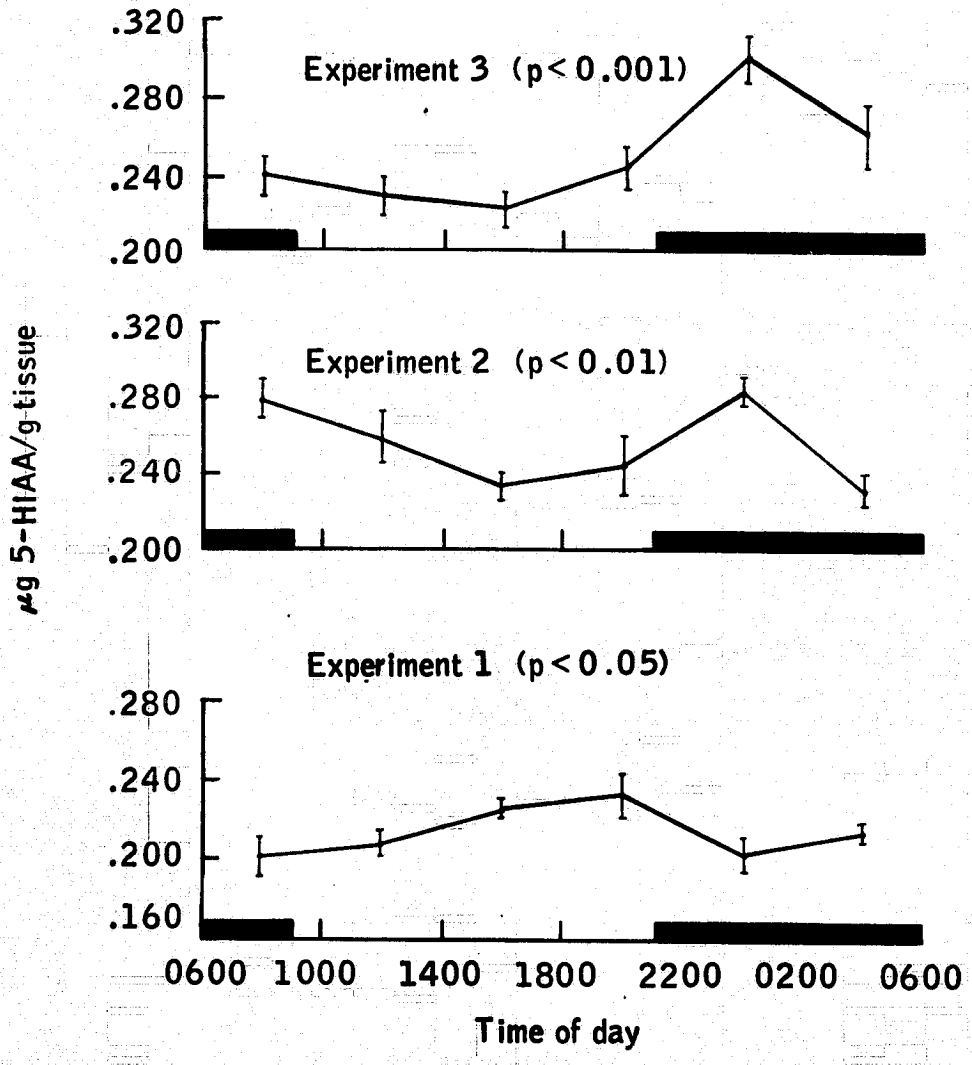

Figure 6-2.- Comparison of daily rhythmic changes in the content of 5-HIAA in the cerebral cortex of Balb/C mice (experiment 1) and AJAX mice (experiments 2 and 3). Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

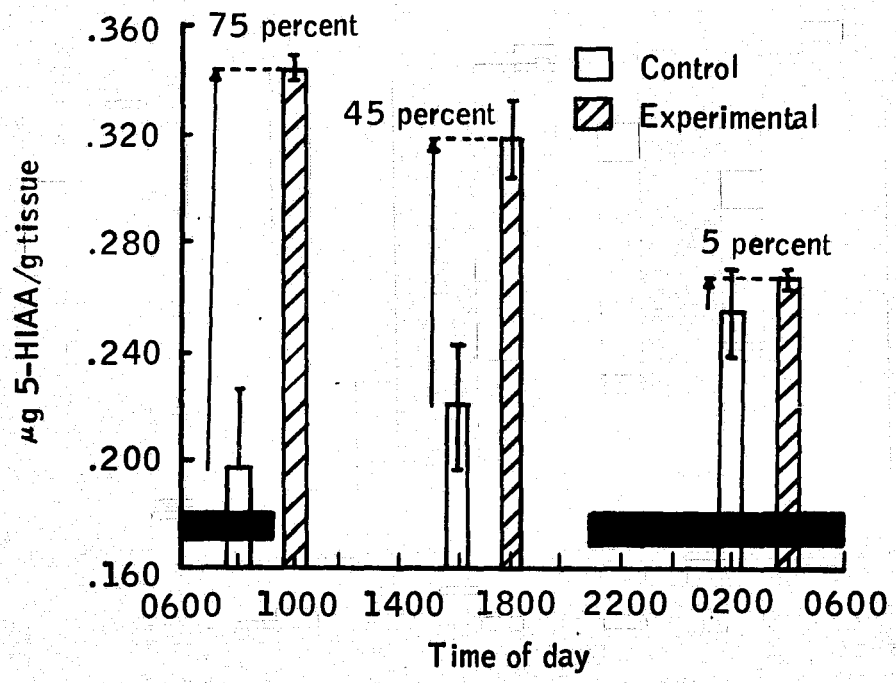

Figure 6-3.- Changes with time of day in probenecid-induced 5-HIAA accumulation in the cerebral cortex of mice. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

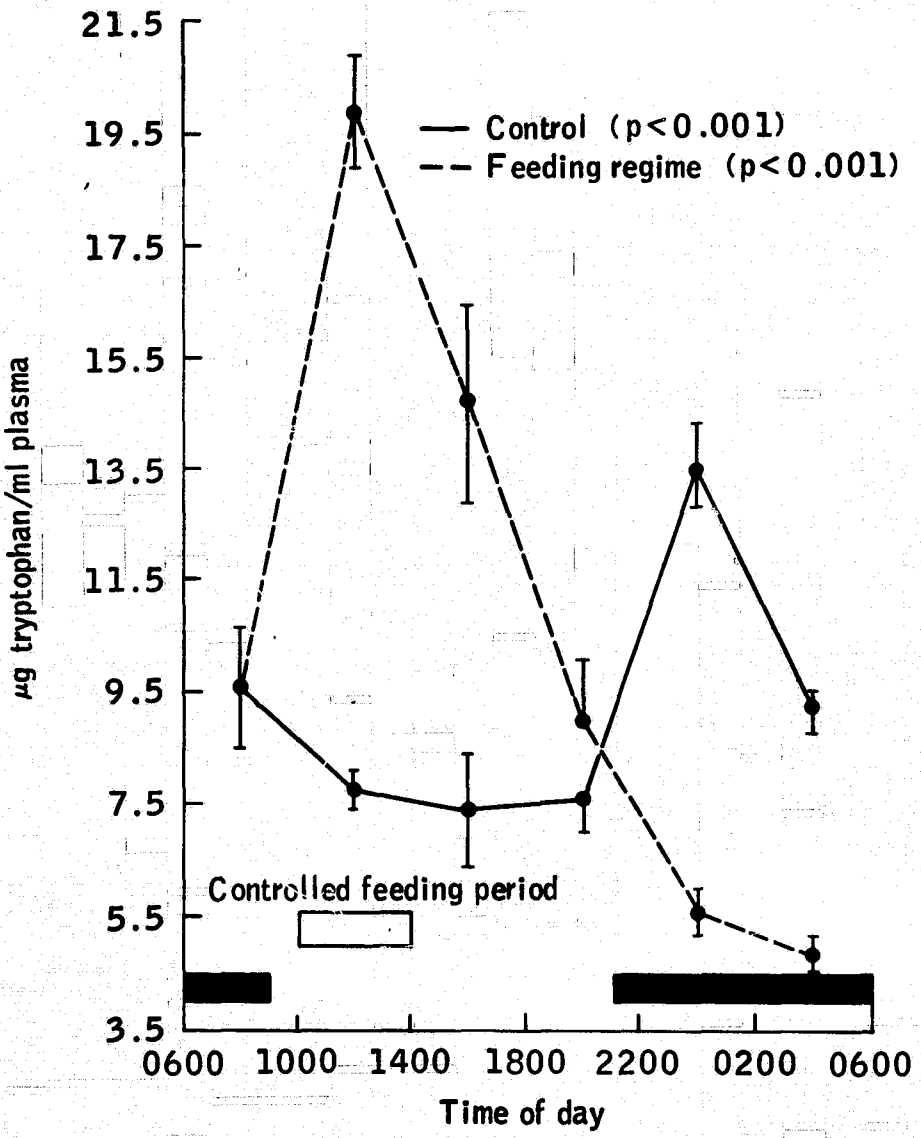

Figure 6-4.- Effect of a controlled feeding regime on the diurnal variation of plasma tryptophan content in the mouse. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

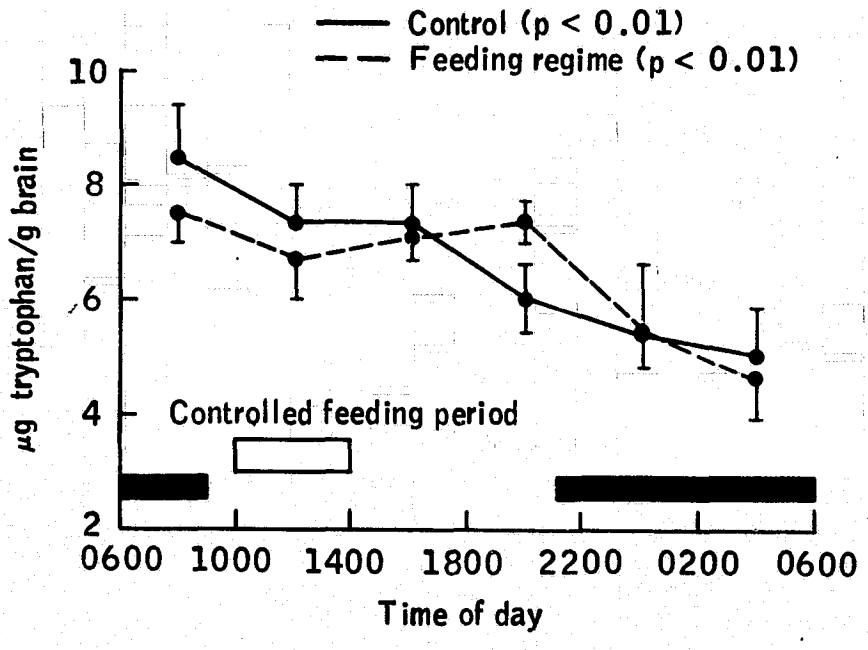

Figure 6-5.- Effect of a controlled feeding regime on the diurnal variation of tryptophan content in the mouse brain. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

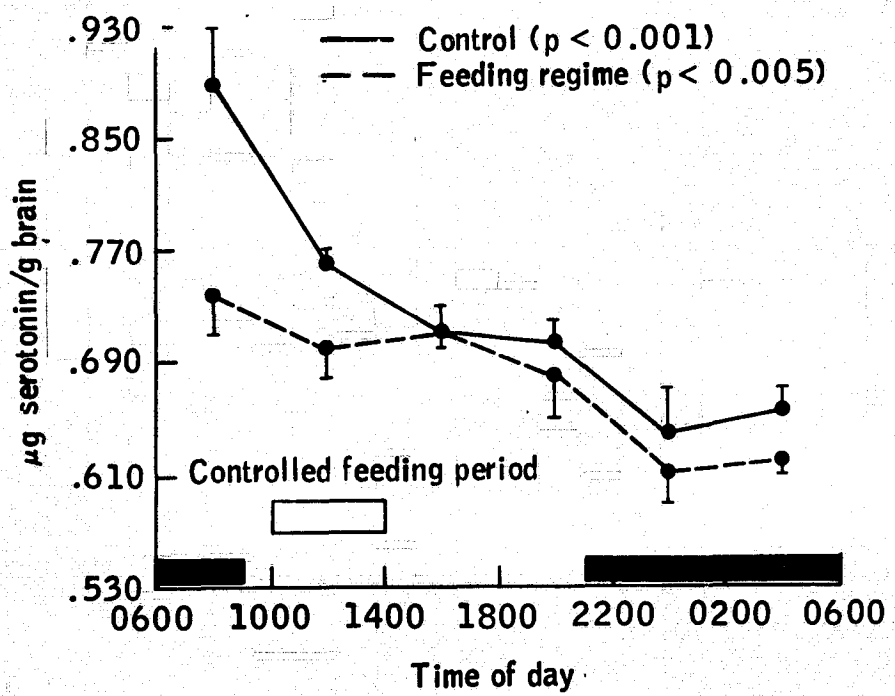

Figure 6-6.- Effect of a controlled feeding regime on the diurnal variation of serotonin content in the mouse brain. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

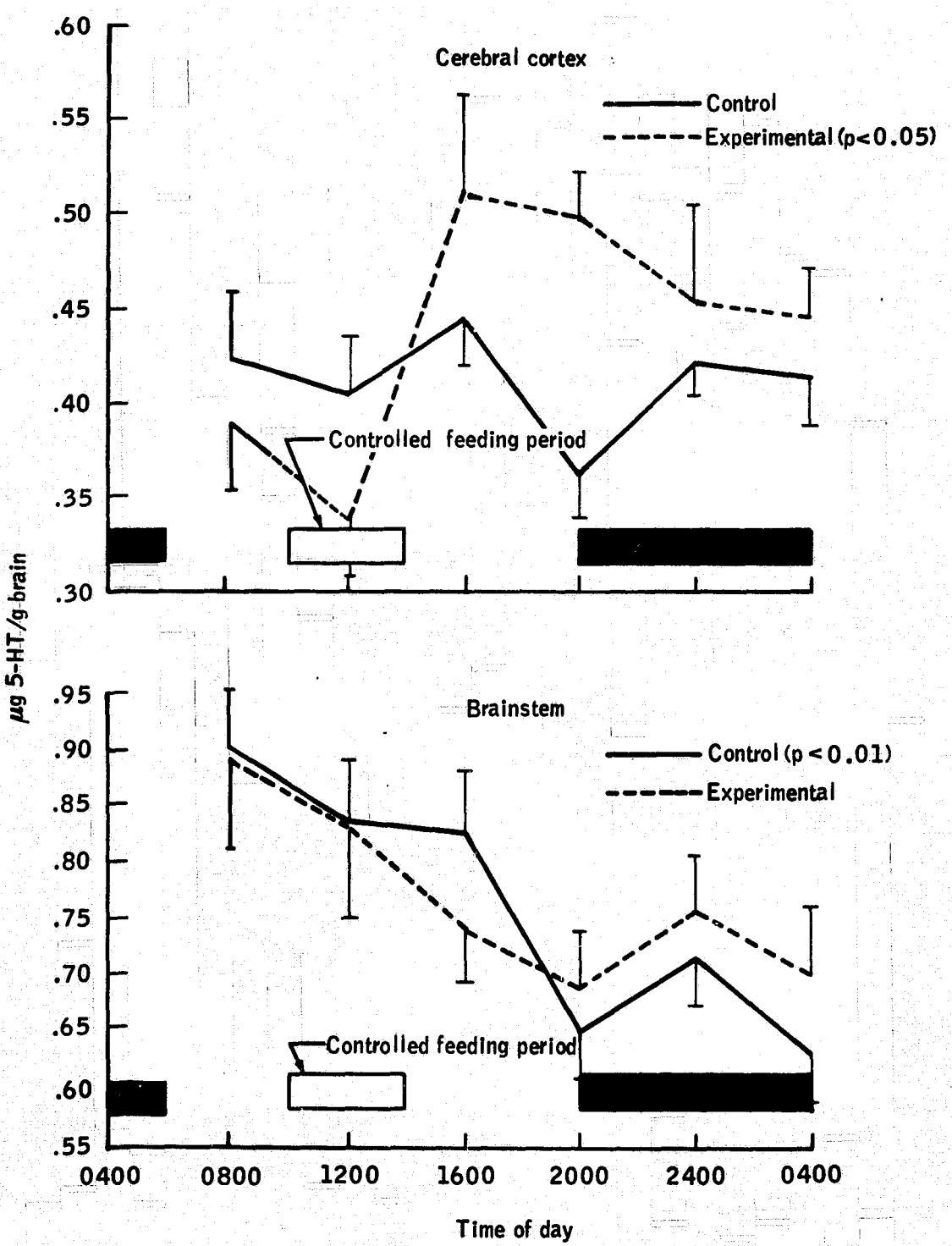

Figure 6-7.- Circadian changes in the content of 5-HT in the cerebral cortex and brainstem of control rats fed ad libitum and of experimental rats fed only during a daily 4-hour period. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

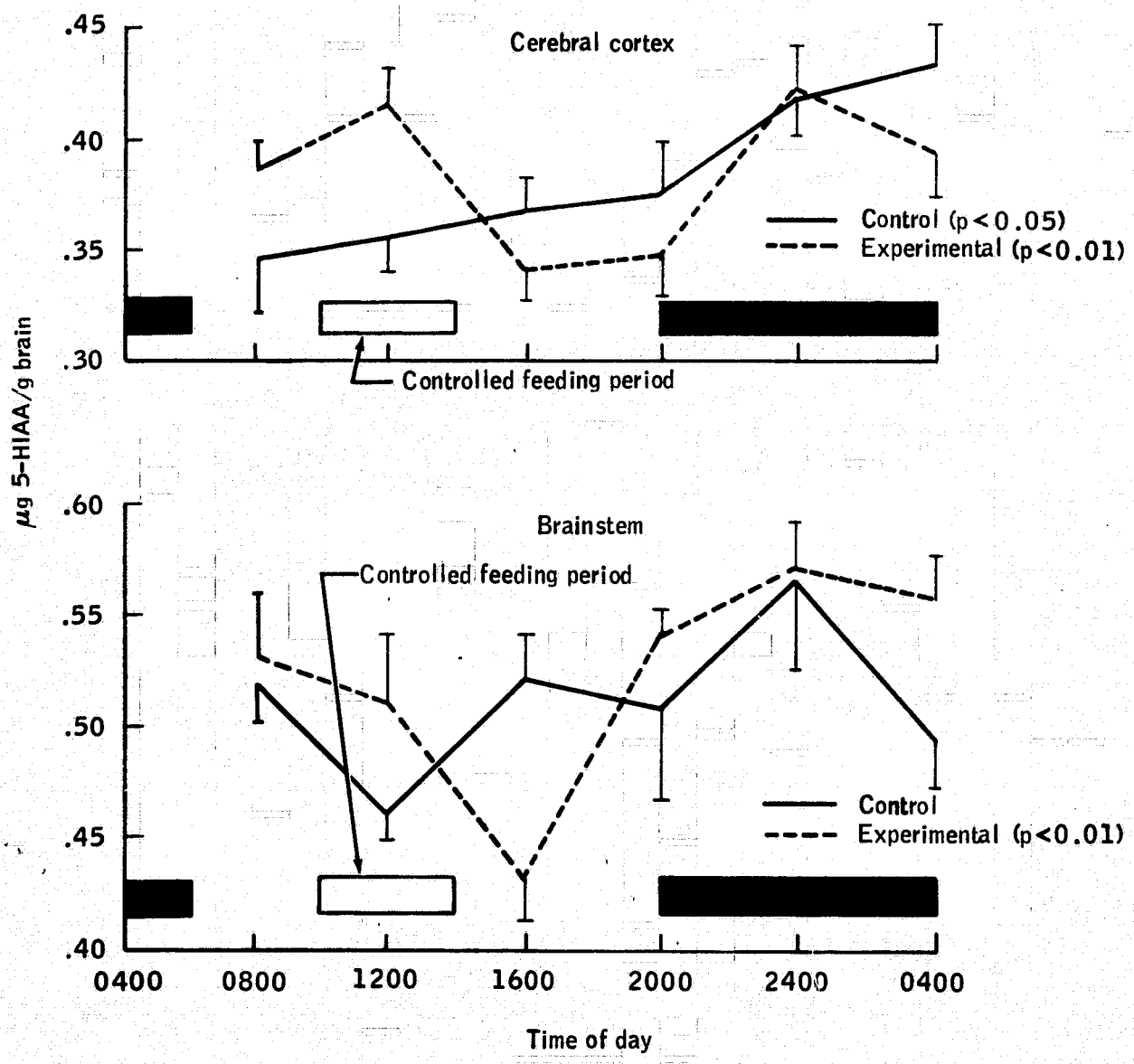

Figure 6-8.- Circadian changes in the content of 5-HIAA in the cerebral cortex and brainstem of control rats fed ad libitum and of experimental rats fed only during a daily 4-hour period. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

