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MANNED SPACECRAFT CENTER

ENDOCRINE PROGRAM CONFERENCE

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16. Abstract

This document contains the proceedings of the 1971 Manned Spacecraft Center Endocrine Program Conference. Subjects covered include: endocrine studies on Apollo 14 and 15 crewmen; the relationships between medical aspects of space flight and nuclear medicine; excretion and secretion of vasopressin during space flight; adrenocorticotrophic hormone levels; the renin-aldosterone system and sodium homeostasis during simulated weightlessness; measurement methods for plasma aldosterone and catecholamine concentrations; parathyroid hormone, calcitonin, and vitamin D; dissociation effects of prolonged confinement (heart rate, body temperature, cortisol, insulin, thyroxine, and triiodothyronine); and nutrition and musculoskeletal function experiments (series M070) for the Skylab Program.

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PROCEEDINGS OF THE 1971

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ENDOCRINE PROGRAM CONFERENCE

INTRODUCTION

By Carolyn S. Leach, Ph. D.

In October, 1970, the first conference on endocrine data collected in support of manned space flight was conducted at the NASA Manned Spacecraft Center, Houston, Texas. As a part of the continuing effort to fully understand and to evaluate the endocrine changes observed during previous manned missions and, thereby, to further understanding of the physiological adaptation of man to the space-flight environment, the Second Annual NASA Manned Spacecraft Center Endocrine Program Conference was conducted in December, 1971. 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 Manned Spacecraft 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

The attendees of this Second Annual NASA Manned Spacecraft 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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APOLLO MISSIONS 14 and 15

By Carolyn S. Leach, Ph. D.

INTRODUCTION

During the year since the last Manned Spacecraft Center endocrine conference, the endocrine laboratory has continued its major role in support of the NASA manned space flights. Because all of the flight data through Apollo 13 were reviewed at the conference last year (ref. 1), this report will discuss the two missions (Apollo 14 and 15) that have occurred in the interim. Although both missions were lunar landing missions of approximately the same duration and both involved three crewmen, there were fundamental differences in the mission parameters (table I.). It is still an open question as to whether these differences influenced the mission medical results; however, there is no doubt that the Apollo 15 crewmen reacted to space flight differently than did the crewmen of previous missions.·

Both Apollo 14 and 15 were successful in their basic objectives of lunar exploration and physical science research experiments (refs. 2 and 3). The biomedical information is obtained for the purpose of ensuring crewmember safety and for obtaining information on the biomedical effects of space flight. The medical results from these two flights were more detailed than the data obtained on any previous Apollo flights, facilitating a more complete interpretation of the physiological effects of space flight.

The endocrine laboratory participated in the medical examination of the crewmen by the collection of biological samples and their analyses in order to determine the effects of space flight on the hormonal compounds that regulate endocrine/metabolic homeostasis.

METHODS

Analyses were performed on 45 cubic centimeters of venous blood that was drawn 27 (F-27), 15, and 5 days before the missions in order to ascertain the health status of the crewmen and to establish baseline values for postmission comparison. Comparable amounts of blood were drawn as

soon as possible (ASAP) after recovery, 1 day (17 hours), 6 days, and 16 days (+13 on Apollo 15) after recovery on both Apollo 14 and 15; in addition, blood was drawn on the second day after recovery on Apollo 15. All blood samples were obtained while the crewmembers were fasting, except for those samples drawn immediately following splashdown (crewmen had not eaten during the preceding 4 hours).

Beginning on day 27 preflight, 24-hour urine samples were collected from each crewman on the same day that blood was drawn. Twenty-four-hour urine samples were collected 1, 2, 3, and 6 days postflight. These samples were aliquoted, stabilized, and frozen for later analysis.

Because of the need for crewmen health stabilization, the crewmen for both Apollo 14 and 15 were confined to the NASA John F. Kennedy Space Center crew quarters for health stabilization for the 21 days immediately preceding the missions and the Apollo 14 crew, in addition, underwent a 21-day quarantine postflight.

During the pre- and postflight periods, the crewmen consumed the diet of their choosing but inflight adhered to the provided Apollo diet. Sodium intake averaged 5 grams per day and potassium intake averaged 3 grams per day. Fluids were available ad libitum.

Analyses on the blood (plasma or serum) samples included hydrocortisone, triiodothyronine (T_3), thyroxine (T_4), angiotensin I, insulin, parathormone, sodium, potassium, chloride, osmolality, and others. In addition, adrenocorticotrophic hormone (ACTH) was measured in the samples from the Apollo 15 crewmen.

The 24-hour urine samples were analyzed for hydrocortisone, epinephrine, norepinephrine, antidiuretic hormone (ADH), aldosterone, sodium, potassium, chloride, and osmolality. In addition, the samples from the Apollo 15 crewmen were analyzed for 17-ketosteroid and titratable acidity.