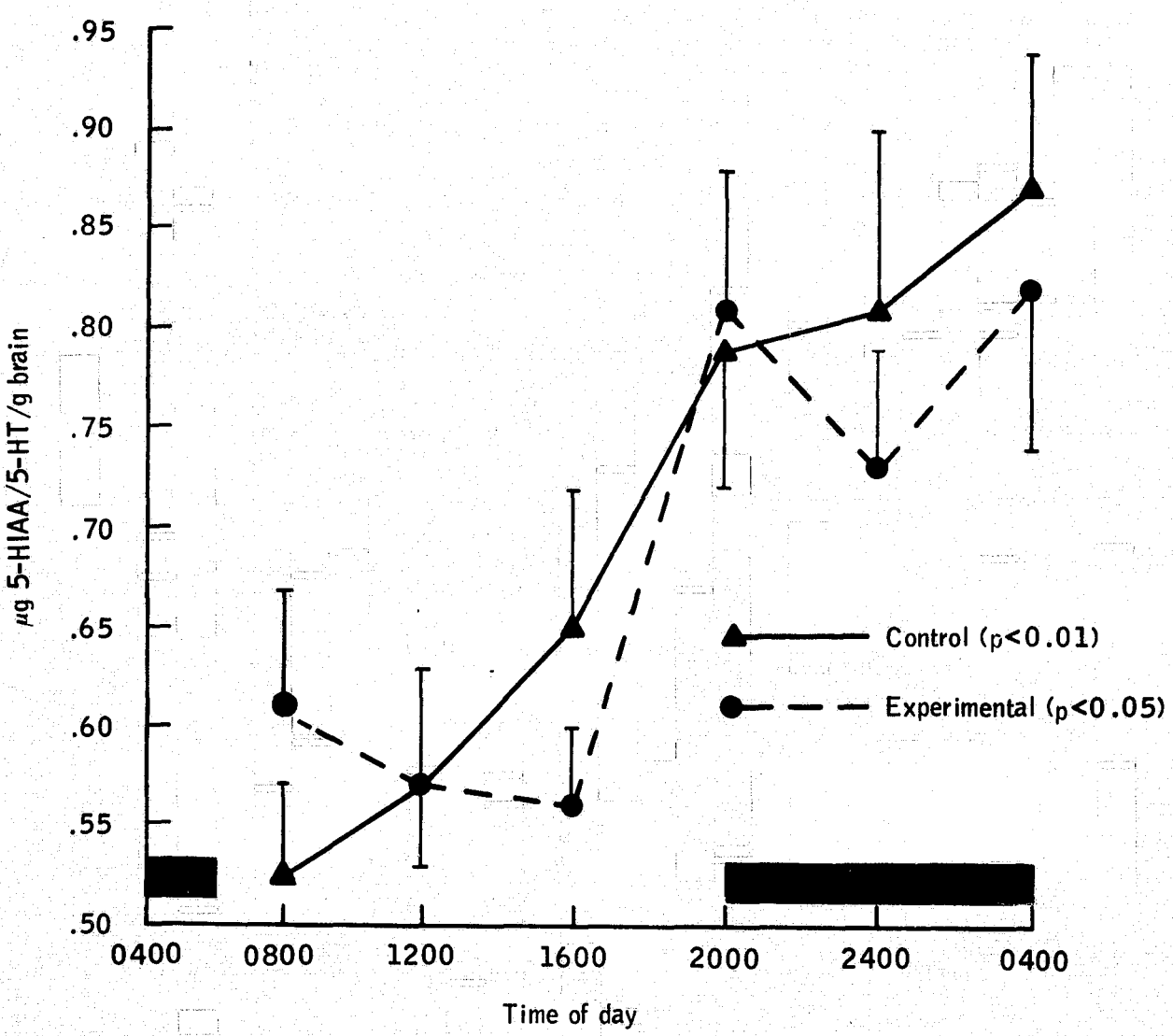

Figure 6-9.- Circadian changes in the ratio of 5-HIAA to 5-HT in the brainstem of control rats fed ad libitum and of experimental rats fed only during a daily 4-hour period. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

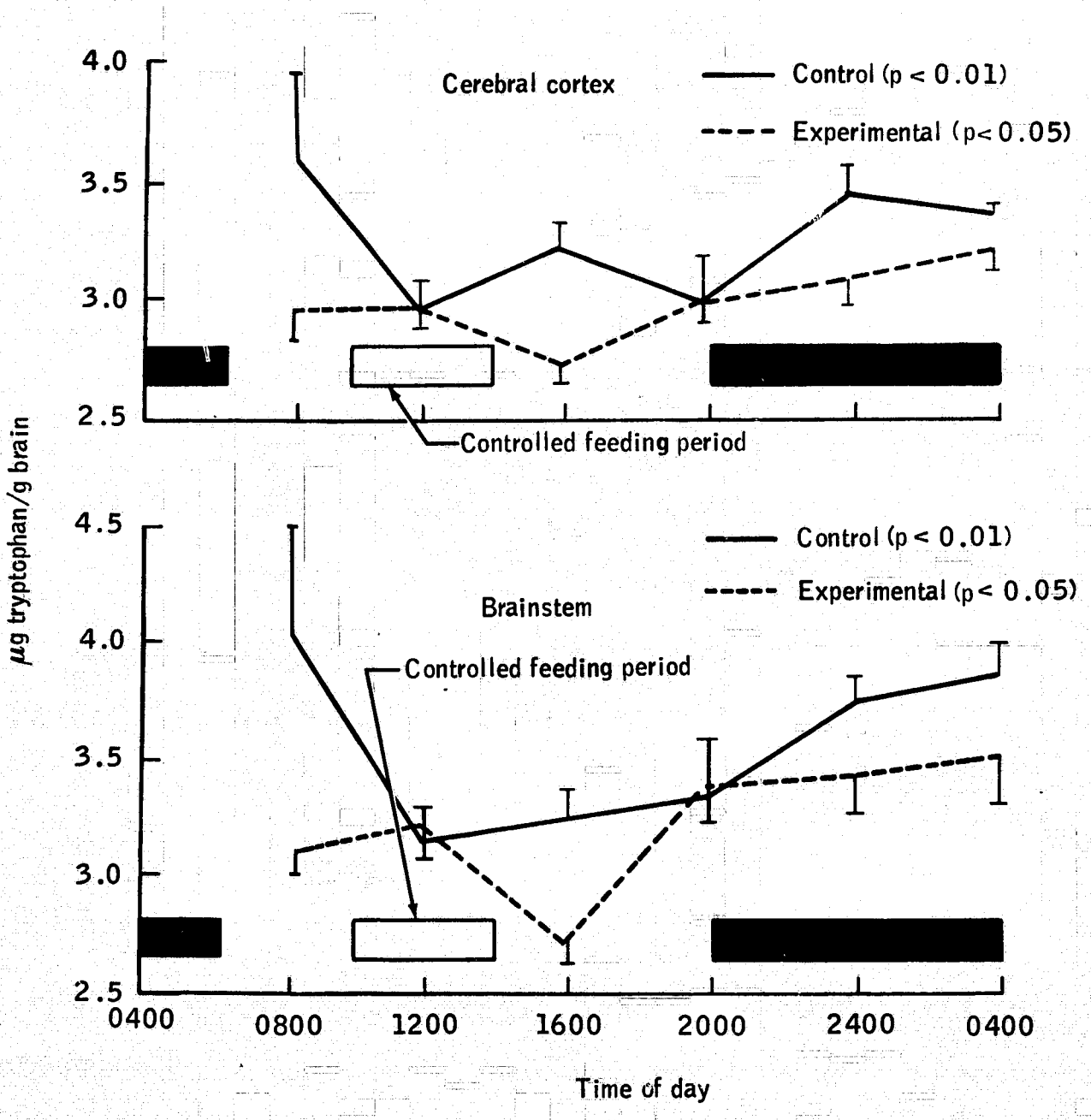

Figure 6-10.- Circadian changes in the content of tryptophan in the cerebral cortex and brainstem of control rats fed ad libitum and of experimental rats fed only during a daily 4-hour period. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

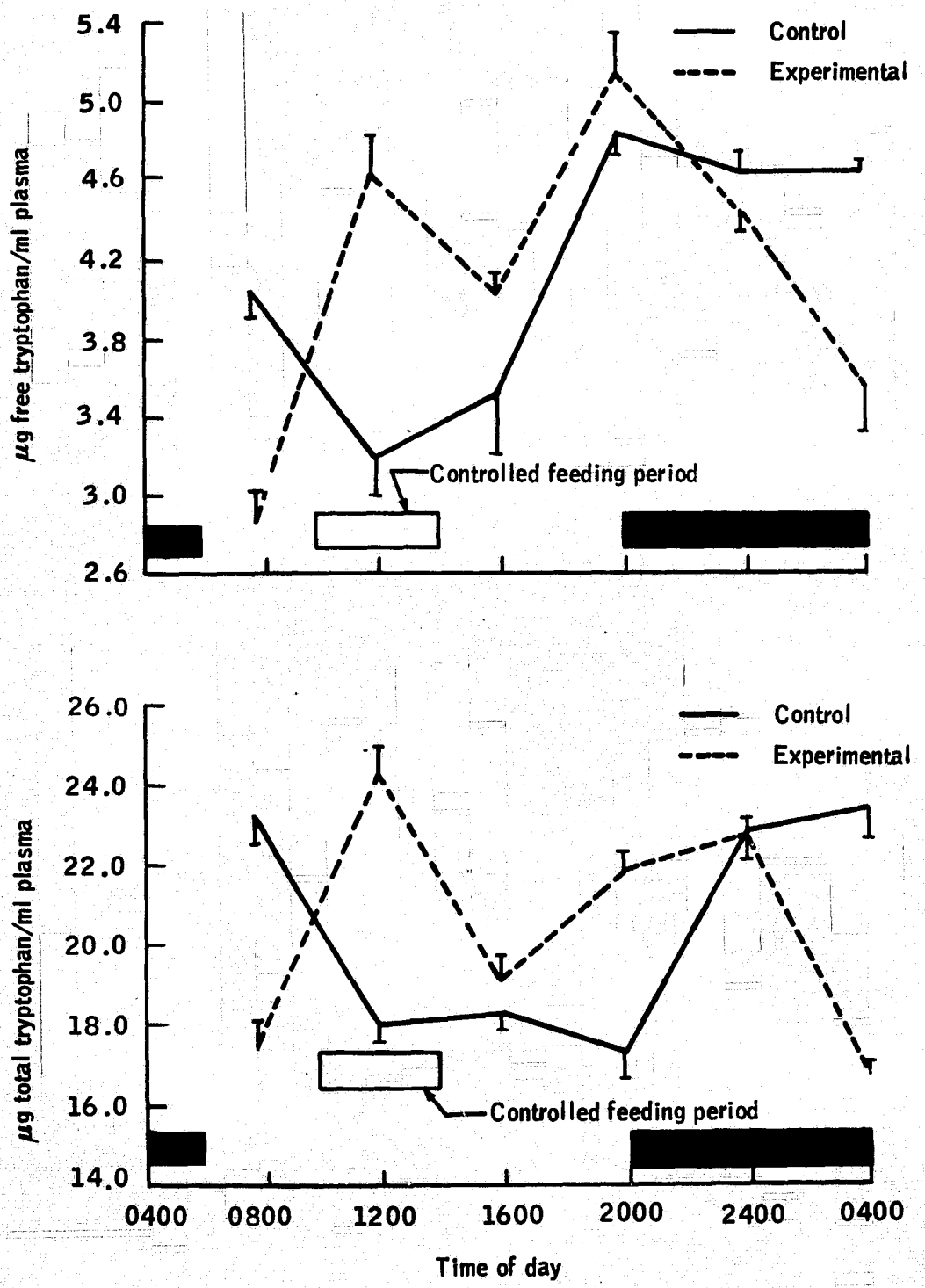

Figure 6-11.- Circadian changes in the content of free and total tryptophan in the plasma of control rats fed ad libitum and of experimental rats fed only during a daily 4-hour period. Brackets represent mean ± SE; black bars represent dark phase of lighting regime.

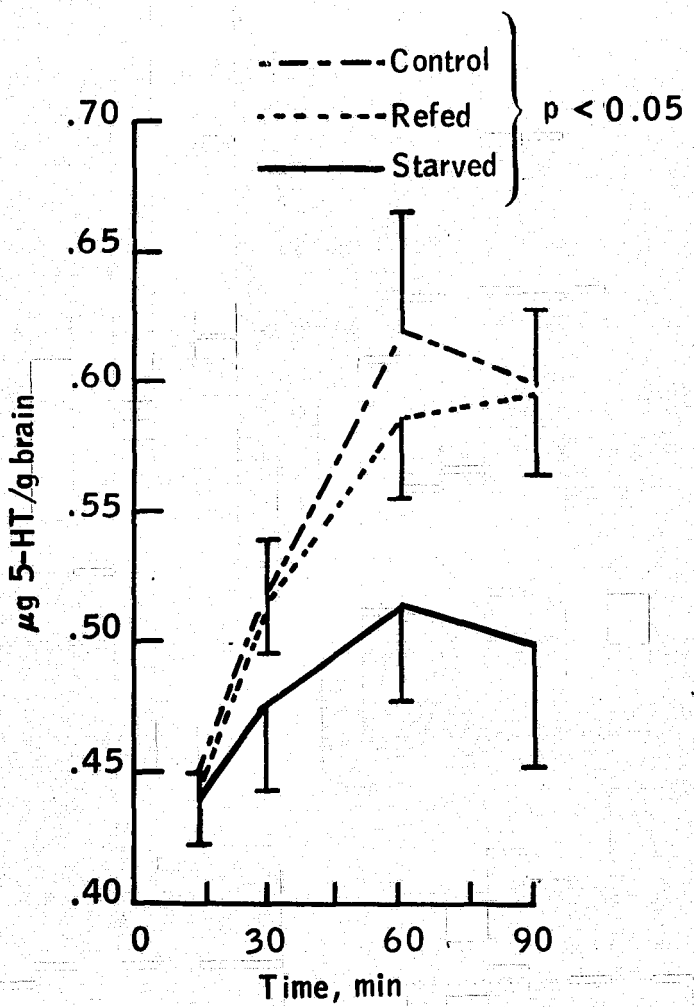

Figure 6-12.- Accumulation of 5-HT in the whole brain of control, 48-hour-starved, and 48-hour starved-and-then-refed hamsters sacrificed at 15, 30, 60, and 90 minutes after the inhibition of monoamine oxidase by pargyline (75 mg free base/kg). Brackets represent mean ± SE.

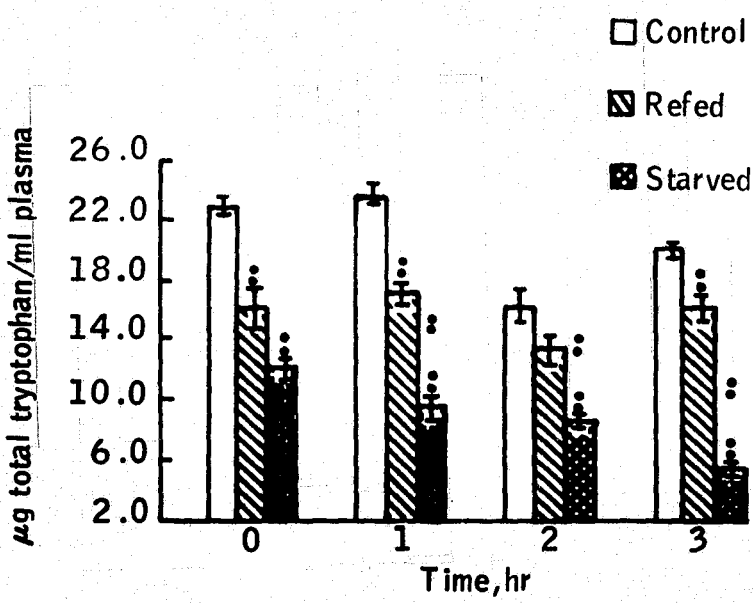

Figure 6-13.- Levels of plasma total tryptophan in control, starved, and refed hamsters starting 48 hours after food deprivation and continuing at 1, 2, and 3 hours after food was given to the refed hamsters. Brackets represent mean ± SE. Lower set of double dots over a bar indicates the statistical significance of that value from controls. Upper set of dots located only above the starved bar indicates the statistical significance of the starved mean from the refed mean ($p < 0.001$).

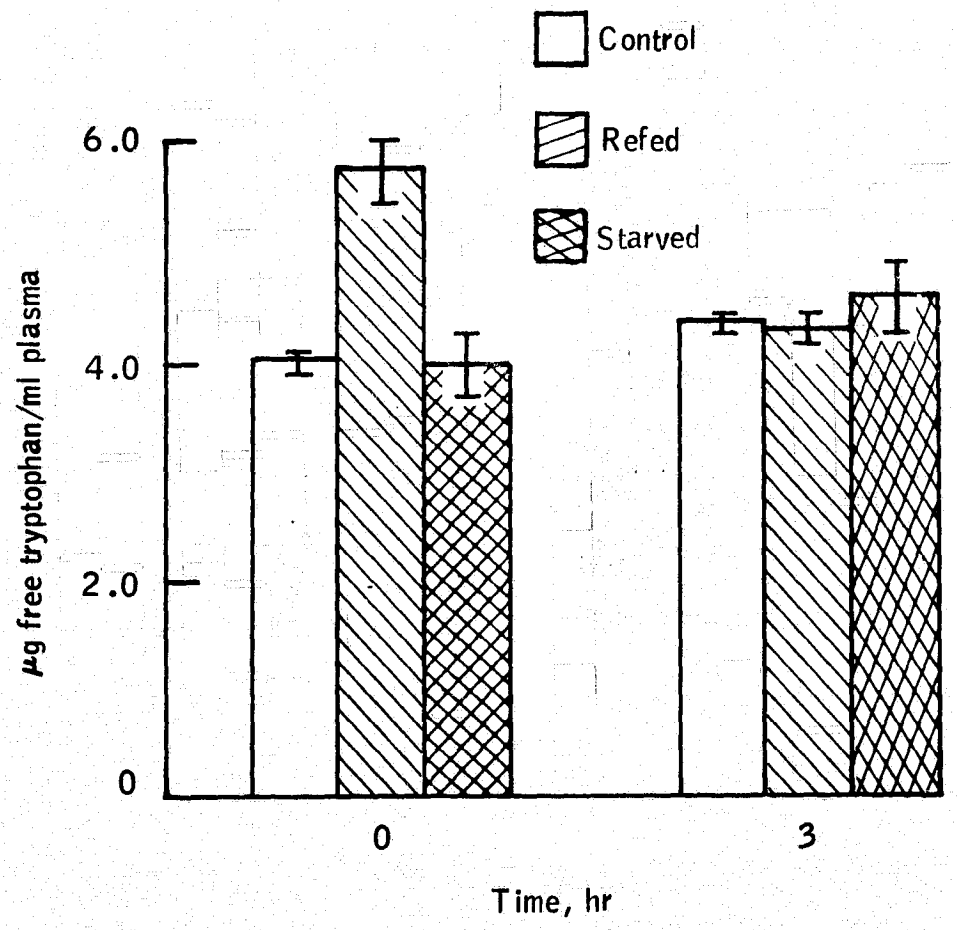

Figure 6-14.- Level of plasma free tryptophan of control, starved, and refed hamsters. Brackets represent mean ± SE of eight animals ($p < 0.001$).

7. THE ROLE OF BRAIN BIOGENIC AMINES IN THE CONTROL OF PITUITARY-ADRENOCORTICAL ACTIVITY

By Roger P. Maickel, Ph. D.

INTRODUCTION

Pretreatment of rats with the monoamine oxidase (MAO) inhibitor pargyline prevents the metabolic destruction of 5-hydroxytryptamine (5-HT) and norepinephrine released in the brain by a subsequent dose of reserpine, and high levels of the amines remain in the brain. Under these conditions, the hypersecretion of adrenocorticotropic hormone (ACTH) usually evoked by reserpine does not occur. The elevated levels of brain 5-HT and norepinephrine produced by MAO inhibition do not cause ACTH hypersecretion nor do they prevent the ACTH hypersecretion caused by exposure to cold or by administration of sedative doses of chlorpromazine.

Previous reports have indicated that sedative doses of reserpine evoke a hypersecretion of ACTH in rats, similar to that produced by exposure to low environmental temperatures (refs. 7-1 and 7-2). The characteristic response includes decreased adrenal ascorbic acid and increased plasma corticosterone, liver tryptophan pyrrolase (TPO), and adrenal gland weight. Similar effects were produced in rats by sedative doses of the Rauwolfia alkaloids, raunescine and rescinnamine, the reserpine analogue syrosingopine, or the benzoquinolizine Ro 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9, 10-dimethoxy-1,2,-3,6,7-hexahydrobenzo(a)quinolizine). In contrast, isoreserpine, the pharmacologically inactive stereoisomer of reserpine, failed to evoke a pituitary-adrenal response.

The ACTH hypersecretion evoked by reserpine was shown to be independent of the sedation caused by the drug (ref. 7-3). For example, pretreatment of animals with desmethylimipramine antagonized the reserpine-induced sedation without preventing the decline in brain amines or the hypersecretion of ACTH. This report explores the antagonism of reserpine-induced ACTH hypersecretion by the MAO inhibitor pargyline (MO 911, N-methyl-N-benzyl-2-propynylamine). Evidence is presented that this antagonism is related to the level of brain biogenic amines maintained during the course of action of the drug. Pretreatment with MAO inhibitors does not affect the ACTH hypersecretion evoked by exposure to cold or chlorpromazine, lending further support to the hypothesis that reserpine-induced ACTH hypersecretion is related to brain amine changes.

MATERIALS AND METHODS

Experimental procedures were performed with unanesthetized adult male Sprague-Dawley rats that weighed 250 to 300 grams. The animals were allowed food and water ad libitum and maintained as previously described (ref. 7-1). Reserpine, as the lyophilized phosphate salt, was dissolved in 0.5 milliliter of water and injected into the tail vein. Chlorpromazine, pargyline, and Ro 4-1284 were dissolved in distilled water and injected intraperitoneally. Control animals were injected with saline or distilled water. Animals were stunned and then immediately decapitated. Blood was collected in beakers containing heparin, transferred to tubes, and centrifuged immediately. Plasma samples for corticosterone assay were stored at 263.15 K (-10° C). For the assay of liver TPO, adrenal ascorbic acid, and brain 5-HT, norepinephrine, and MAO, tissues were removed and stored at 263.15 K (-10° C).

Chemical Methods

The level of adrenal ascorbic acid was determined by the Maickel method (ref. 7-4); plasma corticosterone levels were determined by the Guillemin method (ref. 7-5); the level of liver TPO was determined by the Knox method (7-6); amounts of brain 5-HT and norepinephrine were determined by the method of Maickel et al (ref. 7-7); and the amount of brain MAO activity was established by the method of Weissbach et al. (ref. 7-8).

Parameters of Pituitary-Adrenal Stimulation

The following indices of pituitary-adrenal stimulation were measured: a fall in the level of adrenal ascorbic acid, a rise in the level of plasma corticosterone, and an increased activity of liver TPO. The limitations and advantages of each of these indices have been discussed in a previous paper (ref. 7-1). In some experiments, the changes in plasma corticosterone levels were used as the sole index of pituitary-adrenal hyperactivity.

RESULTS

Effects of Pargyline on Brain Levels of 5-HT and Norepinephrine on Pituitary-Adrenal System

The administration of a single dose of pargyline (25 mg/kg, intraperitoneally) or two doses 18 hours apart did not affect the levels of adrenal ascorbic acid, plasma corticosterone, or liver TPO (table 7-I). Brain levels of 5-HT and norepinephrine were markedly increased, and brain MAO activity was more than 95 percent inhibited. The extent of MAO activity was determined by the method of Weissbach et al. (ref 7-8).

Effects of Pretreatment With Pargyline on Reserpine-Induced ACTH Hypersecretion

In a previous report, the pretreatment of rats with pargyline was determined to have prevented both the sedation and the ACTH hypersecretion induced by reserpine (ref. 7-3). The results in table 7-II show that administration of reserpine to rats pretreated with pargyline (25 mg/kg, intraperitoneally) caused little change in brain 5-HT and norepinephrine levels in 6 hours. The animals did not show the sedation characteristic of reserpine, and there was no evidence of ACTH hypersecretion.

When rats were pretreated with smaller doses of pargyline, the subsequent administration of reserpine produced a variety of results (table 7-III). In some animals (group 1), MAO inhibition was not sufficient to prevent the immediate metabolism of the amines released by reserpine. These animals became deeply sedated in 3 hours. At that time, brain levels of 5-HT and norepinephrine had fallen markedly, and there was a pronounced hypersecretion of ACTH. In other animals (group 2), the MAO inhibitor was only partially effective in protecting the released amines; in such animals, the sedative action of reserpine was delayed. In the second group, sedation was apparent in 6 hours; however, the brain levels of 5-HT and norepinephrine had not fallen to minimal values, and no ACTH hypersecretion was observed. Finally, a third group of animals was observed in which the MAO inhibitor protected most of the released amines from destruction; 6 hours after doses of reserpine, the animals showed only a slight sign of sedation. In this third group of animals, reserpine had no significant effect on pituitary-adrenocortical activity.

Dose-Response Relationship of Pargyline Antagonism of Reserpine-Induced ACTH Hypersecretion

Previous papers have shown that a number of compounds similar to reserpine lower brain amine levels and cause ACTH hypersecretion in rats (refs. 7-1 and 7-2). One striking similarity in these data is that levels of amines in the brain must be depleted below 50 percent of the normal level in order to observe hypersecretion of ACTH. When rats pretreated with varying doses of pargyline were given reserpine, only those animals in which the level of brain amines fell below 50 percent of that in the control animals showed ACTH hypersecretion (table 7-IV). If the dose of pargyline was sufficient to protect the reserpine-released amines to the extent that the levels of 5-HT did not fall to values below 50 percent of normal, there was no measurable pituitary-adrenal stimulation.

Lack of Effect of Pargyline Pretreatment on ACTH Hypersecretion Induced by Other Stressful Stimuli

Previous reports from the Endocrine Laboratory of the Lyndon B. Johnson Space Center have described the ACTH hypersecretion evoked by exposure to cold or administration of chlorpromazine (refs. 7-1 and 7-9). Pretreatment of rats with pargyline had no effect on the ACTH hypersecretion induced by exposure to

cold or chlorpromazine (table 7-V). This absence of effect is in striking contrast to the antagonism of reserpine action produced by pargyline and lends further weight to the hypothesis that the action of reserpine on the pituitary-adrenocortical system is mediated through an effect of the drug on brain amines.

DISCUSSION

The studies presented in this paper further support the hypothesis that the ACTH hypersecretion caused by reserpine and drugs similar to reserpine is related to the effects of the drugs on the storage of MAO in the brain. A previous paper (ref. 7-2) noted that these drugs produce ACTH hypersecretion only when given in doses that lower the amine stores below 50 percent of normal, regardless of whether this lowering effect was achieved by administration of a single large dose or repeated smaller doses. In animals pretreated with pargyline (in doses that block brain MAO activity by 95 percent), administration of reserpine does not lower the level of brain amines. Although reserpine impairs the storage of 5-HT and norepinephrine, the liberated amines are protected from the degradative action of MAO; because of their poor lipid solubility, the amines can diffuse across the blood-brain barrier into the bloodstream only very slowly. Thus, high levels of free amines remain in the brain for a long period of time.

The relationship of pituitary-adrenal hyperactivity to depletion of brain amines to values below 50 percent of normal, first noted with reserpine alone, has also been observed in pargyline-treated animals. The possible significance of this relationship is not immediately apparent. In the rat, the whole brain is used for the determination of 5-HT and norepinephrine; the assay method measures the amines as if they were uniformly distributed throughout the entire brain. Perhaps this level of amines in a brain homogenate is equivalent to a complete depletion of the amines in one or more specific areas of the brain, or perhaps this amine level is related to an imbalance of amines and, consequently, to neural function in some specific neuronal circuits.

The observation that pretreatment of rats with an MAO inhibitor does not prevent the stimulatory effects of exposure to cold temperatures or chlorpromazine on the pituitary-adrenal axis may also suggest that the hypersecretion of ACTH evoked by reserpine is caused by interference with storage processes for brain biogenic amines.

REFERENCES

7-1. Maickel, Roger P.; Westermann, E. O.; and Brodie, B. B.: Effects of Reserpine and Cold-Exposure on Pituitary-Adrenocortical Function in Rats. J. Pharmacol. Exp. Therap., vol. 134, no. 2, Nov. 1961, pp. 167-175.

7-2. Westermann, E. O.; Maickel, R. P.; and Brodie, B. B.: On the Mechanism of Pituitary-Adrenal Stimulation by Reserpine. J. Pharmacol. Exp. Therap., vol. 138, no. 2, Nov. 1962, pp. 208-217.

7-3. Martel, R. R.; Westermann, E. Q.; and Maickel, R. P.: Dissociation of Reserpine-Induced Sedation and ACTH Hypersecretion. Life Sci., vol. 1, no. 4, Apr. 1962, pp. 151-155.

7-4. Maickel, R. P.: A Rapid Procedure for the Determination of Adrenal Ascorbic Acid. Application of the Sullivan and Clarke Method to Tissues, Anal. Biochem., vol. 1, no. 6, Dec. 1960, pp. 498-501.

7-5. Guillemin, R.; Clayton, G. W.; Lipscomb, H. S.; and Smith, J. D.: Fluorometric Measurement of Rat Plasma and Adrenal Corticosterone Concentration. J. Lab. Clin. Med., vol. 53, 1959, pp. 830-832.

7-6. Knox, W. E.: Two Mechanisms Which Increase in Vivo and Liver Tryptophan-peroxidase Activity: Specific Enzyme Adaptation and Stimulation of the Pituitary-Adrenal System. Brit. J. Exp. Path., vol. 32, 1951, pp. 462-469.

7-7. Maickel, Roger P.; Cox, Raymond H., Jr.; Saillant, Jean; and Miller, F. P.: A Method for the Determination of Serotonin and Norepinephrine in Discrete Areas of Rat Brain. Int. J. Neuropharmacol., vol. 7, 1968, pp. 275-282.

7-8. Weissbach, Herbert; Smith, Thomas E.; Daly, John W.; Witkop, Bernhard; and Udenfriend, Sidney: A Rapid Spectrophotometric Assay of Monoamine Oxidase Based on the Rate of Disappearance of Kynuramine. J. Biol. Chem., vol. 235, no. 4, Apr. 1960, pp. 1160-1163.

7-9. Smith, Robert L.; Maickel, Roger P.; and Brodie, Bernard B.: ACTH-hypersecretion Induced by Phenothiazine Tranquilizers. J. Pharmacol. Exp. Therap., vol. 139, 1963, pp. 185-190.

TABLE 7-I.- EFFECTS OF PARGYLINE ON BRAIN LEVELS OF 5-HT AND NOREPINEPHRINE ON PITUITARY-ADRENAL PARAMETERS[a]

[Each value is mean ± standard deviation]

Group	No. of animals	Adrenal ascorbic acid, mg/100 g	Plasma corticosterone, µg/ml	Liver TPO, µmole/g liver/hr	Brain amine	
					5-HT, µg/g	Norepinephrine, µg/g
Control	20	447 ± 41	0.15 ± 0.05	3.2 ± 0.36	0.45 ± 0.04	0.46 ± 0.04
Pargyline (one dose)[b]	13	451 ± 40	.16 ± .05	2.9 ± .38	[c].69 ± .06	[c].60 ± .04
Pargyline (two doses 18 hours apart)[b]	15	453 ± 46	.19 ± .04	3.0 ± .27	[c].80 ± .06	[c].72 ± .07

[a]Measurements were made 18 hours after the last dose of pargyline.

[b]25 mg/kg, injected intraperitoneally.

[c]Value is significantly different from control ($p < 0.05$).

TABLE 7-II.- ANTAGONISM OF RESERPINE-INDUCED SEDATION AND ACTH HYPERSECRETION BY PARGYLINE[a]

[Each value is mean ± standard deviation]

Group	No. of animals	Plasma corticosterone, μg/ml	Brain amine		Sedation
			5-HT, μg/g	Norepinephrine, μg/g	
Control	20	0.15 ± 0.05	0.45 ± 0.04	0.46 ± 0.04	--
Pargyline	15	.19 ± .04	[b].80 ± .06	[b].72 ± .07	No
Reserpine	11	[b].44 ± .04	[b].08 ± .02	[b].07 ± .03	Yes
Pargyline and reserpine	9	.12 ± .03	[b].76 ± .03	.55 ± .05	No

[a]Animals were pretreated intraperitoneally with pargyline (25 mg/kg) at 18 and 36 hours before the intraventricular administration of reserpine (1 mg/kg), and brain amines and plasma corticosterone were measured 6 hours after the injection of reserpine.

[b]Value is significantly different from control ($p < 0.05$).

TABLE 7-III.- EFFECT OF A BORDERLINE DOSE OF PARGYLINE ON RESERPINE-INDUCED SEDATION AND ACTH HYPERSECRETION[a]

[Each value is mean ± standard deviation]

Group	No. of animals	Sedation		Plasma corticosterone, μg/ml	Brain amine	
		3 hours after reserpine	6 hours after reserpine		5-HT, μg/g	Norepinephrine, μg/g
Control	20	--	--	0.15 ± 0.05	0.45 ± 0.04	0.46 ± 0.04
Pargyline	6	--	--	.16 ± .03	[b].62 ± .03	[b].57 ± .04
Reserpine	11	Deep	Deep	[b].44 ± .04	[b].08 ± .02	[b].07 ± .03
Pargyline and reserpine[c]						
Group 1	10	Deep	Deep	[b].39 ± .03	[b].20 ± .03	[b].19 ± .04
Group 2	10	Slight	Deep	.18 ± .05	.40 ± .03	[b].34 ± .03
Group 3	11	None	Slight	.17 ± .05	.53 ± .03	.45 ± .05

[a]Animals were given a single intraperitoneal dose of pargyline (18 mg/kg) 18 hours before an intraventricular administration of reserpine (1 mg/kg), and all animals were killed 6 hours after injections of reserpine were administered.

[b]Value is significantly different from control ($p < 0.05$).

[c]Groups were selected by visual observation of degree of sedation at 3 hours after injection of reserpine.

TABLE 7-IV.- DOSE-RESPONSE OF PARGYLINE ANTAGONISM OF RESERPINE-INDUCED ACTH HYPERSECRETION[a]

[Each value is mean ± standard deviation]

No. of animals	Pargyline, mg/kg	Reserpine, mg/kg	Plasma corticosterone, µg/ml	5-HT, µg/g	Norepinephrine, µg/g	Sedation
20	--	--	0.15 ± 0.05	0.45 ± 0.04	0.46 ± 0.04	--
6	10	--	.14 ± .03	[b].58 ± .03	[b].50 ± .02	--
6	10	1	[b].37 ± .03	[b].21 ± .02	[b].16 ± .02	Yes
13	25	--	.13 ± .03	[b].69 ± .04	[b].60 ± .02	--
9	25	1	.15 ± .04	[b].57 ± .03	[b].49 ± .03	No
15	[c]25	--	.14 ± .04	[b].80 ± .06	[b].72 ± .07	--
9	[c]25	1	.12 ± .03	[b].76 ± .03	[b].55 ± .05	No

[a]Reserpine was administered intraventricularly 18 hours after intraperitoneal injection of pargyline; animals were killed 6 hours after reserpine injections.

[b]Value is significantly different from control ($p < 0.05$).

[c]Administered for 2 days.

TABLE 7-V.- FAILURE OF PRETREATMENT WITH PARGYLINE TO BLOCK ACTH HYPERSECRETION INDUCED BY EXPOSURE TO COLD OR TO CHLORPROMAZINE[a]

[Each value is mean ± standard deviation]

Group	No. of animals	Plasma corticosterone, μg/ml
Control	20	0.15 ± 0.05
Control (exposed to cold)	7	[b].42 ± .04
Control (chlorpromazine)	10	[b].39 ± .04
Pargyline	15	.14 ± .04
Pargyline (exposed to cold)	13	[b].40 ± .03
Pargyline (chlorpromazine)	9	[b].38 ± .03

[a]Animals were exposed to cold (277.15 K (4° C)) or given chlorpromazine (15 mg/kg) 18 hours after pretreatment with pargyline (two doses, 25 mg/kg, 18 hours apart), then killed 3 hours after chlorpromazine or after 2 hours of exposure to cold.

[b]Value is significantly different from corresponding control ($p < 0.05$).

8. STUDIES OF ACID-BASE HOMEOSTASIS DURING SIMULATED WEIGHTLESSNESS: APPLICATION OF THE WATER IMMERSION MODEL TO MAN

By Murray Epstein, M.D.

INTRODUCTION

Previous reports from the author's laboratory at the University of Miami School of Medicine have proposed the use of a model of water immersion as an investigative tool for studying the circulatory mechanisms of plasma volume control and sodium homeostasis during weightlessness (refs. 8-1 to 8-3). These studies have demonstrated that the redistribution of blood volume that accompanies "head out" water immersion (to the neck) results in a significant and reproducible natriuresis and a suppression of the renin-aldosterone system (refs. 8-1 and 8-3). Since volume expansion is known to decrease bicarbonate reabsorption in the proximal tubule (ref. 8-4), and since aldosterone deficiency impairs distal hydrogen secretion (ref. 8-5), it was anticipated that water immersion would exert significant effects on acid-base homeostasis. This study was undertaken to assess the effects of water immersion on acid-base homeostasis under carefully controlled conditions.

DISCUSSION

Studies of renal acidification were carried out on seven healthy male subjects, each consuming a diet containing 150 meq sodium and 100 meq potassium. Control and immersion studies were carried out on each subject on the fourth and sixth days, respectively, of dietary equilibration, by which time all subjects had achieved sodium balance. The experimental protocols on study days were similar (except for the amount of water administered) and were carried out as described in the following paragraphs.

Following 10 hours of overnight dehydration, the subject was awakened at 0700 and instructed to sit quietly for 1 hour. Beginning at 0730, an oral water load totaling 800 milliliters was administered during control. Since water immersion predictably induces a diuresis (ref. 8-2), the water load was omitted during immersion in the present study to match the urine flow rates during control and immersion, thereby obviating the effect of increased urine flow on urinary pH (ref. 8-6). At 0800, venous blood was drawn for the determination of serum electrolytes, creatinine, plasma hematocrit, pH, and partial pressure of carbon dioxide (pCO_2). The venous sample was obtained in the following manner. Blood for electrolyte determinations was first drawn into a 20-milliliter

syringe, the tourniquet was released immediately and without stasis, and a 2-milliliter heparinized syringe was transferred to the needle for aspiration of the sample for pH and pCO_2 determinations. After completely emptying his bladder, the subject again assumed a seated position for 6 hours (0800 to 1400). During control, the subject sat quietly outside the immersion tank for the 6-hour period. During immersion, the subject sat in the study tank immersed in water to the neck for 5 hours (0900 to 1400), preceded by 1 hour of quiet sitting outside the tank (prestudy immersion hour).

Each subject voided spontaneously at hourly intervals during the study. During immersion, the subject stood briefly on a platform in the immersion tank to void. All urine samples were collected under mineral oil.

To maintain an adequate urine flow during control, 150 milliliters of water were administered orally every hour. Sodium, potassium, and creatinine were measured in aliquots of the hourly urine collections. Blood was collected at the midpoint and end of each study. All subjects were weighed every morning at 0700 after voiding, and before and after each study.

Immersion was carried out in a waterproof tank described in detail in reference 8-2. A constant water temperature of 307.15 ± 0.5 K (34 ± 0.5° C) was maintained by two heat exchangers with a combined output of 4 kilowatts (13 500 Btu/hr), controlled by an adjustable temperature-calibrated control meter, with input derived from two thermistors immersed at different water levels.

Blood and urine pH and pCO_2 were measured within 5 minutes of collection on a Radiometer Acid-Base cart ABC-1. Ammonium was measured by the method of Seligson (ref. 8-7); titratable acidity was measured by titrating the urine to blood pH with 0.1 N sodium hydroxide using a Radiometer Automatic Titrator, Type TTT11b. Blood bicarbonate concentration was calculated from the Henderson-Hasselbalch equation, assuming a pK_a of 6.10 and a solubility coefficient of 0.0301; urine values were calculated similarly, using a pK_a of $(6.33 - 0.5\sqrt{\mu}$ (ref. 8-8) and a solubility coefficient of 0.0309. Net acid excretion was calculated as the sum of ammonium (NH_4) and titratable acid (TA), less bicarbonate (HCO_3) excretions:

$$\text{Net acid excretion} = U_{NH_4}V + U_{TA}V - U_{HCO_3}V$$

The effects of 5 hours of water immersion on sodium and potassium excretion are shown in table 8-I. During quiet sitting (control), the rate of sodium excretion ($U_{Na}V$) was constant, ranging from 33 to 43 μeq/min. Immersion resulted in a highly significant increase in $U_{Na}V$ as compared to both the control periods and the prestudy hour, beginning at hour 1 of immersion

($P < 0.005$). As shown in table 8-II, the absolute quantity of the sodium excreted during 5 hours of immersion was fourfold greater than that excreted during the control period ($P < 0.001$). Figure 8-1 illustrates the effect of immersion on urine pH and on the rate of bicarbonate excretion ($U_{HCO_3}V$). During control, urine pH was constant, ranging from 5.21 to 5.52. Immersion resulted in a highly significant increase in urine pH, beginning at hour 1 ($P < 0.01$). By hour 5, mean urine pH had increased from 5.31 to 6.77 ($P < 0.001$). During control, the urine of all subjects was bicarbonate free. Immersion was associated with an increase in $U_{HCO_3}V$ during hours 2, 4, and 5. Concomitantly with these changes, net acid excretion was markedly suppressed.

Urine pCO_2 was constant during control, ranging from 36 to 40 mm Hg (fig. 8-2). Beginning with the initial hour of immersion, there was a significant increase in urine pCO_2 compared to the level at the prestudy hour. The peak level of pCO_2 of 73 ± 4 mm Hg was attained during the final hour of study.

These changes in the absolute value of urine pCO_2 cannot automatically be interpreted to indicate increased generation of CO_2 from carbonic acid (H_2CO_3) formed within the tubular lumen. Since CO_2 diffuses freely across cell membranes, alterations in plasma pCO_2 will change the pCO_2 in urine without any associated change in intraluminal H_2CO_3 production. To correct for this diffusion, pCO_2 has been expressed as the difference between urine and plasma CO_2 tension (ref. 8-9). As shown in figure 8-2, urine minus plasma pCO_2 paralleled the changes in urine pCO_2. Of note, the urine pCO_2 has already increased by hour 2 of immersion at a time when blood pCO_2 had not changed from the preimmersion value.

Titratable acid excretion significantly decreased during the final 2 hours of immersion (table 8-I). Urinary ammonium excretion also decreased during the final 4 hours of immersion compared to control. As a consequence of the simultaneous decrease in TA and NH_4 excretion and the concomitant bicarbonaturia, net acid excretion decreased during immersion from 38 ± 3 μeq/min during the prestudy hour to 1 ± 7 μeq/min during hour 5 ($p < 0.005$) (fig. 8-1).