Results and Discussion: Apollo 14

In the assessment of the endocrine/fluid electrolyte status of the Apollo 14 crewmen, the two areas emphasized were endocrine-metabolic cost and endocrine control of fluid balance. The plasma/serum results from Apollo 14 are given in table II, and the 24-hour urine results are given in table III. The results from Apollo 14 were similar to those results from the previous Apollo missions and further support the previously proposed hypothesis regarding endocrine/fluid electrolyte responses both to space flight and to return to 1-g (ref. 4). However, several points deserve specific mention.

During the preflight phase, all three crewmen were affected by a stress of undetermined origin that was sufficient to cause a catecholamine and hydrocortisone response. This was significant because these hormones are responsible for many metabolic processes. Consequently, these elevated hormone levels necessitated an interpretation of the preflight medical data, with consideration given to these abnormal values.

Immediately postflight, there was a decrease in plasma hydrocortisone similar to that noted in previous missions. Several explanations have been proposed for this phenomenon. Among these are a reduction in adrenal glucocorticoid activity induced by weightlessness or, more likely, changes in metabolism of the steroids that were caused by alteration in liver blood flow. Plans for future Apollo missions include additional urine and plasma assays for specific compounds that will help resolve this problem. However, no definitive information will be available until inflight urine samples collected during Skylab missions are returned for analysis.

The analysis conducted to ascertain metabolic response to the conditions of space flight included T_3, T_4, and insulin. Although it was not possible to analyze for these hormones at each sample period, it is evident that no significant changes occurred when the postflight results are compared to the preflight results. Furthermore, insulin concentration did not differ significantly in the comparison of the ASAP results to the preflight values.

Parathormone was considered in relation to calcium and phosphorus metabolism. The concentration of this hormone was not significantly different during the postflight period, although there appeared to be a decreasing concentration trend.

In determinations of the fluid/electrolyte status of the three crewmen, it was noted that the lunar module pilot (LMP) and command module pilot (CMP) showed changes of greater magnitude than the commander (CDR). From the graphs depicting these data, it can be observed that the LMP and CMP showed a decrease in 24-hour urine volume postflight (fig. 1). This finding, in conjunction with the determinations for the electrolytes sodium (Na), potassium (K), and cloride (Cl) and with the endocrine results, suggest a retention in fluids and electrolytes (fig. 2). Although the CDR showed a slight general increase in aldosterone, he reacted to a much lesser degree than the other two crewmen (fig. 3). The increase in aldosterone is suggestive of renal tubular activity to maintain sodium homeostasis. Angiotensin I, a measure of renin activity, was elevated significantly only in the CMP postflight (fig. 4).

In summary, two distinct patterns of response to space flight can be observed in the crewmen of Apollo 14. Although the CMP was

considerably more reactive than either of the other two crewmen, both he and the LMP were characterized by significant changes in all relevant parameters measured. The CDR seemed nonreactive to the environmental and physiological stresses associated with space flight and essentially remained in a compensated state. The marked changes of the CMP postflight that were made in an attempt to adapt to the absence of gravity or reintroduction to earth gravity, were reflective of increased homeostatic activity. These data support further the hypothesis of this laboratory that the physiological cost of adaptation may, in some instances, exceed the available reserves, thereby progressing to a state that becomes clinically important.

Results and Discussion: Apollo 15

The results of the plasma analyses from crewmen of Apollo 15 are shown in table IV, and the 24-hour urine results are given in table V. Plasma hydrocortisone was elevated on F-5 in the CDR and LMP; however, as found in previous missions, the immediate postflight concentration appeared to be decreased in two crewmen and not changed in the third. ACTH was measured, and the findings ranged from normal to decreased for the three crewmen immediately postflight. The 24-hour urine results showed elevations in hydrocortisone during the first few days postflight. These findings have been relatively constant for the Apollo flight (ref. 5) and are unexplained. It is thought that steroid metabolism may not be normal during space flight. This would account for the postflight changes if there was an immediate reaction to the effects of gravity by changes in blood flow to the adrenal, liver, and kidney.

The catecholamines demonstrated significant elevations in all three crewmen during the postflight period. These elevations occurred generally on the same days as those observed for hydrocortisone; the two different compounds (steroids and catecholamines) are indicative of both adrenal cortical response to stress and the reaction of the sympathetic nervous system to the condition imposed on the crewmen as part of the readaptation to the earth environment.

Insulin and T_3 were measured as indicators of metabolic activity before and after space flight. There were no significant changes in T_3 or in insulin in the CDR or CMP. Immediately after recovery, the LMP exhibited an elevated insulin level that returned to preflight values by the next sampling period. This is not thought to be related to food consumed by this crewman prior to recovery because the blood glucose was normal. It could have been a response to stress.

Parathormone analysis demonstrated no significant changes in the three crewmen. These results are consistent with other studies on these

men that demonstrated no change in calcium or phosphorus excretion post-
flight. Furthermore, there were no changes in bone mineral mass as
measured by photon absorptometry on this mission (ref. 6), indicating
no change in body composition.