The possibility that other effects related to the experimental design (other than redistribution of blood volume) might explain the alterations in urinary pH and the increase in $U_{HCO_3}V$ must be considered first. Since an increase in urine volume will result in an increase in the urine pH of an acid urine (ref. 8-6), it was important to ensure that changes in urine flow were responsible for the pH changes. As shown in table 8-I, the modification of the water load during control and the omission of the water load during immersion

were successful in the matching of the urine flow rates during control and immersion. Urine flow during immersion was not significantly different from comtrol during each hour of study. Similarly, urine osmolality during each hour of immersion did not differ from the comparable control hour. Although potassium has been demonstrated to exert a regulatory role over renal HCO_3 reabsorption independent of any effect attributable to volume (ref. 8-10), it appears unlikely that changes in the state of body potassium stores during immersion could have induced the observed changes in HCO_3 reabsorption. It is well established that potassium depletion enhances HCO_3 reabsorption at any level of sodium excretion. Thus, if the small (albeit significant) increase in potassium excretion during immersion observed in the present studies played a role in altering HCO_3 handling, an increased proximal reabsorption of HCO_3, rather than the observed bicarbonaturia would have been favored.

Finally, although plasma CO_2 tension is a determinant of HCO_3 reabsorption (ref. 8-11), it is unlikely that the decrease in venous pCO_2 at the termination of the present study could be invoked to explain the observed alterations. The encountered increases in urine pCO_2 and $U_{HCO_2}V$ were in evidence as early as hour 2 of immersion, at a time when blood pCO_2 had not changed from the preimmersion value (fig. 8-2). The differences encountered must therefore represent true changes induced by immersion to the level of the neck.

The mechanism whereby immersion alters renal acidification is not yet clear. As noted earlier in this report, several possibilities must be considered, including decreased HCO_3 reabsorption in the proximal tubule, and impairment of distal tubular hydrogen-ion gradient generation. Although immersion is associated with a decrease in aldosterone excretion (refs. 8-1 and 8-3), it would appear unlikely that a decrease in aldosterone is the sole cause of the encountered bicarbonaturia. The increase in HCO_3 excretion in the present study was accompanied by a significant increase in urinary pCO_2, which presupposes a significant degree of hydrogen secretion in the distal tubular segment. Furthermore, the bicarbonaturia occurred as early as the second hour of immersion (fig. 8-1). Previous studies have suggested that even after changes in aldosterone have occurred, there is a 1- to 2-hour lag before they are reflected in changes in renal tubular reabsorptive and secretory processes (refs. 8-12 and 8-13). These data suggest that the initial increase in bicarbonate excretion occurred before any aldosterone-mediated changes in distal hydrogen secretion. The present results, considered as a whole, suggest that suppression of distal hydrogen secretion mediated by suppression of aldosterone cannot be the sole factor responsible for the prompt bicarbonaturia observed. Rather, it is more likely that the bicarbonaturia was primarily attributable to an increased proximal rejection of filtered HCO_3

secondary to an increase in central blood volume. However, only additional studies carried out during HCO_3 infusion to assess alterations in net tubular reabsorption of HCO_3 will definitely resolve this point.

Although the precise mechanisms for the encountered bicarbonaturia remain to be elucidated, the demonstrated ability of water immersion to alter renal acidification suggests that this model may constitute a tool for further assessing the effects of increases in central blood volume on regulating renal HCO_3 handling in humans. Furthermore, the similarities in renal sodium and potassium handling between the present study and manned space flight suggest that consideration be given to a systematic assessment of parameters of renal acidification during future manned space flights. Such studies may help to elucidate further the mechanisms mediating the perturbations in sodium homeostasis during weightlessness.

REFERENCES

8-1. Epstein, Murray; and Saruta, Takao: Effect of Water Immersion on Renin-Aldosterone and Renal Sodium Handling in Normal Man. J. Appl. Physiol., vol. 31, no. 3, Sept. 1971, pp. 368-374.

8-2. Epstein, Murray; Duncan, D. C.; Fishman, L. M.: Characterization of the Natriuresis Caused in Normal Man by Immersion in Water. Clin. Sci., vol. 43, Aug. 1972, pp. 275-287.

8-3. Epstein, Murray; Katsikas, James L.; and Duncan, David C.: The Role of Mineralocorticoids in the Natriuresis of Water Immersion in Man. Circ. Res., vol. 32, no. 2, Feb. 1973, pp. 228-236.

8-4. Kurtzman, Neil A.: Regulation of Renal Bicarbonate Reabsorption by Extracellular Volume. J. Clin. Invest., vol. 49, 1970, pp. 586-595.

8-5. Kurtzman, Neil A.; White, Martin G.; and Rogers, Philip W. (With the technical assistance of Peter M. Meserol): Aldosterone Deficiency and Renal Bicarbonate Reabsorption. J. Lab. Clin. Med., vol. 77, no. 6, June 1971, pp. 931-940.

8-6. Tannen, R. L.: The Relationship Between Urine pH and Acid Excretion - The Influence of Urine Flow Rate. J. Lab. Clin. Med., vol. 74, no. 5, Nov. 1969, pp. 757-769.

8-7. Seligson, D.; and Seligson, H.: A Microdiffusion Method for the Determination of Nitrogen Liberated as Ammonia. J. Lab. Clin. Med., vol. 38, 1951, pp. 324-330.

8-8. Hastings, A. B.; and Sendroy, J., Jr.: The Effect of Variation in Ionic Strength on the Apparent First and Second Dissociation Constants of Carbonic Acid. J. Biol. Chem., vol. 65, 1925, pp. 445-455.

8-9. Kennedy, T. J., Jr.; Orloff, J.; and Berliner, R. W.: Significance of Carbon Dioxide Tension in Urine. Am. J. Physiol., vol. 169, no. 3, June 1952, pp. 596-608.

8-10. Kurtzman, N. A.; White, M. G.; and Rogers, P. W.: The Effect of Potassium on Renal Bicarbonate Reabsorption. Clin. Res., vol. 18, 1970, p. 507.

8-11. Kurtzman, Neil A.; White, Martin G.; and Rogers, Philip W.: Pathophysiology of Metabolic Alkalosis. Arch. Intern. Med., vol. 131, no. 5, May 1973, pp. 702-708.

8-12. Ross, E. J.; Reddy, W. J.; Rivera, A.; and Thorn, G. W.: Effects of Intravenous Infusions of dl-aldosterone Acetate on Sodium and Potassium Excretion in Man. J. Clin. Endocrin., vol. 19, 1959, pp. 289-296.

8-13. Crabbe, J.: Stimulation of Active Sodium Transport by the Isolated Toad Bladder with Aldosterone in vitro. J. Clin. Invest., vol. 40, Nov. 1961, pp. 2103-2110.

TABLE 8-I.- EFFECT OF IMMERSION ON URINARY EXCRETORY PATTERNS

[Results are mean ± SE[a] for seven subjects]

Parameter/Group (b)	Prestudy	Results, after -				
		1 hr	2 hr	3 hr	4 hr	5 hr
V, ml/min						
Control	0.7 ± 0.1	0.9 ± 0.2	1.6 ± 0.6	1.3 ± 0.2	1.8 ± 0.4	1.5 ± 0.4
Immersion	.7 ± .1	1.2 ± .4	2.3 ± .5	1.6 ± .2	1.4 ± .2	1.6 ± .3
U_{Osm}, mOsm/kg H_2O						
Control	794 ± 62	694 ± 100	542 ± 103	483 ± 80	396 ± 83	439 ± 76
Immersion	808 ± 56	558 ± 88	435 ± 89	496 ± 68	553 ± 64	516 ± 67
$U_{Na}V$, μeq/min						
Control	43 ± 11	36 ± 8	38 ± 11	33 ± 8	35 ± 9	34 ± 6
Immersion	55 ± 12	[c]88 ± 18	[c]121 ± 24	[c]147 ± 26	[c]161 ± 21	[c]191 ± 19
$U_{TA}V$, μeq/min						
Control	19.0 ± 3.3	14.2 ± 1.7	17.1 ± 4.1	17.5 ± .9	19.9 ± 1.1	22.3 ± 1.6
Immersion	15.0 ± 2.2	11.0 ± 4.5	6.4 ± 2.9	6.9 ± 2.9	[d]6.1 ± 2.6	[c]5.6 ± 2.1
$U_{NA_4}V$, μeq/min						
Control	33.1 ± 3.7	26.7 ± 3.5	29.4 ± 2.5	27.4 ± .4	31.6 ± 3.2	28.8 ± 4.1
Immersion	30.7 ± 7.5	17.6 ± 2.3	[d]15.4 ± 1.7	[d]14.9 ± 2.0	[c]14.3 ± 1.4	[d]12.3 ± .5

[a]SE = standard error.

[b]V = urine volume flow; U_{Osm} = urine osmolality.

[c]$p < 0.005$ difference from control.

[d]$p < 0.05$ difference from control.

TABLE 8-II.- CHANGES IN CUMULATIVE URINARY SODIUM AND POTASSIUM EXCRETION DURING 5-HOUR WATER IMMERSION

[Results are mean ± SE for seven subjects]

Group	Sodium Excretion, meq/5 hr (a)	Potassium excretion, meq/5 hr (b)
Control	10.5 ± 2.3	15.2 ± 0.8
Immersion	42.0 ± 5.8	23.5 ± 2.0

[a] $p < 0.001$.

[b] $p < 0.01$.

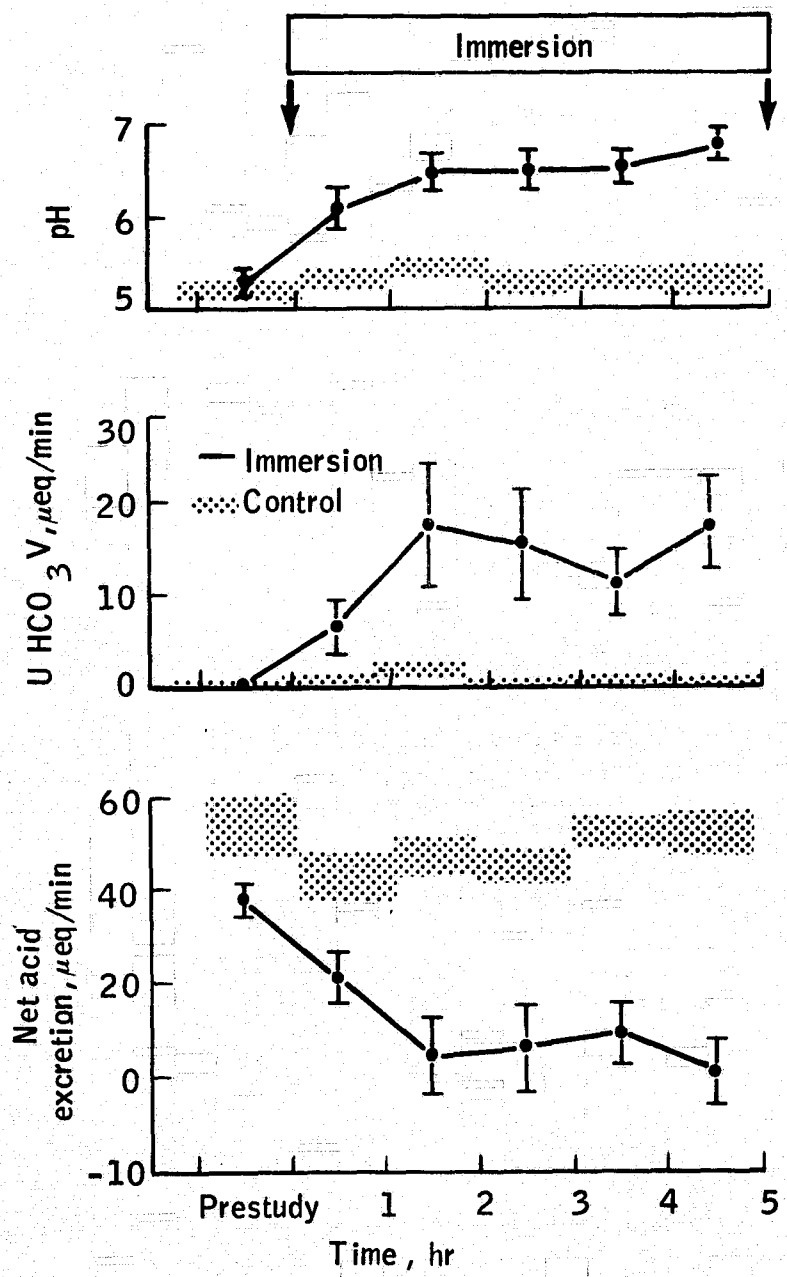

Figure 8-1.- Effect of immersion on urinary pH, the rate of bicarbonate excretion ($U_{HCO_3}V$), and net acid excretion in normal subjects. The shaded area represents mean ± SE during control; brackets represent mean ± SE for immersion group.

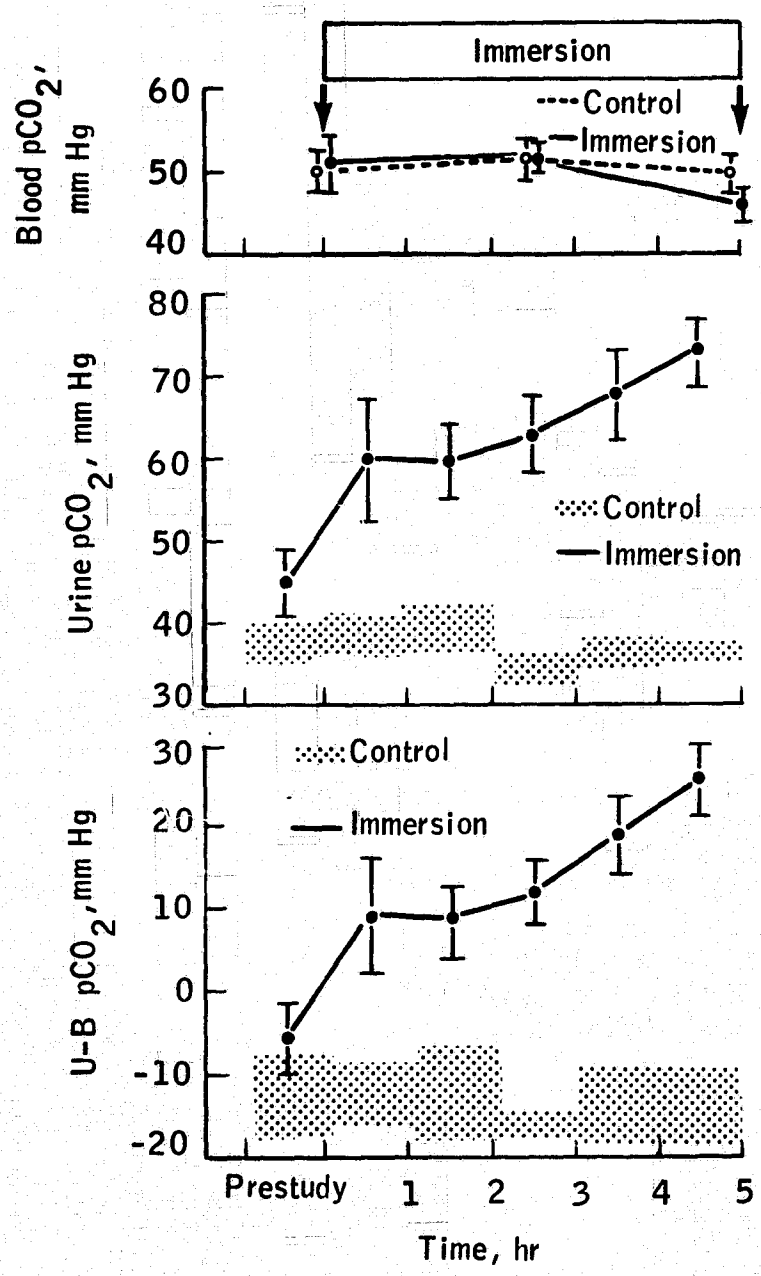

Figure 8-2.- Effect of immersion on blood and urine pCO_2 in normal subjects. The lower panel depicts the difference between the urine and plasma pCO_2 (U-B pCO_2). The shaded area in the two lower panels represents mean ± SE during control; brackets represent mean ± SE.

9. PARATHYROID HORMONE, CALCITONIN, AND VITAMIN D 1974: PRESENT STATUS OF PHYSIOLOGICAL STUDIES AND ANALYSIS OF CALCIUM HOMEOSTASIS

By John T. Potts, Jr., M.D., and K. G. Swenson

The ultimate purpose of the research efforts of the authors and NASA has been to define the role of parathyroid hormone, calcitonin, and vitamin D in the control of calcium and bone metabolism. Particular emphasis was placed on the physiological adaptation to weightlessness and, as a potential model for this purpose, on the immobilization characteristic of space flight or prolonged bed rest.

Recent studies of prolonged bed rest have shown a negative calcium balance; and, in the 50- to 60-day Skylab missions, an increase in urinary calcium has been detected consistent with at least incipient negative calcium balance. It is of practical importance to define the factors responsible for this disorder in calcium homeostasis in order to prevent or reverse this trend to negative calcium balance so that longer duration space flights could be undertaken without the risk of serious bone mineral loss.

Very little information is now available concerning the nature of changes, if any, in the relative rates of secretion or metabolism of parathyroid hormone (PTH), calcitonin, or vitamin D under conditions of prolonged weightlessness or immobilization. The determination of the importance of alterations in the normal endocrine homeostasis of PTH, calcitonin, and vitamin D in response to prolonged immobilization will require considerable basic research into the physiology and the techniques of measuring these hormonal agents. This determination must take into consideration present investigations into the biosynthesis, control of secretion, and metabolism of these hormonal agents.

BONE MINERAL AND CALCIUM LOSS AND HORMONAL CHANGES DURING PROLONGED IMMOBILIZATION

The studies of Hulley et al. (ref. 9-1) have indicated that prolonged bed rest leads to a negative balance as great as 200 to 300 milligrams per day during the second month, as well as a substantial reduction in bone mineral as estimated by bone densitometry. Recently, attempts have been made to reverse bone mineral loss by the administration of calcitonin, the application of a regimen of intermittent compression and exercise, and/or the administration of large amounts of supplemental calcium and phosphate. Preliminary findings,

although not conclusive, are encouraging because they suggest that calcium and phosphate supplementation and a combination of compression and exercise may help retard bone mineral loss (ref. 9-2). However, extensive study will be required to firmly establish the value of any countermeasures to retard negative calcium balance.

Very little information is presently available concerning potential circadian rhythms or diurnal fluctuations in the rate of production, secretion, or turnover of biologically active forms of PTH, calcitonin, or vitamin D. One report (ref. 9-3) has suggested the existence of a circadian rhythm in PTH concentrations in normal humans. In otherwise healthy, normal, male and female subjects, an approximate twofold increase in PTH concentration was detected in short-term studies in association with sleep. Such changes in immunoreactive PTH concentrations during sleep are difficult to interpret, however, because much of the immunoreactive hormone in blood represents biologically inert fragments whose rate of metabolic turnover may change with the reduced renal blood flow occurring during sleep and with the reduced fluid intake at night.

PARATHYROID HORMONE

Physiologists and clinicians concerned with improved understanding of the homeostatic role of PTH in calcium and bone metabolism and with improved methods of diagnosis and treatment in disease states associated with disorders of the parathyroid function have been understandably excited by the many advances in fundamental research on PTH in the last decade. The amino acid sequence of the biologically active amino terminal portion of human PTH has recently been determined; the complete amino acid sequence of the bovine and porcine PTH was determined earlier. Synthesis of biologically active subfragments of PTH has permitted a systematic analysis of structure/activity relationships in PTH and has provided much material for detailed biochemical, physiological, and immunochemical studies.

Paradoxically, however, these fundamental advances have led not only to improved understanding of parathyroid physiology but also to confusion and uncertainty in parathyroid-related physiological and clinical research because of a recognition of the hitherto unappreciated complexity in hormone biosynthesis and metabolism.

Biosynthesis

Recent advances in the knowledge of the chemistry of PTH have greatly aided studies of its biosynthesis (refs. 9-4 to 9-9). The successful development of an in vitro system for study of PTH biosynthesis, using slices of bovine parathyroid glands, was reported by Cohn et al. (ref. 9-10). These studies indicated the presence of a rapidly synthesized peptide that was larger than PTH but was biologically active. The peptide produced hypercalcemia in rats and bone resorption in vitro, and was chemically similar to PTH in that the larger peptide reacted with antisera to PTH. This peptide was referred to

as "a nonparathyroid calcemic fraction (calcemic fraction A)" while awaiting proof that it was a true biosynthetic precursor. Subsequently, it was conclusively demonstrated that this larger peptide was indeed a precursor of PTH; that is, a prohormone, or proparathyroid hormone (proPTH) (refs. 9-10 to 9-12).

Shortly after the existence of proPTH was conclusively established, Cohn et al. (ref. 9-10) undertook large-scale purification of the prohormone and isolated a sufficient quantity (approximately 1 milligram) to permit, in collaboration with the authors and others, a determination of proPTH primary amino acid sequence (refs. 9-13 and 9-14). The additional hexapeptide sequence at the amino terminus of the hormone is heavily basic; four of the six additional amino acids are positively charged (three lysine and one arginine) (fig. 9-1). It has not yet been established conclusively whether the 90 amino acid prohormone is the direct product synthesized from the messenger ribonucleic acid (RNA) for PTH. Larger, as yet unidentified, precursor polypeptides of PTH may exist.

In a recent study (ref. 9-15), the author and others greatly extended the sensitivity of the radioactive-microsequencing approaches. The amino terminal sequence of the human proPTH was determined after tissue slices were pulsed with radioactive amino acids and structural analysis was performed by Edman degradation. The sequence studies indicated that the amino terminal prohormone specific sequence of the human hormone is identical with that of the bovine molecule; that is, the amino terminal sequence analysis indicated the structure Lys-Ser-Val-Lys-Lys-Arg, which was followed by the amino terminal sequence of the human hormone Ser_1-Val_2-Ser_3, already established (ref. 9-14).

Until recntly, very little was known about cellular mechanisms involved in the biosynthesis, transport, and storage of PTH or about the critical points of intracellular control of hormone production. Recent studies, however, are now providing considerable information about the intracellular sites of proPTH synthesis and conversion and the processes that lead to the storage and secretion of PTH.

Figure 9-2 summarizes and schematically depicts proposed rate-limiting points in the parathyroid cell biosynthetic machinery. These points are those at which calcium or other agents may exert a regulatory influence on the synthesis, transport, cleavage, or storage as well as secretion of PTH. Little information exists regarding the role of calcium at specific control points, but some conclusions are possible.

No effect of Ca++ ion on the activity of the cleavage enzyme (step 4 of fig. 9-2) has been shown. Preliminary studies (ref. 9-16) suggest that, although low calcium stimulates and high calcium suppresses the overall synthesis of proPTH, there is no evidence of a direct effect on transcription or translation (steps 2 and 3 of fig. 9-2). There is evidence, however, of a considerable intracellular turnover of PTH or proPTH (steps 6 and 7 of fig. 9-2) and evidence that high Ca++ stimulates this process and low Ca++ inhibits intracellular degradation.

A more definitive picture of the normal controlling processes within the parathyroid cell involved in regulation between the initial steps of proPTH synthesis and the eventual release of PTH from storage granules will be of fundamental interest and will serve as a model for evaluating the defects in cellular control that are involved in excessive PTH secretion. Hence, defects such as the release of predominantly prohormone (lack of specific cleavage enzyme) or predominantly fragments (uncontrolled proteolytic degradation) might be found in pseudohyperparathyroidism.

In the development of a radioimmunoassay for proPTH, immunization with synthetic peptides containing the prohormone specific hexapeptide sequence has resulted in the production of uniquely useful antibodies (ref. 9-17). Assays based on this antiserum readily detect intact prohormone and synthetic peptides incorporating the prohormone sequence, but do not detect the hormone or hormonal fragments, such as the prohormone hexapeptide sequence alone or the amino terminal peptides of the hormone itself (fig. 9-3). Prohormone appears to be rapidly degraded in blood by proteolytic activity, and no conclusions have yet been reached on the secretion of bovine or human prohormone. However, the development of human prohormone-specific antisera (now that the structure of the human precursor is known) and the continuing efforts to block prohormone degradation in plasma seem promising. Therefore, crucial issues in these areas of biosynthesis investigation include the completion of analysis of the intracellular processes and controlling features of hormone biosynthesis, storage, and release; the detection of the secretion of prohormone or prohormone-related (hexapeptide) peptides; and the development of routinely applicable immunoassays for measurement of these precursor molecules. Advances in these investigative areas should provide unique approaches for clinical and radioimmunoassay studies aimed at improving the detection of abnormalities in parathyroid function.

Secretion and Metabolism

Recent studies have led to an important new area of investigation in the metabolism and peripheral turnover of PTH. It has become apparent, for the first time, that the fate of PTH after release from the gland involves a much more complex process than a simple uptake by receptors or an all-or-nothing removal from the circulation. It is also apparent that an appreciation of the physiological significance of this process of peripheral clearance or metabolism of PTH requires the development of new techniques to assess the nature of the cleavage, the site or sites of occurrence of the cleavage, and the biological and chemical properties of the circulating fragments. Efforts in this laboratory have been concentrated on the application of region-specific antibodies to characterize the fragments detected in blood. Since the minimum sequence of amino acids required for biological activity has been determined, immunochemical testing can be applied to determine whether or not any fragment in the circulation has the structural features required for biological activity.

It has been possible to modify antisera by absorption methods so that recognition is limited exclusively to certain regions of the sequence, such as

the amino (N assay) or carboxyl terminal end (C assay) of the hormonal molecule (fig. 9-4) (refs. 9-18 and 9-19). Thus far, studies of endogenous hormone in the circulation of humans and of normal cows, as well as hormone injected into calves and dogs, have established through gel filtration analysis that the large peak of immunoreactive material (which elutes at a position corresponding to a molecular weight of approximately 6000) is detected using antisera that recognize antigenic determinants "carboxyl terminal to position 30" (MC fragment). Existing information about the structural requirements for biological activity makes it probable that any fragment that does not contain the amino-terminal residues 2-27 in intact form will be bilogically inert. Since this large fragment in the peripheral circulation lacks the critical amino terminal sequence required for biologic activity, it must be biologically inactive.

A smaller fragment that was biologically active in an in vitro renal adenyl cyclase assay has been found in concentrates of human plasma. The larger fragment (which appears to be equivalent to the large, middle plus carboxyl terminal fragment (MC fragment) that we have identified) was found to be inactive, corresponding to the predictions based on immunochemical analyses of the MC fragment.

Recent investigations by the authors involving several different approaches have helped to identify more precisely the site of cleavage. Chemical evidence was found to confirm that peripheral cleavage of the hormone could result in the production of an active fragment (ref. 9-19). In preliminary animal studies, ^{125}I-labeled bovine PTH (BPTH), with radioactive iodine linked to the tyrosine residue at position 43, was infused into dogs. As serial blood samples are analyzed, the peak corresponding to the elution position of the MC fragment increases in concentration with time. This large 6000-molecular-weight fragment was separated by gel filtration from other peaks of radioactivity. The MC fragment was subjected to sequential degradation, using the Edman procedure to remove, step-wise, amino acids from the amino terminal end of the peptide. The use of this procedure has made it possible to determine the number of amino acids present in the sequence of the MC fragment, beginning at the amino terminus of the fragment and extending to the radioactive tyrosine at position 43 (fig. 9-5).

Thus, the determination of the probable site or sites of cleavage in the hormone is possible. These studies have shown the appearance of radioactive tyrosine after 7 and 10 steps of degradation, indicating that the 6000-molecular-weight fragment isolated from the circulation of the dog probably consists of at least two fragments closely related in size. One fragment presumably consists of sequence 34 to 84, and the other of sequence 38 to 84. Both fragments must be biologically inactive since they lack the amino-terminal 25 amino acids required for activity. In these studies, a variety of region or sequence-specific antisera have also been applied to the analysis of the rate of disappearance and of formation, respectively, of intact hormone, and the analysis of the rate of formation of the MC fragment following injection of unlabeled, biologically active hormone (fig. 9-6). The results achieved by

this approach are in close agreement with the stuides based on radiochemical approaches. Similar estimates are found by each method for the rate of disappearance of the intact hormone and for the formation of the MC fragment (resulting from cleavage of the injected hormone) (fig. 9-7).

These studies (sequence analysis) confirm that the MC fragment is indeed a cleavage product and that peripheral cleavage of intact hormone entering the circulation is the origin of the MC fragment. The similarity of results seen by the immunochemical approaches using nonradioactive, biologically active hormone have validated the use of radiolabeled peptide to investigate the metabolism of endogenous hormone. Furthermore, these results indicated, by two independent methods, that if fragmentation results from a single site-specific cleavage, such as that which would occur by the action of an endopeptidase, the smaller amino terminal fragment (presumably residues 1-33) resulting from cleavage could be biologically active since it would contain the structural region necessary for activity. This concept, however, is still speculative.

Many broad issues of great interest need to be resolved concerning the peripheral cleavage of PTH. The site, nature, and physiological significance of the cleavage must be defined. If all fragments generated are inactive, then the cleavage process reflects simply metabolic degradation of the hormone. However, if one or more of the fragments are biologically active and if they are cleaved in the vicinity of receptors in the target organs, such fragments, even though they need not be present in the general circulation, could constitute a major, if not the sole, mediator of PTH actions. Because the activity of the intact hormone even in the in vitro assays might reflect prior conversion to active fragments, it must be considered that there is no conclusive evidence that the intact hormone is the active molecular species in vivo. Thus, the issue of hormonal metabolism is critical in present efforts to understand the mode of action of the hormone (the homeostatic regulation of expression of hormonal effects such as the precise control of hormone secretion) and in efforts to develop radioimmunoassays that can measure the most appropriate and reliable index of hormonal activity.

The parathyroid cell is the site of initial cleavage-prohormone to hormone conversion, and peripheral metabolism is the second site of cleavage conversion of secreted hormone into one or more peptide subfragments. When the issues concerning the nature of these hormonal products released into the circulation (fig. 9-8) can be resolved and eventually analyzed by appropriate immunoassays, a new era in the use of special laboratory aids for improved diagnosis of parathyroid disorders should be opened. In particular, the possibility exists for a more meaningful analysis of the details of the normal secretory patterns of biologically active PTH, including circadian rhythms, as a background for the investigation of homeostatic perturbations induced by prolonged immobilization.

VITAMIN D METABOLISM

The authors' efforts have continued to focus on the development of assay methods and selected animal studies to provide techniques suitable for clear

delineation in humans (in vivo) of essential features of the metabolism of vitamin D and to detect specific disorders in disease states.

Vitamin D is now known to be stored in the body after formation in the skin or absorption from the diet and then converted to 25-OH-vitamin D (25-OH-D) by a specific hydroxylase in the liver. This compound must be further hydroxylated in the kidney before it can act on its target tissues in vivo. One renal metabolite ($1,25\text{-}(OH)_2D$) stimulates calcium transport by the intestine and mobilizes bone calcium in vitamin D-deficient rats and may be the principal hormonal form of the vitamin. The physiological roles of a second dihydroxy metabolite ($24,25\text{-}(OH)_2D$) and a recently discovered trihydroxy metabolite ($1,24,25\text{-}(OH)_3D$) are as yet unclear (ref. 9-20).

The metabolic activation of vitamin D appears to be a regulated process in both the liver and the kidney. In vitro and in vivo experiments in the rat had suggested product inhibition of the liver hydroxylase. Using the competitive binding assay for vitamin D and OH-D, the authors have found wide variations (tenfold or greater) in the serum OH-D levels in D-deficient rats with little or no change in serum calcium. In contrast, small changes in OH-D levels with large changes in serum calcium were found. Indeed, in these latter, clinically deficient animals with hypocalcemia, stunted growth, and abnormal bones, the OH-D levels were reduced by one-third to one-half, but were still detectable (>1 ng/ml) for at least 4 to 5 weeks after the onset of clinical deficiency (fig. 9-9).

In a group of normal human subjects, no apparent correlation was found between serum 25-OH-D levels and the absorptive efficiency for calcium. Some of the subjects had very efficient absorption with low levels of 25-OH-D; in others, the converse was found. In serial studies over a 2-year period in a few of these subjects, calcium absorption and 25-OH-D content in serum seemed to vary independently, whereas serum calcium, phosphorus, and alkaline phosphatase remained unchanged. Thus, in humans and in rats, no direct correlation was found between serum 25-OH-D levels and the consequences of vitamin D metabolism, which might have been expected if strict product inhibition of the 25-hydroxylase in the liver was operative over the normal range of vitamin D intake. Although blood 25-OH-D levels may be an important parameter for assessing overall status of vitamin D intake and metabolism, the results clearly emphasize the need to focus on concentrations of dihydroxy metabolites to examine the precisely regulated homeostasis of vitamin D (ref. 9-21). The renal hydroxylase, in contrast to the hepatic hydroxylase, seems to be tightly controlled by one or more metabolic variables related to or varying with calcium or skeletal metabolism.

In contrast to the hepatic hydroxylase, the renal hydroxylase is subject not to simple product inhibition but rather to complex metabolic regulation. The nature of the metabolic control has not yet been clarified and is, in fact, the subject of some dispute. Omdahl and DeLuca (ref. 9-22) have shown that formation of $1,25\text{-}(OH)_2D_3$ was favored in hypocalcemic states in animals with intact parathyroid glands and by the administration of parathyroid hormone to

thyroparathyroidectomized animals. They also reported that formation of 1,25-$(OH)_2D_3$ was favored in thyroparathyroidectomized animals who had been phosphate-depleted prior to surgery and were thus relatively hypophosphatemic. Most work with subcellular fractions has confirmed this finding, but other investigations of subcellular fractions of renal tissue and whole animal experiments were contradictory. Larkins, et al. (ref. 9-23) found that 1,25-$(OH)_2D_3$ formation was suppressed in some animals following administration of PTH and that 24,25-$(OH)_2D_3$ formation was favored. This finding is apparently a direct contradiction of the experiments of Omdahl and DeLuca. The examination of the control of 1,25 di-OH-D production continues in numerous laboratories. It seems imperative, however, to develop an efficient and sensitive assay for 1,25-$(OH)_2D$ in humans.

To develop selective assays for 1,25-$(OH)_2D$ and to further examine the general issue of the plasma transport of vitamin D, the authors have analyzed vitamin D transport proteins in several species. In vitro studies have conclusively demonstrated a single transport protein for vitamin D and OH-D in rat serum and have shown that it binds only vitamin D and D metabolites and not other steroids or sterols. The authors have now shown that 25-hydroxylated forms of vitamin D are preferentially bound, whereas other changes in the basic vitamin D molecule, either on the side chain or on the A ring, seem to reduce the binding affinity (ref. 9-24) (fig. 9-10). In other species, the authors have demonstrated two vitamin D transport proteins (one binding vitamin D alone and the other binding vitamin D and OH-D) but have not yet studied other vitamin D analogues in these systems except in the case of the chick. In this case, D_2 and OH-D_2 are poorly bound by chick binding proteins (when compared to D_3 and OH-D_3), which may explain the differential potency of D_2 and D_3 in curing rickets in the chick (ref. 9-25) (figs. 9-11 and 9-12). (D_2 and D_3 are equipotent on a weight basis in the line test using vitamin D-deficient rats, whereas D_2 is only 1/100 as potent as D_3 in the line test using vitamin D-deficient chicks.)

The authors have continued to investigate the method of delivery of 1,25-$(OH)_2D$ to receptor sites in bone and intestine from production sites in the kidney. The presence of 1,25-$(OH)_2D_3$ in plasma is estimated to be at low concentrations (pg/ml); that is, in very small amounts compared to the concentrations of circulating vitamin D and 25-OH-D (ng/ml). Thus, plasma transport could be accomplished by either a large excess of a single vitamin D/OH-D/$(OH)_2D$ protein or, if the concentration of the binding protein is rate limiting, by a preferential binding of 1,25-$(OH)_2D_3$ over vitamin D and 25-OH-D. When isotopic 1,25-$(OH)_2D_3$ is added to rat plasma, most of the activity appears to be associated with the vitamin D/OH-D transport protein. Isotopic 1,25-$(OH)_2D_3$ could be displaced from plasma by nonisotopic 1, 25-$(OH)_2D_3$; but, on a weight basis, 25-OH-D is bound 10 times as tightly as the dihydroxy compound.

Further studies are required to clarify the nature of 1,25-$(OH)_2D_3$ transport in plasma, but these studies suggest that a single vitamin D/OH-D/1,25-$(OH)_2D$ binding protein may perform this transport function in rats.

Transport of the dihydroxy metabolite is probably accomplished by a large excess concentration of the transport proteins. The authors have found an undersaturation of the plasma transport proteins in the rat. Although normal rats contain a total of approximately 50 ng/ml of vitamin D and 25-OH-D, the total binding capacity measured with an in vitro assay can be shown to be a minimum of 1200 to 1500 ng/ml and as great as 2500 to 3000 µg/ml in animals with combined calcium and vitamin D deprivation. Hence, no separate binding protein need be postulated for carraige of the dihydroxy metabolites in rat plasma.

The situation is somewhat different for human plasma. When isotopic 1,25-$(OH)_2D_3$ is added to human plasma, only a small amount of activity is associated with the vitamin D/OH-D transport protein; the bulk of the activity is associated with another distinct fraction that is clearly not identical with other vitamin D or 25-OH-D binding proteins noted previously. If ^{14}D-D_3 and ^{3}H-1,25-$(OH)_2D_3$ are added to human plasma, additional 25-OH-D displaces the ^{14}C-D_3, but not the ^{3}H-1,25-$(OH)_2D_3$, from plasma binding, consistent with the existence of a separate dihydroxy transport or binding protein (ref. 9-26). Such a separate binding protein would be of great physiologic importance and practical interest, since a specific rapid assay for the dihydroxy metabolite might be readily developed with the 1,25-$(OH)_2D$ binding protein. Dihydroxy transport proteins continue to be studied to clarify the importance of plasma transport in normal vitamin D metabolism and in disorders of calcium or skeletal homeostasis, such as sarcoidosis, in which vitamin D responsiveness is abnormal.

Studies are now underway to purify the suspected binding protein that selectively binds 1,25-$(OH)_2D$ and other dihydroxy-metabolites of vitamin D in human plasma. This protein migrates with the D/OH-D transport protein when human plasma is fractionated by gel filtration over Sephadex G-200, but is separable from this latter protein on acrylamide gel electrophoresis. For assay purposes, the separation of 1,25 di-OH-D binding protein from the D/OH-D transport protein would be very important because the latter inferferes with assay of 1,25-$(OH)_2D$. Currently, attempts are underway to separate the proteins on the basis of charge with either preparative acrylamide gel electrophoresis or the use of chromatography.

Application of the 25-OH-D assays to physiological studies in humans has been difficult because D_3 and 25-OH-D_3 (resulting from photoactivation of precursor in the skin and the physiologic form in humans) and D_2 and 25-OH-D_2 (from dietary sources) are not equally reactive in the assay system with the rat binding protein, although they are equipotent biologically. The authors

discovered, however, that the vitamin D binding protein in chick sera is relatively insensitive to D_2 and 25-OH-D_2 and may provide the basis for development of selective assays for vitamin D_2 and D_3 separately. This finding would provide, for the first time, the opportunity to accurately assess the contribution of dietary as compared to environmental factors in the provision of vitamin D in normal humans.

The ultimate aim of these investigations is to provide a battery of highly selective saturation analysis assays with which to assess the concentration of D_2, D_3, 25-(OH)D_2 and 25-(OH)D_3, and the dihydroxylated metabolites of vitamin D. By this means, one can adequately evaluate vitamin D metabolism patterns in humans under normal physiological conditions as a prelude to screening for homeostatic perturbations either in various calcium deficiency states in humans or under unusual physiological stress states, such as prolonged immobilization or weightlessness and space flight.

CALCITONIN

The development and application of sensitive and specific radioimmunoassays for the calcitonins have been useful in defining the control of secretion of this hormone (ref. 9-27). Bioassay techniques of much greater sensitivity than bioassays for parathyroid hormone have been applicable to analysis of gland content of hormone; and they have been applicable to analysis of secreted hormone in certain species, with concentration of blood or in thyroid effluent blood. However, the biological assays are at the borderline of necessary sensitivity and are technically difficult or cumbersome for multiple analyses.

The first immunoassay developed was for porcine calcitonin (ref. 9-28). This assay was sufficiently sensitive to detect peripheral concentrations of the peptide in both the rabbit and porcine species and in human medullary thyroid tumors grown in vitro (ref. 9-29). Initial studies demonstrated that calcitonin is continuously secreted at physiological concentrations of blood calcium. Measurements made during calcium infusion in these animals demonstrated that the concentration of the hormone rises within minutes of induced hypercalcemia. Bioassay studies of thyroid effluent blood and immunoassay of mixed venous blood have shown that the secretion of the hormone is under the directly proportional control of blood calcium.

The importance of calcitonin in calcium and skeletal homeostasis in normal humans has not been established. The study of the secretion of calcitonin in humans has been most extensively done in patients with medullary thyroid carcinoma who have elevated concentrations of the peptide in peripheral blood. Because of methodological problems, the reliable measurement of calcitonin in humans other than those with calcitonin-secreting tumors of the thyroid has not been possible (fig. 9-11). Until calcitonin can be shown to be secreted in humans, no hormonal or physiological role can be ascribed to the peptide. Earlier reports based on bioassay measurements of human calcitonin in the blood of normal subjects led to estimates varying from 200 to 1700 pg/ml (refs. 9-30

and 9-31). Later, however, these reports were shown to be incorrect; spurious factors and artifacts were shown to be present in the methods used (ref. 9-32). The development (ref. 9-33) and subsequent application (refs. 9-34 and 9-35) of immunoassays for human calcitonin also led, initially, to conflicting results. However, subsequent to the demonstration of artifacts in immunoassay methods (ref. 9-36), the preliminary estimates that calcitonin is secreted normally in humans at a concentration of 50 to 400 pg/ml have been revised sharply downward. Studies with a sensitive immunoassay for human calcitonin (ref. 9-36) have shown the peptide to be undetectable (less than 100 pg/ml) not only in the peripheral plasma of many normal subjects but also in thyroid venous blood taken from patients (primarily with hypercalcemia) during selective venous catheterization or at surgery (fig. 9-12). These findings suggest that the concentration of calcitonin in the peripheral blood of humans, if present at all, must be much lower than that found in other mammals. Development of much more sensitive assays will be required to determine whether calcitonin does circulate but, perhaps analogous to adrenocorticotropic hormone, at very low concentrations or whether the hormone simply is not secreted under physiological conditions in normal humans and is, therefore, vestigial in humans in contrast to other mammals.

Recently, however, calcitonin has been detected after calcium infusion by radioimmunoassay in the peripheral blood of patients with diseases associated with chronic hypocalcemia (ref. 9-37). These findings increase the possibility that calcitonin is secreted in normal human subjects and have spurred efforts by the authors and by others to develop more sensitive assays for calcitonin. Affinity chromatography methods, in particular, show promise for meaningful interpretations of calcitonin secretion dynamics in humans. Questions of adaptive responses to protect skeletal calcium stores during prolonged immobilization can then be evaluated with appropriate assays of requisite sensitivity.