The electrolyte and osmolality analyses were conducted by the
clinical laboratory in support of the operational medicine work as well
as for a specific fluid/electrolyte study. Serum sodium was unchanged
postflight in the CDR and LMP, but the CMP showed a slight decrease
postflight when compared to preflight values. Serum chloride remained
unchanged in all three crewmen. However, serum potassium was decreased
significantly immediately postflight in all three crewmen (figs. 5 and 6).

Twenty-four hour urine electrolyte data demonstrated a retention
of Na, K, and Cl postflight (fig. 7) that had volumes ranging from normal
to increased (fig. 8). The urine had increased titratable acidity and
the osmolality was decreased slightly; immediately postflight, urinary
antidiuretic hormone levels were normal to slightly elevated (fig. 9).

Renin activity was measured as angiotensin I in the blood samples
pre- and postflight. Although these postflight results did not differ
statistically from the preflight analysis, there is an apparent increase
postflight. This increase is substantiated by the significant postflight
increase in urinary aldosterone (fig. 10), which is thought to be con-
tributory to the decrease in serum K observed on this mission (fig. 6).

The hormonal fluid/electrolyte data discussed above give evidence
of inflight changes in body fluid and electrolyte balance initiated
by the null gravity environment. Redistribution of the circulating blood
volume, particularly from the lower extremities into the abdomen and
thorax, results in a negative water balance with a net loss of water and
electrolytes. Dietary intake of electrolytes cannot be ignored in con-
sideration of fluid/electrolyte loss because it has been variable;
however, the sodium and potassium intakes during this mission appear to
be on the lower side of normal daily intakes.

The loss of fluid/electrolytes is thought to occur early in space
flight, just as in bedrest, and depletes the plasma volume that is then
replenished by an aldosterone response. During flight, an increase in
aldosterone would account for the decrease in exchangeable potassium
measured during this mission.

CONCLUDING REMARKS

Endocrine/metabolic responses to space flight have been measured on the crewmen of Apollo missions 14 and 15. There were significant biochemical changes on the crewmen of both missions immediately postflight. However, the Apollo 15 mission results differed from Apollo 14 and previous Apollo missions by the apparent lack of fluid conservation postflight shown by a normal to increased urine volume with slight increases in antidiuretic hormone. The exact reasons for these findings are not certain but could be related to a potassium deficit at the renal tubular site.

Although Apollo 15 was the first mission in which the exchangeable potassium measurement was made (a decrease), results from other missions were indicative of similar conclusions.

More specific and definitive explanations of the results on these two missions are not possible. The return of urine and blood samples collected during the Skylab flights in 1973 will add the needed data for interpretation of the environmental changes imposed by space flight.

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TABLE 1-I.- MISSION PARAMETERS OF APOLLO 14 AND APOLLO 15

Parameter	Apollo 14	Apollo 15
Duration (hr:min)	217:03	296:45
Landing site	Fra Mauro Highlands	Hadley Rille
Time on lunar surface (hr:min)	34:11	66:55
Lunar EVA, number	2	3
Lunar EVA time, (hr)	9:09	19:09
Nonlunar EVA	No	Yes (38 min)
Postflight quarantine	Yes	No

TABLE 1-II.- APOLLO 14 BLOOD ENDOCRINE/ELECTROLYTE RESULTS

Subject	Sample period	Hydrocortisone, μg/100 cm³	T₃, percent uptake	T₄, μg/cm³	Angiotensin I, mU/cm³/hr	Insulin, mU/cm³	Parathormone, mμg/cm³	Na, meq/m³	K, meq/m³	Cl, meq/m³	Osmolality, mOsmo
CDR	F-27	12.0	32.2	8.2	0.86	1.0	0.25	142	4.1	106	289
	-15	16.8	28.5	10.0	1.05	<1.0	.39	141	4.0	105	292
	-5	17.8	NDᵃ	ND	.53	7.5	.33	141	4.4	107	294
	ASAP	9.2	30.8	7.6	.47	8.0	<.03	142	4.4	105	290
	R+1	12.8	29.6	7.5	.33	<1.0	ND	140	4.6	103	284
	+6	10.8	32.7	5.6	.65	10.0	.32	146	4.6	109	303
	+16	14.0	28.1	9.0	1.60	4.0	.32	146	4.4	108	305
LMP	F-27	17.6	34.2	5.4	3.06	7.0	.40	142	4.1	107	292
	-15	14.8	ND	7.5	1.43	<1.0	.24	143	4.6	108	294
	-5	22.4	ND	ND	1.02	<1.0	.33	140	4.1	107	285
	ASAP	12.2	30.4	6.9	2.43	2.0	.36	144	4.1	106	297
	R+1	19.6	ND	ND	3.10	2.0	ND	140	4.1	106	282
	+6	22.4	32.7	6.0	3.18	23.0	.30	145	4.1	109	299
	+16	20.2	29.2	5.6	3.55	2.0	.32	144	4.4	110	302
CMP	F-27	17.6	36.5	5.4	.56	<1.0	.49	143	4.0	107	295
	-15	18.4	36.5	7.0	.80	<1.0	.44	141	4.2	104	294
	-5	23.2	ND	7.1	3.60	1.0	.42	139	3.7	102	287
	ASAP	8.0	32.7	9.0	10.93	9.0	.39	142	3.8	104	292
	R+1	19.2	31.5	7.9	1.38	4.0	ND	140	4.6	103	284
	+6	10.4	36.5	5.8	.67	<1.0	.29	146	4.5	108	296
	+16	22.0	31.9	4.7	.48	15.0	.38	145	4.3	110	301

ᵃND = Not done.

TABLE 1-III.- APOLLO 14 URINE ENDOCRINE/ELECTROLYTE RESULTS

Subject	Sample period	Volume	Hydrocortisone, ug/tv	Catecholamines, ug/tv		ADH, mU/tv	Aldosterone, ug/tv	Na, meq/tv	K+, meq/tv	Cl, meq/tv	Osmolality, mOsmo
				Epinephrine	Norepinephrine						
CDR	F-27	1750	17.5	33.7	41.3	10.9	2.4	136	48	138	456
	-15	1600	105.5	25.4	68.3	13.9	7.0	192	80	157	356
	-5	1150	75.2	33.5	23.5	8.7	6.1	138	70	104	667
	ASAP	2250	103.9	45.9	64.1	27.9	16.2	209	85	179	611
	R+1	2325a	109.1	61.3	72.9	24.2	19.1	--	--	--	403
	+6	1845	62.4	27.6	64.2	10.3	14.5	193	54	142	568
	+16	1820	74.6	33.1	48.4	18.6	14.9	255	86	244	638
LMP	F-27	1800	16.2	13.5	48.4	.7	4.2	232	45	205	502
	-15	1870	93.5	19.5	36.7	7.2	10.0	157	62	142	482
	-5	870	71.3	20.3	14.2	1.1	12.1	107	60	110	857
	ASAP	1150	74.3	16.2	58.4	15.9	15.6	165	59	135	923
	R+1	1125	64.4	16.1	61.0	13.3	16.0	195	31	159	1004
	+6	1245	77.4	15.4	44.8	49.7	16.2	195	31	164	724
	+16	1202	99.0	11.0	40.8	36.6	11.9	164	53	151	803
CMP	F-27	1900	39.0	9.8	51.4	0	5.4	207	59	182	465
	-15	2440	100.0	22.0	31.2	0	4.0	303	137	300	537
	-5	1630	109.2	14.8	30.1	5.6	9.1	153	96	176	680
	ASAP	1200	121.0	28.8	41.1	95.4	15.5	96	50	82	832
	R+1	2775	86.0	14.8	52.9	23.7	31.0	131	51	141	458
	+6	1790	43.5	7.0	24.2	9.7	26.2	105	29	102	373
	+16	1600	39.2	3.1	55.2	15.6	12.7	206	51	194	503

aVolume for 6 hours assumed 500 cubic centimeters for calculations.

TABLE 1-IV.- APOLLO 15 BLOOD ENDOCRINE/ELECTROLYTE RESULTS

Subject	Sample period	Hydrocortisone, µg/100 cm³	T_3, percent uptake	Angiotensin I, mU/cm³/hr	Insulin, mU/cm³	Parathormone, ng/cm³	Na, meq/m³	K, meq/m³	Cl, meq/m³	Osmolality, mOsmo	ACTH, pg/cm³
CDR	F-27	12.0	33.1	0.50	<1.0	0.29	139	3.8	105	290	41
	-15	18.4	31.5	2.12	5.0	.27	140	4.1	102	291	45
	-5	29.0	ND[a]	2.14	6.0	.21	141	4.4	105	286	20
	ASAP	7.2	31.2	3.62	8.0	<.20	140	3.6	107	289	54
	RR+1	14.8	31.2	1.85	<1.0	<.20	138	4.6	103	293	44
	+2	23.6	32.3	1.35	<1.0	<.20	140	4.2	105	290	22
	+6	20.8	ND	1.00	5.0	.23	143	4.3	106	290	33
	+13	15.0	ND	.73	9.0	ND	141	4.0	108	ND	47
LMP	F-28	15.0	32.7	.79	<1.0	.27	139	4.2	106	289	48
	-15	14.4	30.4	.70	6.0	.38	140	4.5	107	293	52
	-5	20.8	ND	.40	4.0	.24	141	4.6	108	293	59
	ASAP	7.6	31.9	.97	18.0	.30	140	3.9	106	292	20
	R+1	10.4	31.2	5.30	8.0	<.20	137	4.1	103	291	41
	+2	14.8	ND	2.49	4.0	<.20	140	4.3	104	291	ND
	+6	34.4	ND	1.10	6.0	.34	144	4.4	108	294	50
	+13	12.0	ND	.62	5.0	ND	139	4.4	109	ND	39
CMP	F-28	12.4	31.5	.62	<1.0	.25	142	3.9	107	290	100
	-15	19.0	30.0	.90	14.0	.30	142	4.3	105	298	90
	-5	15.8	ND	4.00	4.0	.23	144	4.0	106	294	90
	ASAP	15.2	30.0	2.19	2.0	.38	139	3.9	106	292	32
	R+1	15.0	ND	1.72	6.0	.31	142	4.4	103	295	59
	+2	28.0	ND	8.20	<1.0	<.20	141	4.1	107	288	68
	+6	23.2	ND	3.90	4.0	<.20	144	3.9	106	293	72
	+13	15.0	ND	1.25	7.0	ND	141	4.0	107	ND	52