REFERENCES

9-1. Hulley, Stephen B.; Vogel, John M.; Donaldson, Charles L.; Bayers, Jan H.; et al: The Effect of Supplemental Oral Phosphate on the Bone Mineral Changes During Prolonged Bed Rest. J. Clin. Invest., vol. 50, 1971, pp. 2506-2518.

9-2. Hartman, D. A.; Vogel, John M.; Donaldson, Charles L.; Friedman, R. J.; et al.: Attempts to Prevent Disease Osteoporosis by Treatment With Calcitonin Longitudinal Compression and Supplementary Calcium and Phosphate. J. Clin. Endocrinol. Metab., vol. 36, no. 5, May, 1973.

9-3. Jubiz, William; Canterbury, Janet M.; Reiss, Eric; and Tyler, Frank H.; with the technical assistance of Frailey, Jacqueline; Bartholomew, Ken; and Creditor, Margaret A.: Circadian Rhythm in Serum Parathyroid Hormone Concentration in Human Subjects: Correlation with Serum Calcium, Phosphate, Albumin, and Growth Hormone Levels. J. Clin. Invest., vol. 51, Aug. 1972, pp. 2040-2046.

9-4. Brewer, H. B., Jr.; and Ronan, R.: Bovine Parathyroid Hormone: Amino Acid Sequence. Proc. Nat. Acad. Sci. U.S.A., vol. 67, 1970, pp. 1862-1869.

9-5. Niall, H. D.; Keutmann, H. T.; Sauer, R.; Hogan, J. L.; et al.: The Amino Acid Sequence of Bovine Parathyroid Hormone I. Hoppe Seylers Zeitschrift Physiol. Chem., vol. 351, Dec. 1970, pp. 1586-1588.

9-6. Potts, J. T., Jr.; Keutmann, H. T.; Niall, H. D.; and Tregear, G. W.: Chemistry of Parathyroid Hormone and the Calcitonins. Vol. 29 of Vitamins and Hormones, Academic Press (New York), 1971, pp. 41-93.

9-7. Woodhead, J. S.; O'Riordan, J. L. H.; Keutmann, Henry T.; Stoltz, M. L.; et al: Isolation and Chemical Properties of Porcine Parathyroid Hormone. Biochemistry, vol. 10, no. 14, July 1971, pp. 2787-2792.

9-8. Niall, H. D.; Sauer, R. T.; Jacobs, J. W.; Keutmann, H. T.; et al.: The Amino-Acid Sequence of the Amino-Terminal 37 Residues of Human Parathyroid Hormone. Proc. Natl. Acad. Sci. U.S.A., vol. 71, Feb. 1974, pp. 384-388.

9-9. Brewer, H. B.; Fairwell, T.; Rittel, W.; Dibella, F.; et al.: Human Parathyroid Hormone. Endocrinology 1973, S. Taylor, ed., William Heinemann Med. Books, Ltd. (London), 1974.

9-10. Cohn, David V.; MacGregor, Ronal R.; Chu, Luke L. H.; Kimmel, Joe R.; and Hamilton, James W.: Calcemic Fraction-A: Biosynthetic Peptide Precursor of Parathyroid Hormone. Proc. Nat. Acad. Sci. U.S.A., vol. 69, no. 6, June 1972, pp. 1521-1525.

9-11. Kemper, B.; Habener, J. F.; Potts, J. T., Jr.; and Rich, A.: Proparathyroid Hormone: Identification of a Biosynthetic Precursor to Parathyroid Hormone. Proc. Nat. Acad. Sci. U.S.A, vol. 69, no. 3, Mar. 1972, pp. 643-647.

9-12. Habener, Joel F.; Kemper, Byron; Potts, John T., Jr.; and Rich, Alexander: Proparathyroid Hormone: Biosynthesis by Human Parathyroid Adenomas. Science, vol. 178, no. 4061, Nov. 1972, pp. 630-633.

9-13. Hamilton, J. W.; Niall, H. D.; Jacobs, J. W.; Keutmann, H. T.; and Cohn, D. V.: The N-Terminal Amino-Acid Sequence of Bovine Proparathyroid Hormone. Proc. Nat. Acad. Sci. U.S.A., vol. 71, Mar. 1974, pp. 653-656.

9-14. Niall, H. D.; Jacobs, J. W.; Sauer, R. T.; Keutmann, H. T.; and Potts, J. T., Jr.: High Sensitivity Automated Sequence Analysis of Polypeptide Hormones. Endocrinology 1973, S. Taylor, ed., William Heinemann Med. Books, Ltd. (London), 1974.

9-15. Jacobs, J. W.; Kemper, B.; Niall, H. D.; Habener, J. F.; and Potts, J. T., Jr.: Structural Analysis of Human Proparathyroid Hormone by a New Microsequencing Approach. Nature, vol. 249, May 1974, pp. 155-157.

9-16. Habener, J. F.; Kemper, B. W.; Potts, J. T., Jr.; and Rich, A.: Calcium-Independent Intracellular Conversion of Proparathyroid Hormone to Parathyroid Hormone. Bio. Chem. Biophy. Res. Commun. (To be published).

9-17. Habener, J. F.; Tregear, G. W.; Van Rietschoten, J.; Hamilton, J. W.; et al.: Bovine Proparathyroid Hormone: Immunological and Biological Studies. Am. Fed. Clin. Res., vol. 21, 1973, p. 37.

9-18. Segre, Gino V.; Habener, Joel F.; Powell, David; Tregear, Geoffrey W.; and Potts, John T., Jr.: Parathyroid Hormone in Human Plasma: Immunochemical Characterization and Biological Implications. J. Clin. Invest., vol. 51, Dec. 1972, pp. 3163-3172.

9-19. Segre, G. V.; Niall, H. D.; Jacobs, J. W.; Sauer, R. T.; et al: Metabolism of Parathyroid Hormone: Analysis by Edman Degradation of Radioiodinated Hormone. J. Clin. Invest. (To be published).

9-20. Lam, Hing-Yat; Schnoes, H. K.; DeLuca, H. F.; and Chen, Tai C.: 24, 25-Dihydroxyvitamin D_3. Synthesis and Biological Activity. Biochemistry vol. 12, Nov. 1973, pp. 4851-4855.

9-21. Clark, M. B.; Bernat, M. J. F.; Belsey, R. E.; DeLuca, H. F.; and Potts, J. T., Jr.: Phsiological Significance of Regulation of Hepatic Hydroxylation of Vitamin D. Clin. Res., vol. 21, 1973, p. 619.

9-22. Omdahl, J. L.; DeLuca, H. F.: Regulation of Vitamin D Metabolism and Function. Physiol. Rev., vol. 53, Apr. 1973, pp. 327-372.

9-23. Larkins, R. G.; Colston, K. W.; Galante, L. S.; Evans, I. M. A.; et al.: Regulation of Vitamin-D Metabolism Without Parathyroid Hormone. Lancet, vol. 2, Aug. 1973, pp. 289-291.

9-24. Belsey, R.; Clark, M. B.; Bernat, M. J. F.; Nold, J.; et al.: The Physiologic Significance of Plasma Transport of Vitamin D and Metabolites. Am. J. Med., vol. 57, July 1974, pp. 50-56.

9-25. Belsey, R. E.; DeLuca, H. F.; and Potts, J. T., Jr.: Selective Binding Properties of Vitamin D Transport Protein in Chick Plasma in vitro. Nature, vol. 247, Jan. 1974, pp. 208-209.

9-26. Nold, J. G.; and Belsey, R. E.: Comparative Studies of Rat, Human and Chick Vitamin D Binding Proteins. Fed. Proc., vol. 32, 1973, p. 917.

9-27. Deftos, L. J.; and Potts, J. T., Jr.: Radioimmunoassay for Parathyroid Hormone and Calcitonin. Brit. J. Hosp. Med., vol. 11, 1969, p. 1813.

9-28. Deftos, L. J.; Lee, M. R.; and Potts, J. T., Jr.: A Radioimmunoassay for Thyrocalcitonin. Proc. Nat. Acad. Sci. U.S.A., vol. 60, 1968, pp. 293-299.

9-29. Grimley, T. M.; Deftos, L. J.; Weeks, J. R.; and Rabson, A. S.: Growth in vitro and Ultrastructure of Cells From a Medullary Carcinoma of the Human Thyroid Gland: Transformation by Simian Virus 40 and Evidence of Thyrocalcitonin and Prostaglandin Production. J. Nat. Cancer Inst., vol. 42, 1969, pp. 663-680.

9-30. Sturtridge, W. C.; and Kumar, M. Ashwine: Assay of Calcitonin in Human Plasma. Lancet, vol. 1, Apr. 1968, pp. 725-726.

9-31. Gudmundsson, T. V.; Galante, L.; Woodhouse, N. J. Y.; Matthews, E. W.; and Osafo, T. D.: Plasma Calcitonin in Man. Lancet, vol. 1, 1969, pp. 443-446.

9-32. Bell, P. H.; Dziobkowski, C.; Barg, W. F., Jr.; and Snedeker, E. H.: Plasma-Calcitonin in Man. Lancet, vol. 2, July 1970, pp. 104-105.

9-33. Clark, M. B.; Byfield, P. G. H.; Boyd, G. W.; and Foster, G. V.: A Radioimmunoassay for Human Calcitonin. Lancet, vol. 2, 1969, pp. 74-77.

9-34. Tashjian, A. H., Jr.; Howland, B. G.; Melvin, K. E. W.; and Hill, C. S., Jr.: Immunoassay of Human Calcitonin. New. Eng. J. Med., vol. 283, 1970, pp. 890-895.

9-35. Deftos, L. J.: Immunoassay for Human Calcitonin. I. Method. Metabolism, vol. 20, no. 12, Dec. 1971, pp. 1122-1128.

9-36. Deftos, L. J.; Bury, A. E.; Habener, J. F.; Singer, F. R.; and Potts, J. T., Jr.: Immunoassay for Human Calcitonin. II. Clinical Studies. Metabolism, vol. 20, no. 12, Dec. 1971, pp. 1129-1137.

9-37. Deftos, L. J.; Murray, T. M.; Powell, D.; Habener, J. F.; et al.: Radioimmunoassays for PTH and Calcitonins. Calcium, Parathyroid Hormone and the Calcitonins, R. V. Talmage and P. L. Munson, eds. Excerpta Medica (Amsterdam), 1972, p. 140.

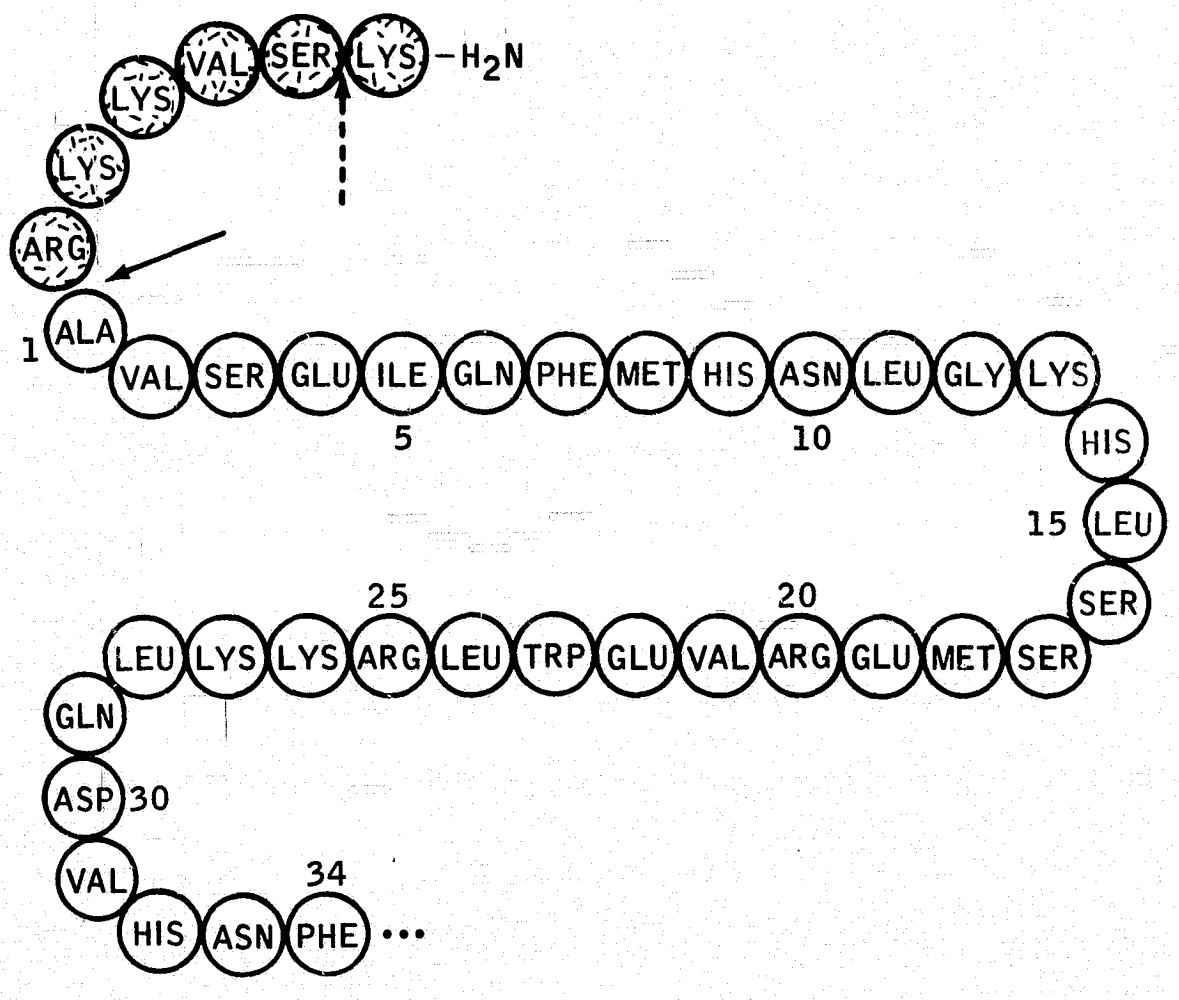

Figure 9-1.- Amino-terminal structure of bovine proparathyroid hormone. Residues 1 to 34 (unshaded circles) represent the amino-terminal sequence of the hormone. The additional six amino-terminal residues found in the prohormone are shown as shaded circles. The continuous arrow shows the cleavage point for the conversion of prohormone to hormone, and the dotted arrow represents a minor cleavage found in some prohormone preparations.

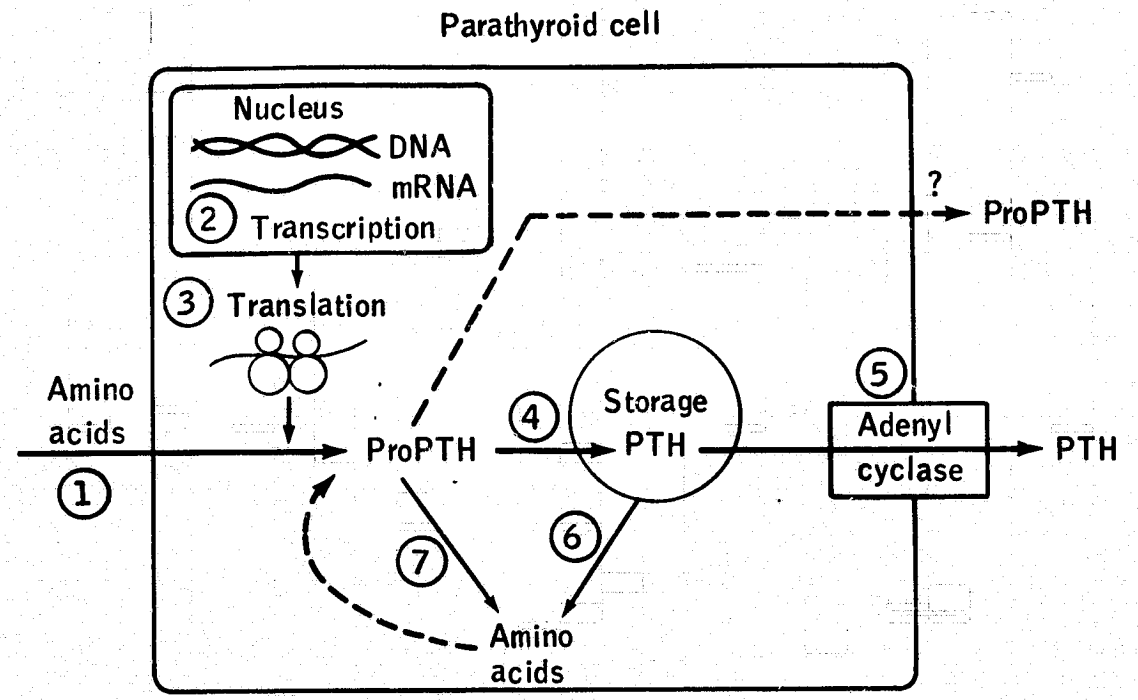

Figure 9-2.- Possible points in biosynthetic machinery of parathyroid cell where calcium may be proposed to exert regulatory effects.

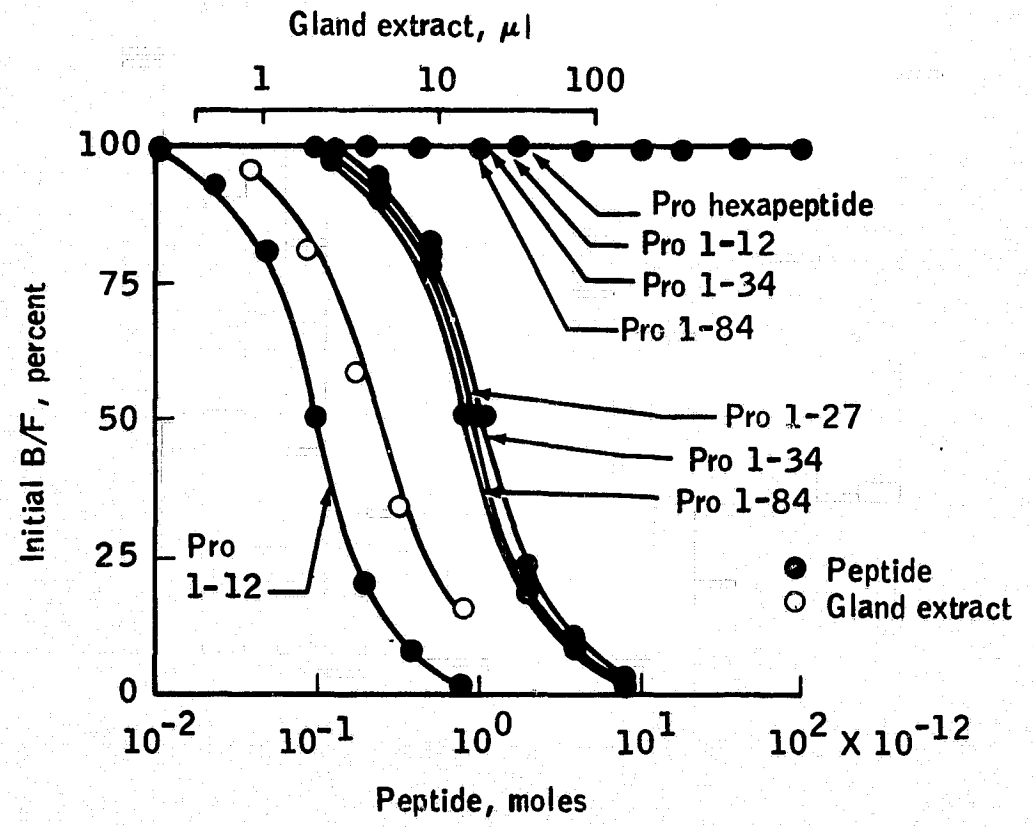

Figure 9-3.- Specific radioimmunoassay for bovine proparathyroid hormone, showing competitive displacement of a ^{125}I-labeled prohormone peptide comprising the prohormone-specific hexapeptide and the first 12 residues of PTH (pro 1-12) by a number of prohormone and hormone peptides. Curves are plotted as a function of molar concentration of peptide versus bound to free ratio (B/F) of labeled pro 1-12 expressed on the basis of percent of B/F value without peptide present. Only prohormone peptides (not the prohormone hexapeptide, bovine PTH (1-84), or PTH fragments) competitively react in assay.

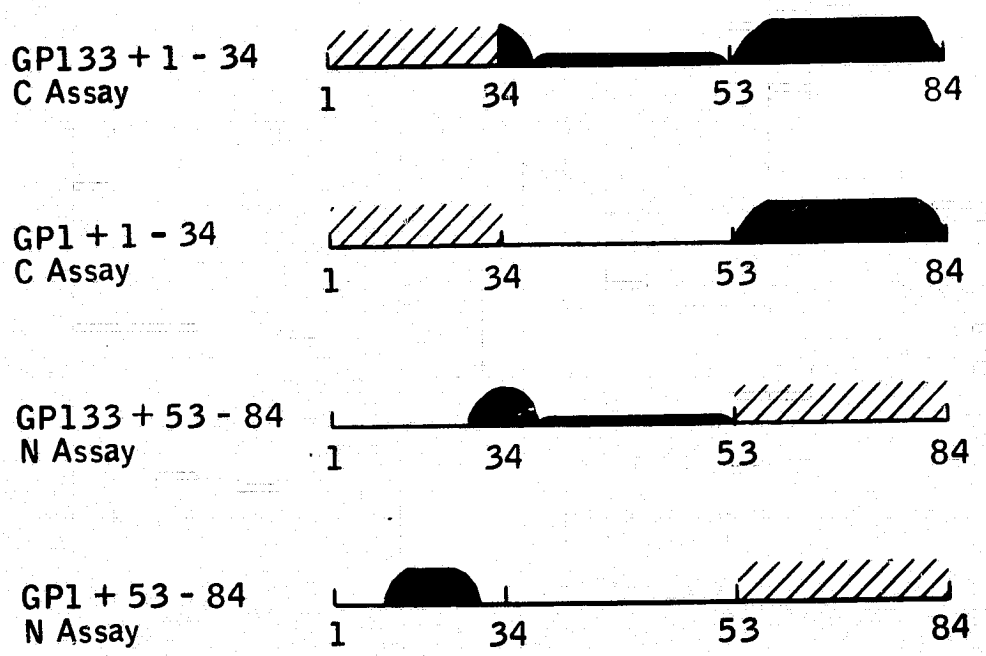

Figure 9-4.- The antigenic recognition sites of two antisera (GP-133 and GP-1) after each has been blocked by addition of excess concentrations of either bovine 1-34 or 53-84 fragment. The region of the molecule blocked by addition of the fragment is indicated by the hatched area and the recognition sites by the heavily shaded areas.