[a]ND = Not done.

TABLE 1-V.- APOLLO 15 URINE ENDOCRINE/ELECTROLYTE RESULTS

Subject	Sample period	Volume	Hydro-cortisone, µg/tv	Catecholamines, µg/tv		ADH, mU/tv	Aldo-sterone, µg/tv	Na, meq/tv	K, meq/tv	Cl, meq/tv	Osmo-lality, mOsmo	17-Keto, mg/tv	Titratable acidity
				Epineph-rine	Norepi-nephrine								
CDR	F-28	1005	75.5	15.9	74.3	26.3	11.6	108	65	91	739	9.0	---
	-15	1265	52.5	27.7	88.2	12.9	15.0	119	134	104	768	7.1	114
	-5	900	74.4	6.7	23.8	87.6	10.4	104	99	98	773	9.2	34
	ASAP	1520	136.8	16.4	85.2	57.8	25.2	150	49	129	783	8.1	473
	R+1	1640	85.3	31.5	67.9	79.6	24.8	146	47	136	624	9.7	256
	+2	1810	101.4	47.1	101.5	29.5	24.8	159	85	159	630	11.6	394
	+6	1420	53.3	17.3	53.7	37.8	15.0	136	43	105	596	9.2	350
	+13	1795	80.8	22.5	93.4		11.8	288	106	241	748	12.6	353
LMP	F-28	655	36.4	16.4	39.8	9.2	13.6	91	65	82	990	6.2	---
	-15	700	46.9	13.7	67.4	18.2	6.8	121	50	112	1010	7.9	278
	-5	1260	94.5	7.9	49.5	12.4	9.2	186	64	165	784	11.9	103
	ASAP	1195	86.0	8.6	33.5	24.4	14.4	105	35	75	679	7.6	229