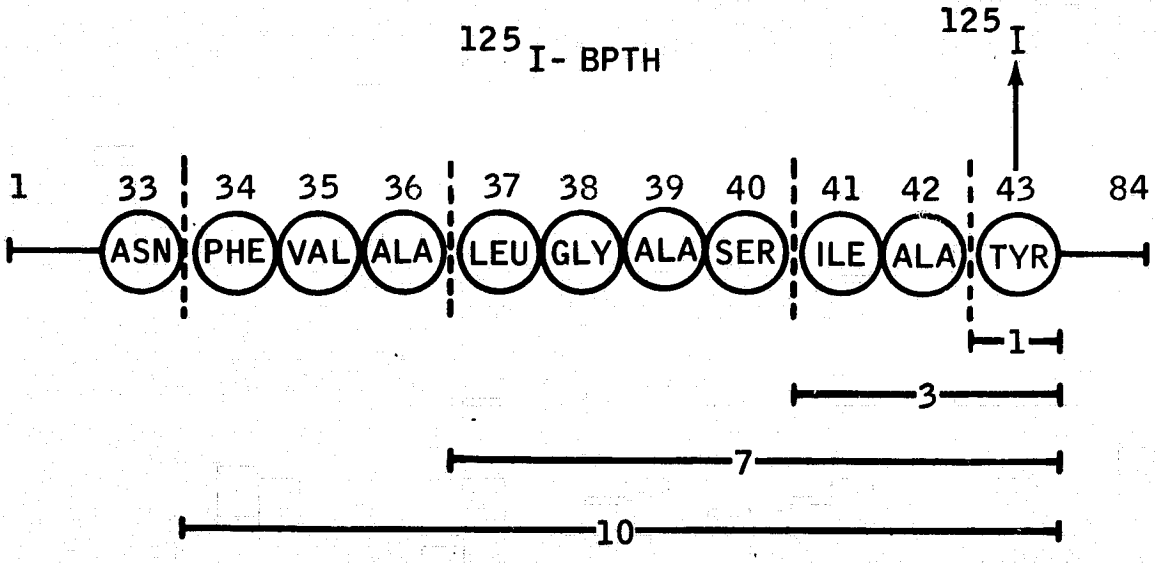

Figure 9-5.- Rationale for sequence analysis of radioiodinated bovine PTH (BPTH) and its fragments. The number of cycles equal to the cleavage site is 43.

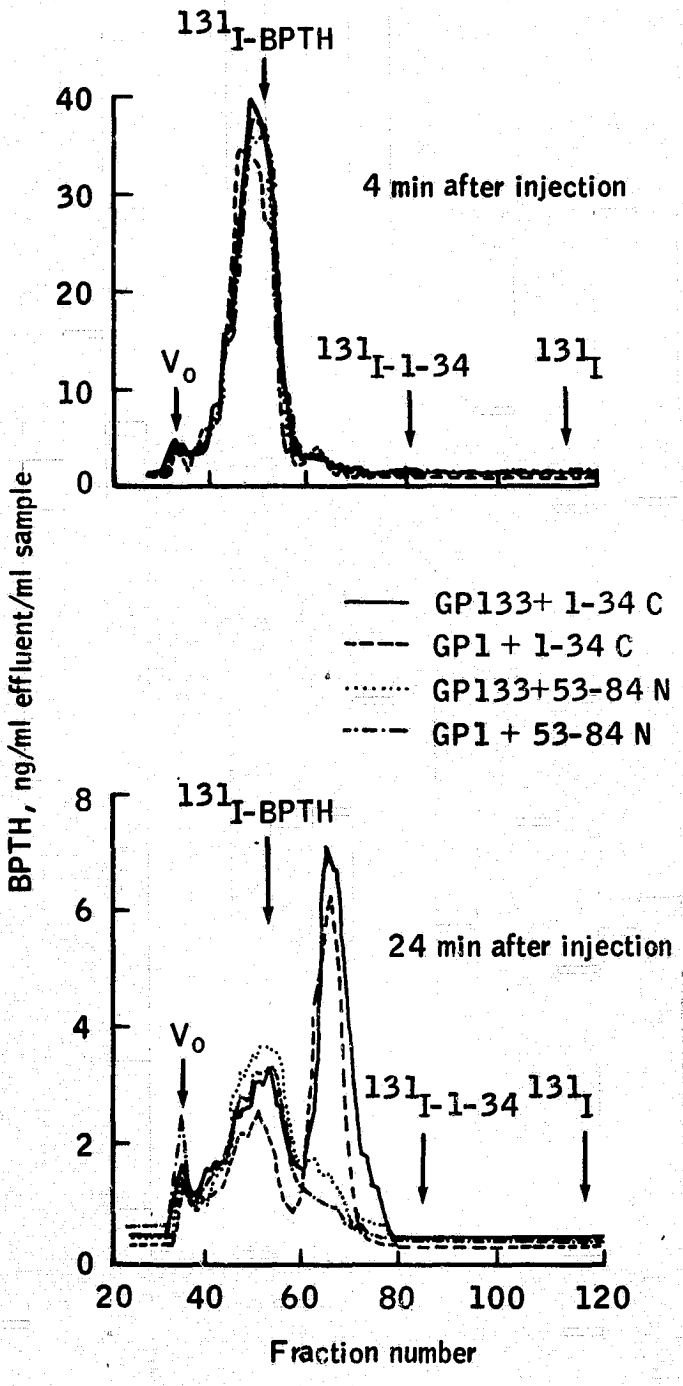

Figure 9-6.- Gel filtration profiles of immunoreactive hormone BPTH in samples taken 4 and 24 minutes after intravenous injection into a dog. Samples were chromatographed on Bio-Gel P-100. Each fraction was assayed in four different sequence-specific radioimmunoassay systems. The elution position of void volume (V_o), intact hormone, bovine hormonal fragment 1-34, and ^{131}I are indicated by the arrows.

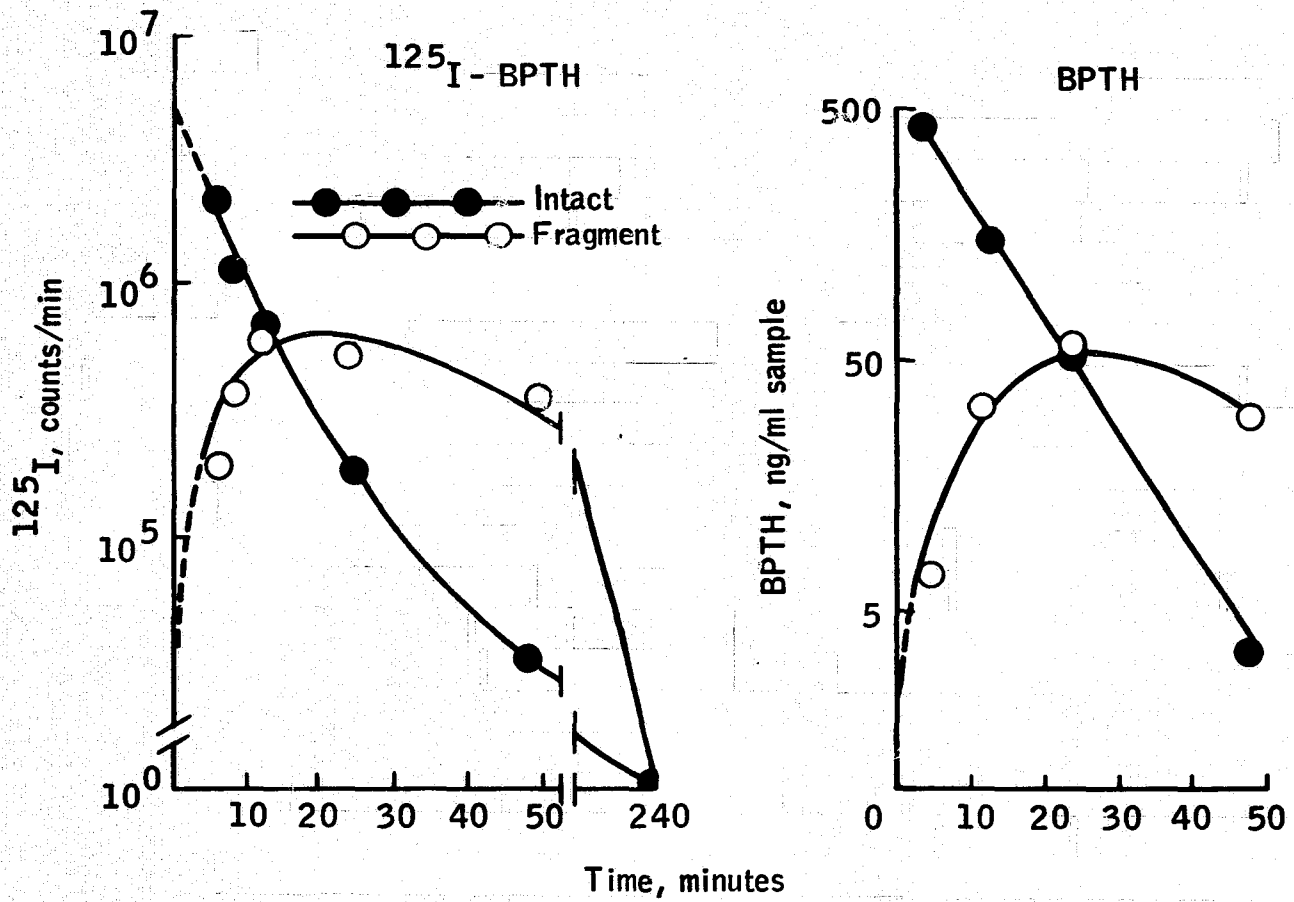

Figure 9-7.--Disappearance curve of intact hormone and appearance and disappearance of hormonal fragment after intravenous injection of BPTH-^{125}I and unlabeled BPTH into dogs.

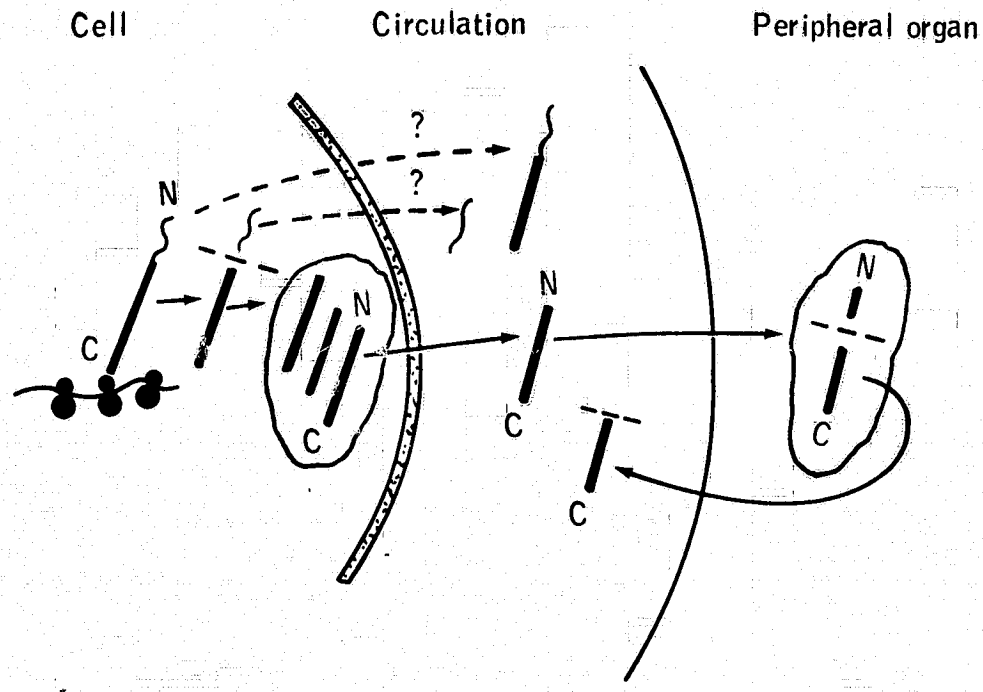

Figure 9-8.- Schematic summary of present knowledge and views concerning the biosynthesis, secretions, and metabolism of parathyroid hormone and the origins of the heterogeneity (known or suspected) for immunoreactive parathyroid hormone in blood. In addition to the release of intact hormone from the parathyroid cell, there may be release of prohormone or the prohormone peptide after cleavage from the precursor (?). Uptake of hormone after secretion occurs in peripheral organs (perhaps target organs) followed by a second cleavage; the larger fragment (C) reenters the circulation but the fate of the smaller fragment (N), which may be biologically active (and may be released adjacent to receptors), is at present uncertain (it may not reenter the circulation in significant amounts, if at all).

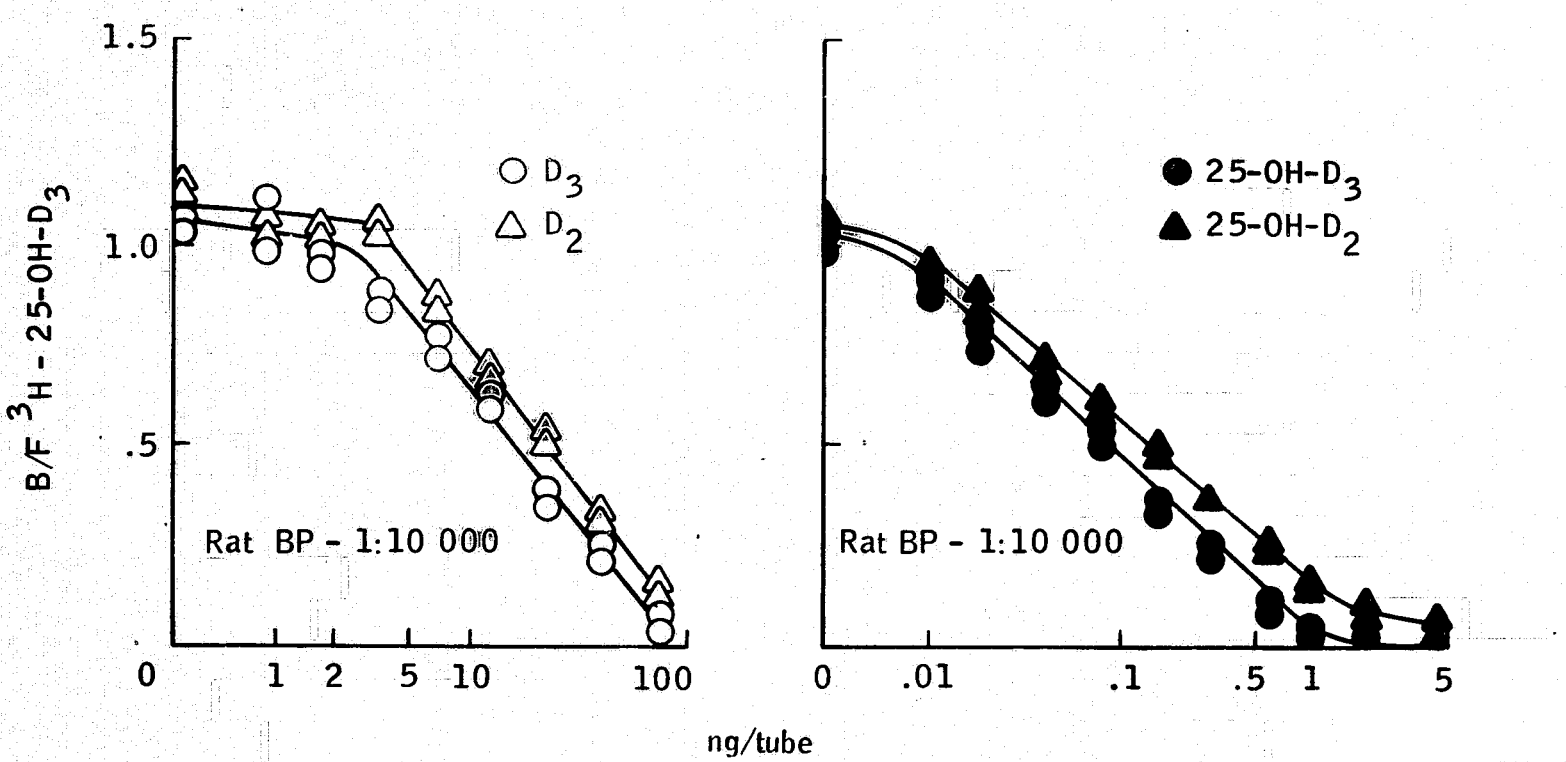

Figure 9-9.- Displacement of ^{3}H-25-OH-vitamin D$_3$ from D-deficient rat plasma with vitamin D analogs. Plasma obtained from D-deficient rats was cleared of nonspecific binding proteins and diluted with barbital acetate buffer, pH 8.6. Increasing amounts of the analogue to be tested with a fixed amount of ^{3}H-25-OH-D$_3$, all in 95 percent ethanol, were added to the diluted binding protein (B) or to buffer only (D). An additional aliquot of the analogue with the ^{3}H-25-OH-D$_3$ was added to a counting vial for determination of the counts/min added to each tube (T-total counts/min). Separation of unbound ^{3}H-25-OH-D$_3$ in both B and D tubes was accomplished with Dextran-coated charcoal (0.25 percent Norit A Charcoal and 0.025 percent Dextran T80). Specific binding was determined by subtracting counts/min in the supernatant in the absence of binding protein (D) from the counts/min with the binding protein present (B) and the bound/free (B/F) ratio calculated: B/F = B--D/T--(B--D).

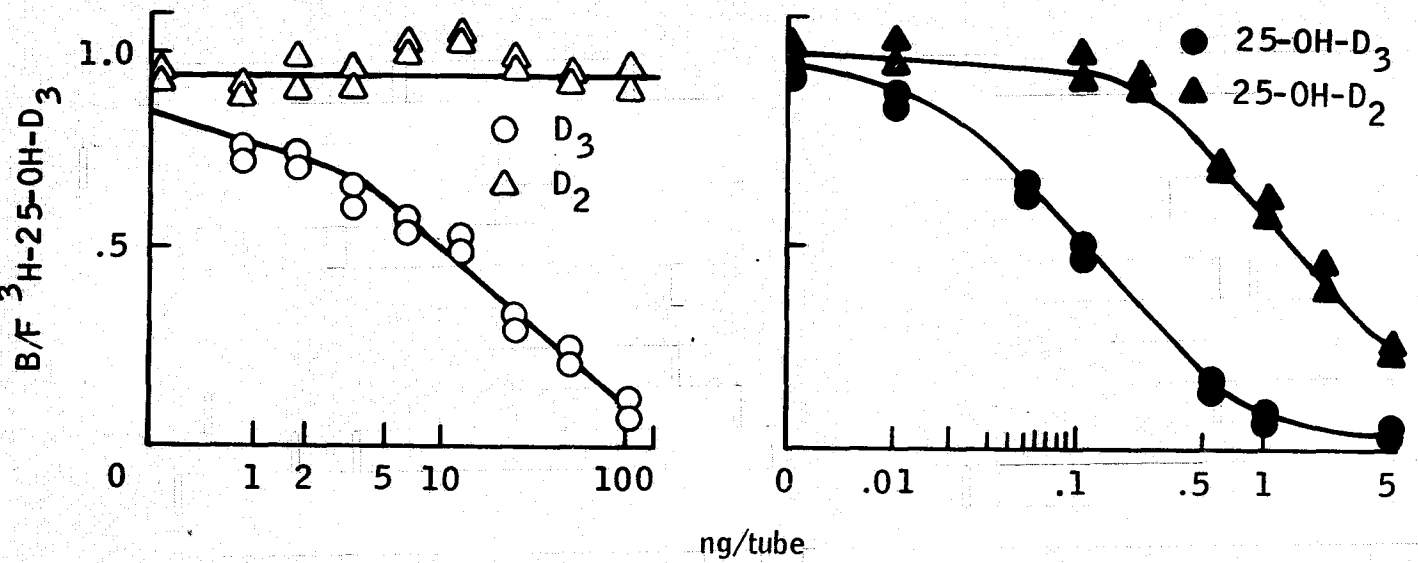

Figure 9-10.- Displacement of ^{3}H-25-OH-vitamin D$_3$ from D-deficient chick plasma with vitamin D analogs. The determination of B/F ratios for comparing binding of D analogs was accomplished by the procedure outlined in fig. 9-9, except that chick plasma was used as a source of the specific D binding protein.

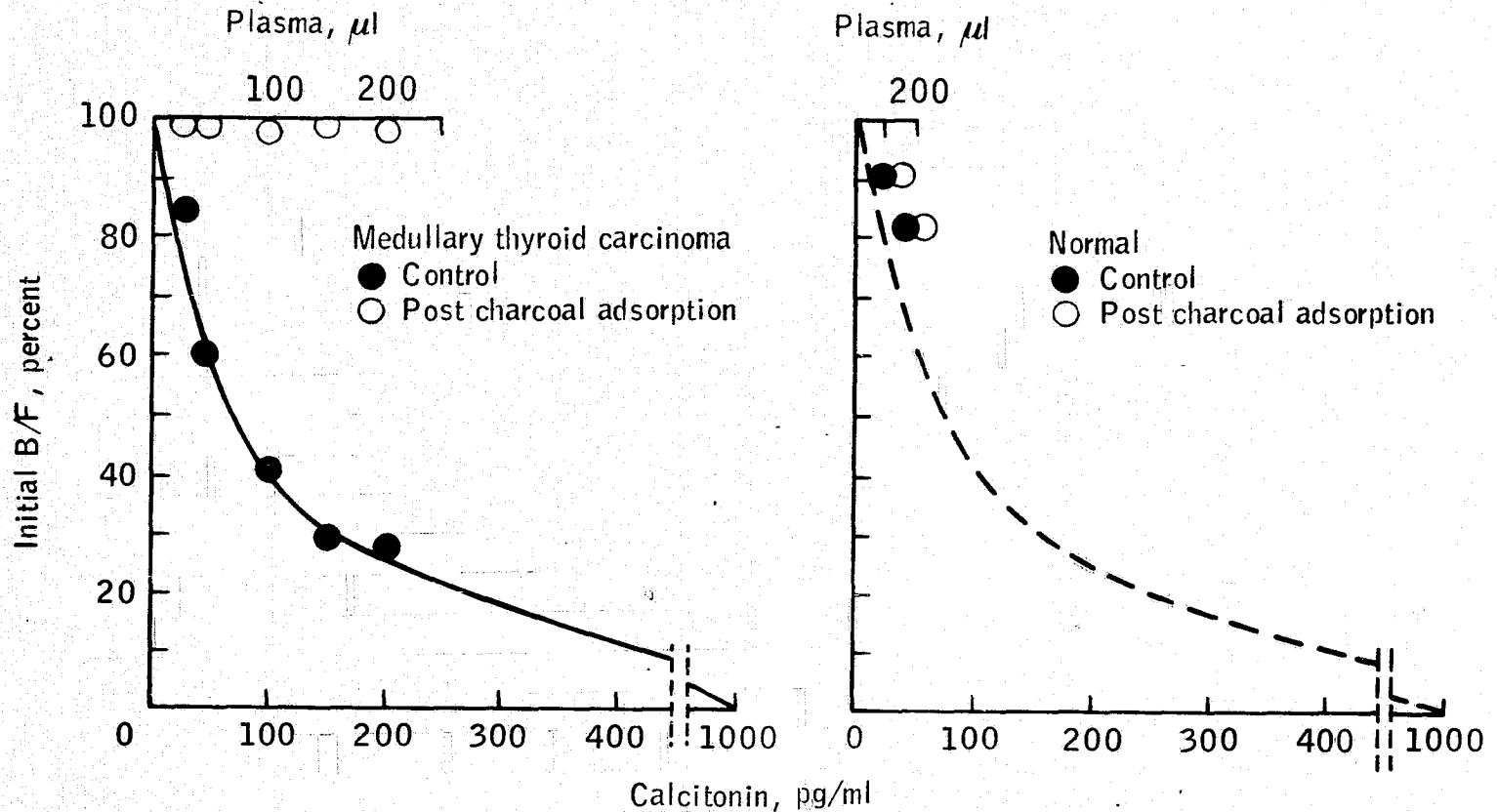

Figure 9-11.- Effect of charcoal adsorption on immunoassayable calcitonin concentration in plasma sample from patient with medullary thyroid carcinoma and on apparent immunoassayable calcitonin concentration in plasma sample from normal adult. The solid line represents the standard curve of tracer displacement produced by human calcitonin assay standard. Charcoal adsorption removes all calcitonin activity from medullary thyroid carcinoma plasma sample and eradicates progressive displacement of tracer from antibody produced by increasing aliquots of this plasma sample before charcoal adsorption. Charcoal adsorption has no significant effect on displacement of tracer produced by aliquots of plasma from normal adult. Because displacement of tracer by normal plasma is not eradicated by charcoal adsorption, it must be concluded that the sample does not contain detectable authentic human calcitonin.

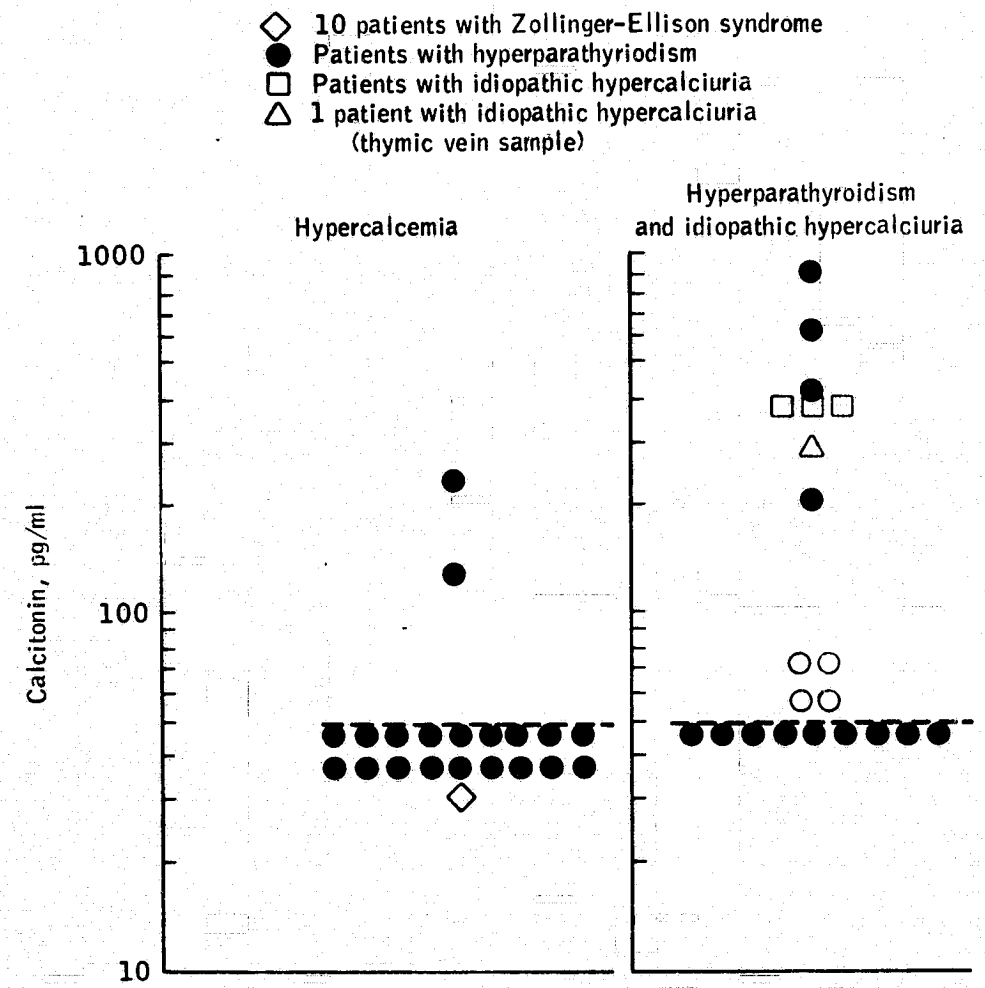

Figure 9-12.- Calcitonin concentration in peripheral plasma samples from patients with hypercalcemia compared to hormone concentration in thyroid vein samples from patients with hyperparathyroidism and idiopathic hypercalciuria and in a thymic vein sample from a patient with idiopathic hypercalciuria. The dashed line represents upper limits of detection; open circle values are too close to detection limits for reliable estimation of concentration.

10. ENDOCRINE CONSIDERATIONS IN THE RED-CELL-MASS AND PLASMA-VOLUME CHANGES OF THE SKYLAB 2 AND 3 CREWS

By Philip C. Johnson, M.D., Carolyn S. Leach, Ph. D., and Theda Driscoll

INTRODUCTION

Decreased red cell mass has been found regularly among crewmembers who return from space flight. This observation was first documented in the crew of the 8-day Gemini V mission and confirmed in the crewmembers of the 14-day Gemini VII mission. This finding was generally not predicted before these missions, since it was not found in ground-based studies designed to simulate weightless flight (ref. 10-1). Studies after four Apollo Moon-landing missions and the first two manned Skylab missions showed a mean decrease in red cell mass of approximately 10 percent. It has been tempting to explain the decrease in red cell mass of Gemini and Apollo crewmen as being due to the effects of hyperoxia. However, the Skylab missions, unlike the Apollo missions, did not have hyperoxic environments; yet, proportionately similar decreases in red cell mass were found. Therefore, hyperoxia cannot be the only cause of the decrease in red cell mass, and other possibilities must necessarily be considered (refs. 10-2 and 10-3).

Accompanying the decreases in red cell mass have been changes in plasma volume. Unlike the red-cell-mass deficit, plasma-volume changes were predicted even before the Mercury flights because plasma-volume decreases had been found consistently in subjects studied during and at the termination of prolonged bed rest (ref. 10-1). It is generally thought that plasma-volume decreases result from a redistribution of the labile portion of the blood volume, which is no longer needed in a weightless environment as circulating blood is freed from the effects of gravity.

Gemini IV crewmembers showed a decreased plasma volume on their return. A mean decrease in plasma volume was measured after an additional Gemini, seven Apollo, and two Skylab missions. One exception to this rule was the crew of Gemini VII (the longest space flight before the Skylab missions), who showed an increase in plasma volume at recovery (ref. 10-3).

Because blood-volume changes are found regularly after each mission, one might expect endocrine responses to the decrease in circulating blood volume when the crewmembers return and experience the effects of gravity. What is unknown is whether these changes in blood volume elicit an endocrine response

during weightlessness. Changes in endocrine function have been shown to occur during weightless flight, and these endocrine changes may have increased, decreased, moderated, or even caused the blood-volume changes. The purpose of this report is to point out how the unknown endocrine changes may have affected the blood volume of the crewmembers. From this preliminary analysis, plans can be made to study this relationship during future missions.

RESULTS AND DISCUSSION

Table 10-I shows the mean percent change in the plasma volume and red cell mass of the returning crewmembers. Except for the Gemini VII crew, all values are negative and considerably different from the controls. The increased plasma volume of the control subjects is believed to be a reflection of the increased environmental temperature that was present in the week before each recovery. The control subjects were aboard sparsely air-conditioned carriers in the tropical waters before the recovery process. The only cold environment occurred during the days before the Skylab 3 recovery. It is therefore not unexpected that the plasma volume of the control subjects for the Skylab 3 mission showed a decrease.

Table 10-II shows the effect of increased hormone levels on plasma volume and red cell mass, and indicates that each hormone has its own effect on circulating blood volume. Levels of antidiuretic hormone (ADH) have been measured in selected Apollo and Skylab missions (refs. 10-4 and 10-5). Generally, ADH activity is lower and osmolality is higher during the flights than in the preflight specimens. Any decrease in ADH should cause a decrease in plasma volume. Dilute urine has never been excreted during space flight, even though adequate water is available for the crewmembers. Characteristically, in-flight urine osmolality is greater than preflight. The Russians use a provocative water-loading test postrecovery. They have found a delayed water diuresis that they interpret as evidence for a continuing negative water balance in the flight (ref. 10-6). Our crews have consistently produced increased urinary ADH levels postrecovery, helping to account for the Russian results (ref. 10-4). The ADH changes are not known to be produced during prolonged bed rest, except during the first 2 days.

Human growth hormone (HGH) levels have been measured during the Skylab missions only (ref. 10-7). The HGH levels were increased on the first flight day and during the first few days after recovery. While prolonged elevations of HGH would be expected to change the blood volume, the short duration of the Skylab increases would not appreciably change the blood volume.

Parathormone and thyrocalcitonin levels have not been found to be increased during or after space flight (refs. 10-4 and 10-7). However, calcium losses do occur, and both calcium and phosphorus tend to be higher in the plasma and urine during the mission than they are premission (refs. 10-5 and 10-7). An increase in serum phosphorus would increase 2,3-diphosphoglycerate and might, in that way, inhibit bone marrow function. This increase could help explain the lowered red cell mass found postmission. Elevated plasma

calcium is often associated with anemia due to early loss of red cells from the circulation. A calcium diuresis resulting from the release of osseous calcium would decrease plasma volume.

Thyroid blood levels were increased in the returning Apollo crewmembers (ref. 10-8). This increase has been observed in Skylab crewmembers when thyroid-stimulating hormone levels were measured also (ref. 10-7). An increase in thyroid function does not occur during bed rest. The changes in thyroid function could have moderated both the plasma-volume and red-cell-mass decreases, because thyrotoxicosis causes increases in both compartments (ref. 10-9).

In diabetics, insulin helps correct the plasma-volume decreases produced by glycosuria. It would not cause changes in subjects without glycosuria. A relative glucose intolerance occurs during bed rest, but insulin levels are unchanged (ref. 10-10). This characteristic contrasts with space flight, during which insulin levels are decreased (ref. 10-7). In the absence of glycosuria, decreased insulin levels would not be expected to change the plasma volume or the red cell mass of crewmembers.

Plasma cortisol was elevated during the Skylab missions (ref. 10-7). Free cortisol excretion was elevated in the urine of the Skylab 2 and 3 crewmembers (ref. 10-5). But 17-hydroxysteroid excretion was decreased in the urine of the Gemini VII and Skylab crewmembers during the missions (refs. 10-5 and 10-11). Increased cortisol excretion was noted on stressful mission days during the Apollo 17 mission (ref. 10-12). Increased cortisol would tend to increase the plasma volume and, to a lesser extent, the red cell mass. However, a change in blood volume would not be expected if tissue levels of this hormone were not actually increased, because the increased urinary cortisol and decreased 17-hydroxysteroid excretion resulted in no net increase in plasma cortisol activity.

There is considerable evidence that aldosterone excretion is increased during a prolonged mission. Increases occurred during the Apollo 15 mission, the second flight week of the Apollo 17 mission, and the Gemini VII mission (refs. 10-11 to 10-13). Increased aldosterone excretion by the crewmembers has been found during the Skylab missions (ref. 10-5). Surprisingly, plasma aldosterone was elevated only during the first 28 days of Skylab 3 (ref. 10-7). Both total-body exchangeable potassium and serum potassium tend to be decreased at the end of a space flight (refs. 10-4 and 10-13). Increased aldosterone activity should increase plasma volume and may be responsible for the increased plasma volume noted in the returning crewmembers of the Gemini VII mission (ref. 10-1). Usually, plasma volume did not increase above preflight levels. This fact suggests that the increased aldosterone excretion is secondary to a prior loss of plasma volume, because adequate or increased aldosterone excretion ordinarily would prevent a decrease in plasma volume unless salt and water intake was inadequate, renal function was impaired, or cortisol excretion was reduced, none of which was likely in the Skylab crewmembers. The increased aldosterone excretion might be a response to the space-flight-induced decrease in ADH. Aldosterone increases are not found in bed-rest subjects until after the end of the bed-rest period (ref. 10-14).

There is no evidence of a change in testosterone levels. However, 17-ketosteroid excretion increased during the Skylab flights (ref. 10-7). Some of the adrenal androgens have a profound effect on red cell mass and may have increased the red cell mass; others have less effect. Fractionation of the 17-ketosteroids will help determine if the excreted steroids might have affected the red cell mass.

Epinephrine and norepinephrine have been measured in the postflight urines of returning Apollo and Skylab crewmembers, and elevated levels have been found (ref. 10-4). In-flight urine of the Skylab crews showed that both norepinephrine and epinephrine decreased (ref. 10-7). An in-flight decrease in catecholamine levels should have increased plasma volume.

The changes in hormone levels noted during examination of plasma and urine of Skylab and Apollo crewmembers may have affected both the plasma-volume and red-cell-mass changes caused by weightless flight. With the exception of the ADH changes, the endocrine changes would tend to increase plasma volume or at least to impede a decrease in plasma volume. In the past, it has been noted that the decreases in plasma volume during space flight have been smaller than the decreases from similar periods of bed rest (ref. 10-1). The differences in aldosterone excretion might account for the smaller decreases after space flight, because this hormone is not excreted in increased amounts during bed rest (ref. 10-14). However, differences in other hormones (for example, ADH) might be involved also. This is true also of red-cell-mass decreases, which may have been moderated by changes in endocrine function. The increase in both thyroid hormone and cortisol levels could stimulate the bone marrow to increase the production of red cells. This effect would be counteracted by the increased plasma-calcium and plasma-phosphorus levels.

Table 10-III summarizes the effects that endocrine changes may have had on the circulating blood volume of the Gemini, Apollo, and Skylab crewmembers.

CONCLUDING REMARKS

The answer to the question of whether the blood-volume changes produced the endocrine changes or whether the endocrine changes followed as a response to the in-flight blood-volume changes is still unknown. The fact that some of the changes were in the wrong direction suggests that changes in endocrine function were not the primary cause of the decreases in the plasma volume and red cell mass.

REFERENCES

10-1. Johnson, Philip C.; Fischer, Craig L.; and Leach, Carolyn S.: Hematologic Implications of Hypodynamic States. Sec. 2 of Hypogravic and Hypodynamic Environments. NASA SP-269, 1971.

10-2. Fischer, Craig L.; Johnson, Philip C.; and Berry, Charles A.: Red Blood Cell Mass and Plasma Volume Changes in Manned Space Flight. JAMA, vol. 200, no. 7, May 1967, pp. 579-583.

10-3. Johnson, Philip C.; Kimzey, S. L.; and Driscoll, T.: Post-Mission Plasma Volume and Red-cell-mass Changes in the Crews of the First Two Skylab Missions. Fifth International Symposium on Basic Environmental Problems of Man in Space, Washington, D.C., Nov. 27-30, 1973.

10-4. Leach, Carolyn S.; Alexander, W. Carter; and Johnson, Philip C.: Fluid/Electrolyte Changes in Apollo Astronauts. Paper presented at the 23rd Congress of the International Astronautical Federation (Vienna, Austria), Oct. 1972.

10-5. Leach, C. S.: Endocrine Responses and Long Duration Manned Spaceflight. Fifth International Symposium on Basic Environmental Problems of Man in Space, Washington, D.C., Nov. 27-30, 1973.

10-6. Balakhovskiy, I. S.; Grigor'ev, A. I.; Dlusskaya, I. G.; Kozyrevskaya, G. I.; et al.: Metabolism and Renal Function of Crewmembers of the Soyuz 6, Soyuz 7, and Soyuz 8 Spacecraft After Flight, Kosmicheskyaya Biologiya i Meditsina, vol. 5, Jan.-Feb., 1971, pp. 37-44.

10-7. Leach, Carolyn S.: Paper presented at the 1974 Lyndon B. Johnson Space Center Endocrine Program Conference (Houston, Tex.), 1974.

10-8. Sheinfeld, M.; Leach, Carolyn S.; and Johnson, Philip C.: Plasma Thyroxine Changes of the Apollo Crewmen. Aerospace Med. (In press).

10-9. Gibson, J. G.; and Harris, A. W.: Clinical Studies of the Blood Volume. V. Hyperthyroidism and Myxedema. J. Clin. Invest., vol. 18, 1939, p. 59.

10-10. Lipman, Richard L.; Schnure, Joel J.; Bradley, Edwin M.; and Lecocq, Frank R.: Impairment of Peripheral Glucose Utilization in Normal Subjects by Prolonged Bed Rest. J. Lab. Clin. Med., vol. 76, no. 2, Aug. 1970, pp. 221-230.

10-11. Lutwak, Leo; Whedon, G. Donald; Lachance, Paul A.; Reid, Jeanne M.; and Lipscomb, Harry S.: Mineral, Electrolyte and Nitrogen Balance Studies of the Gemini VII Fourteen-Day Orbital Space Flight. J. Clin. Endocrin. Metab., vol. 29, no. 9, Sept. 1969, pp. 1140-1156.

10-12. Leach, Carolyn S.; Rambaut, Paul C.; and Johnson, Philip C.: Adrenocortical Responses of the Apollo 17 Crewmembers. Aerospace Med., vol. 45, May 1974, pp. 529-534.

10-13. Leach, Carolyn S.; Alexander, W. Carter; and Johnson, Philip C.: Adrenal and Pituitary Response of the Apollo 15 Crewmembers. J. Clin. Endocrin. Metab., vol. 35, no. 5, 1972, pp. 642-645.

10-14. Hyatt, Kenneth H.; Smith, William M.; Vogel, John M.; Sullivan, Robert W.; et al.: A Study of the Role of Extravascular Dehydration in the Production of Cardiovascular Deconditioning by Simulated Weightlessness (Bedrest). Part I, NASA CR-114808, 1970.

10-12. Leach, Carolyn S.; Rambaut, Paul C.; and Johnson, Philip C.: Adrenocortical Responses of the Apollo 17 Crewmembers. Aerospace Med., vol. 45, May 1974, pp. 529-534.

10-13. Leach, Carolyn S.; Alexander, W. Carter; and Johnson, Philip C.: Adrenal and Pituitary Response of the Apollo 15 Crewmembers. J. Clin. Endocrin. Metab., vol. 35, no. 5, 1972, pp. 642-645.

10-14. Hyatt, Kenneth H.; Smith, William M.; Vogel, John M.; Sullivan, Robert W.; et al.: A Study of the Role of Extravascular Dehydration in the Production of Cardiovascular Deconditioning by Simulated Weightlessness (Bedrest). Part I, NASA CR-114808, 1970.