	R+1	1140	84.4	41.4	39.3	30.3	12.4	88	55	87	799	14.4	394
	+2	1320	110.4	33.3	100.1	49.6	15.6	87	91	108	728	14.4	372
	+6	980	115.6	4.3	56.8	51.0	7.4	136	56	121	783	10.3	421
	+13	890	71.2	16.0	42.4	48.8	7.2	124	59	113	758	6.7	281
CMP	F-28	770	19.3	14.2	53.2	22.6	15.6	149	56	147	929	13.7	---
	-15	740	42.2	17.2	46.3	3.3	5.8	111	61	100	996	13.1	288
	-5	360	22.7	14.1	13.4	9.2	7.2	52	42	55	986	6.9	47
	ASAP	795	62.0	25.5	34.9	38.6	10.6	95	40	53	952	13.2	376
	R+1	1600	136.0	48.9	87.7	58.2	18.0	133	65	130	635	15.2	414
	+2	430	34.8		37.3	12.0	7.0	30	24	30	688	6.2	155
	+6	1480	185.0	60.0	90.2	16.6	22.4	188	87	35	716	30.3	423
	+13	1265	44.3	15.2	42.6	21.2	13.6	106	57	95	418	13.9	129
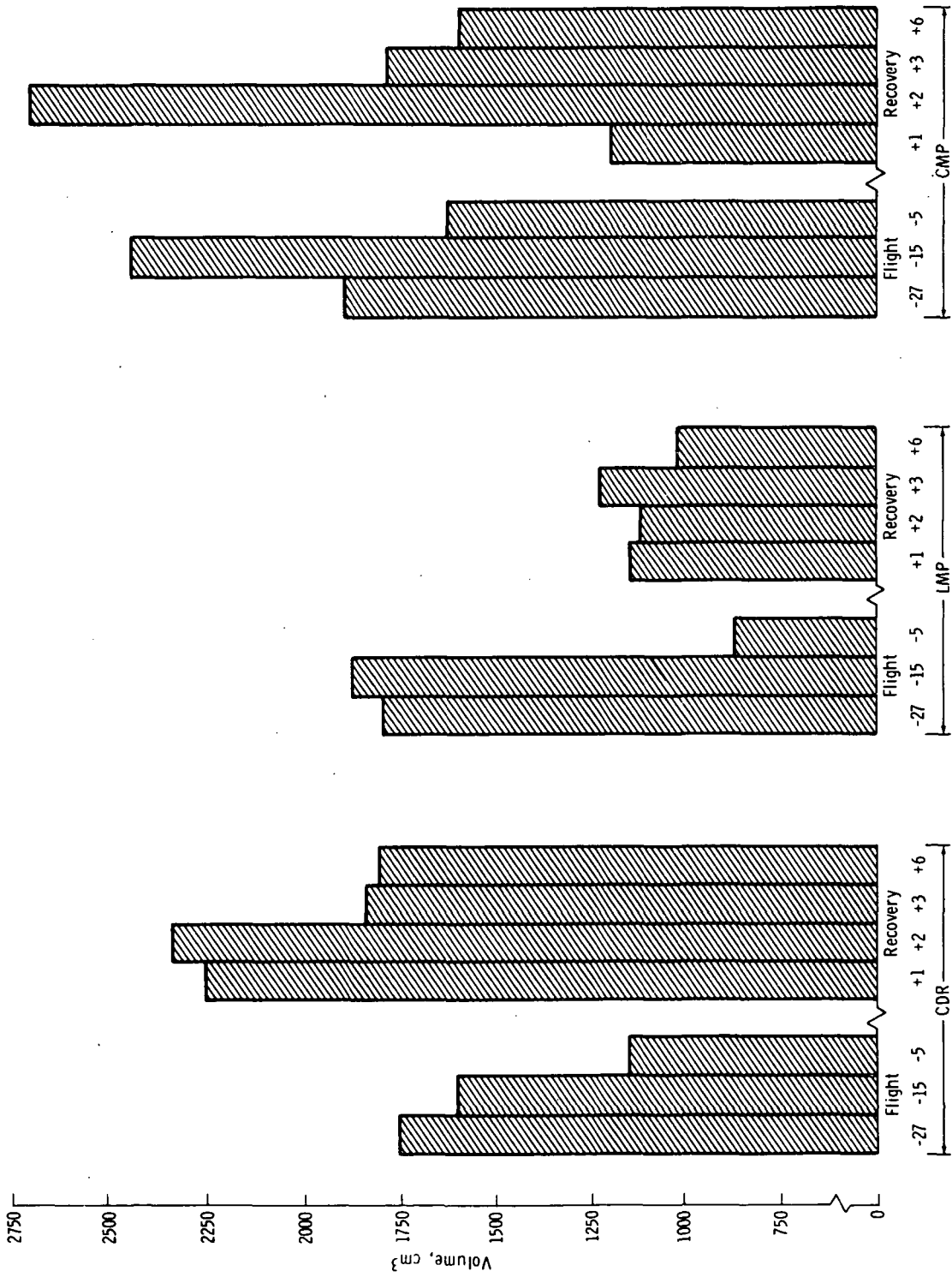
Figure 1-1.- Apollo 14 24-hour urine volume.
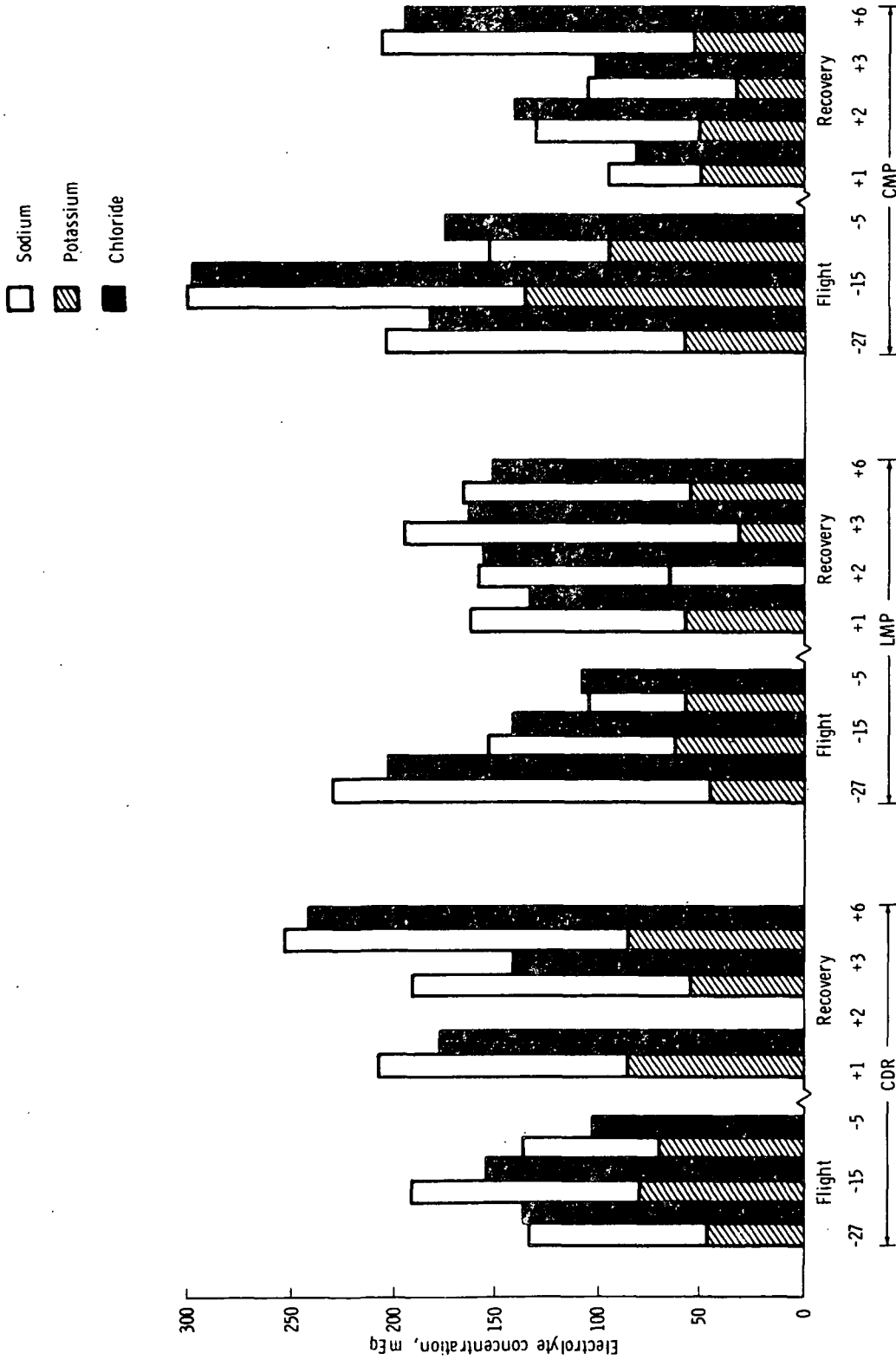
Figure 1-2.- Apollo 14 24-hour urinary electrolytes.
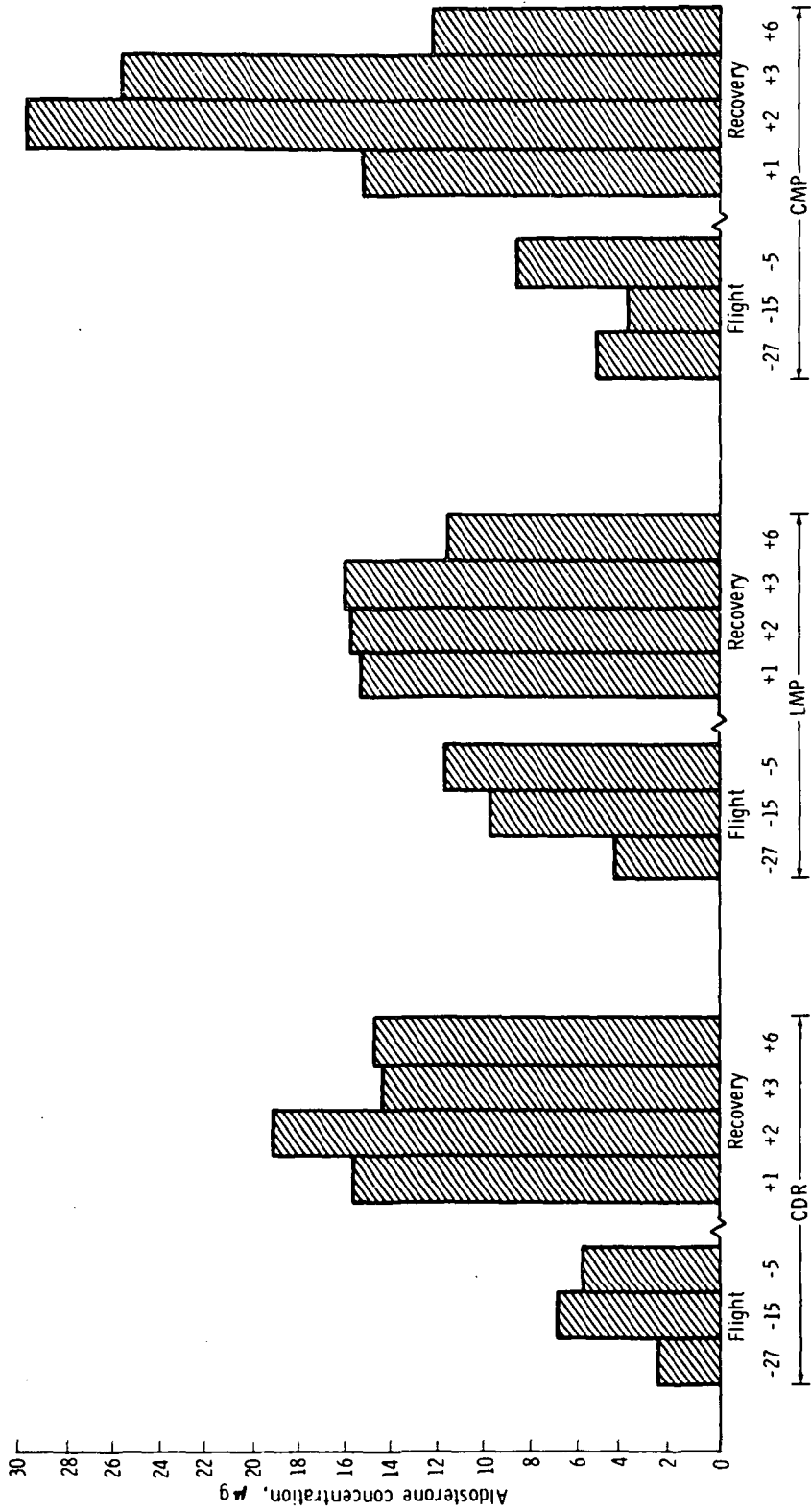
Figure 1-3.- Apollo 14 24-hour urinary aldosterone.
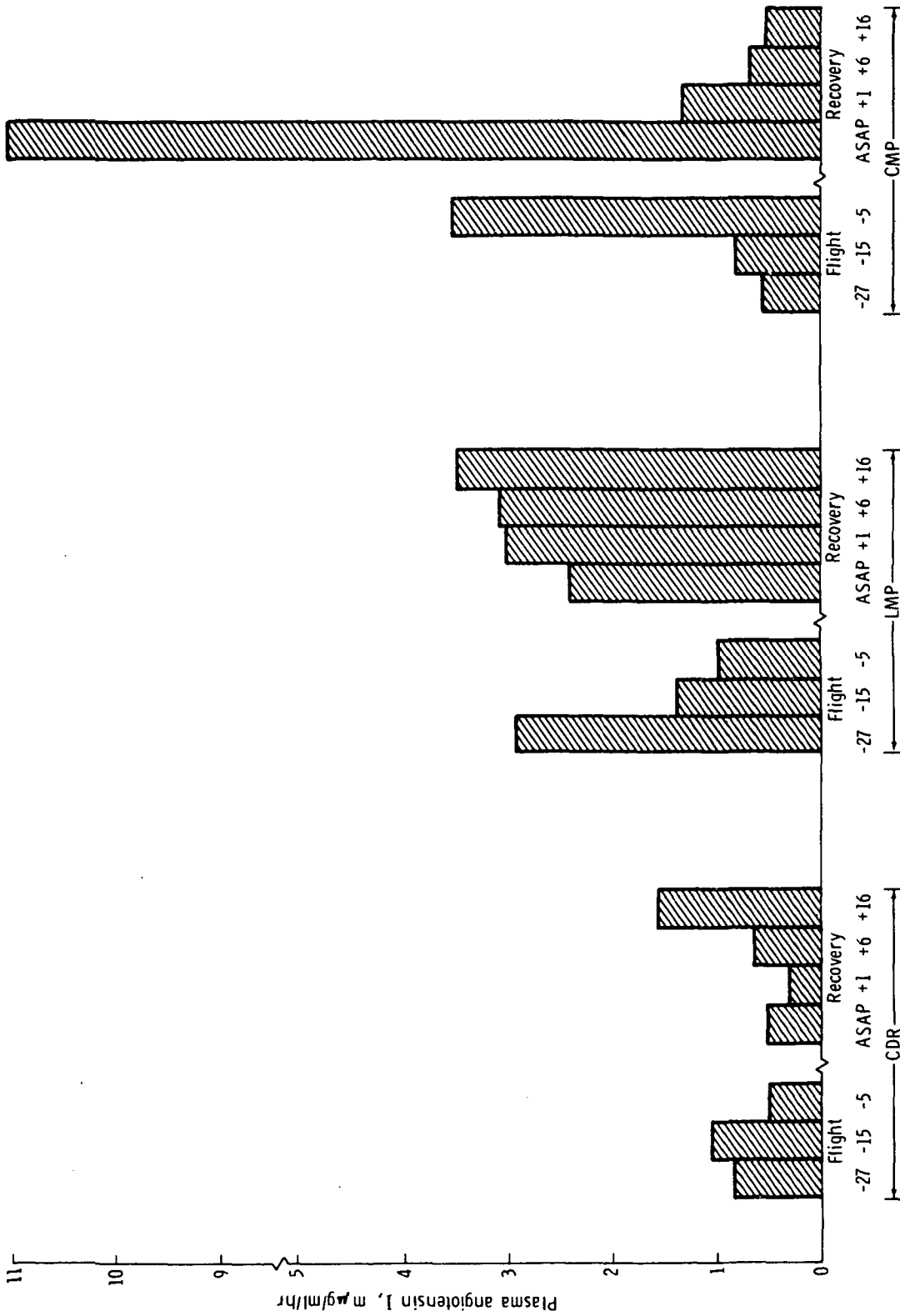
Figure 1-4.- Apollo 14 plasma angiotensin I (renin activity).
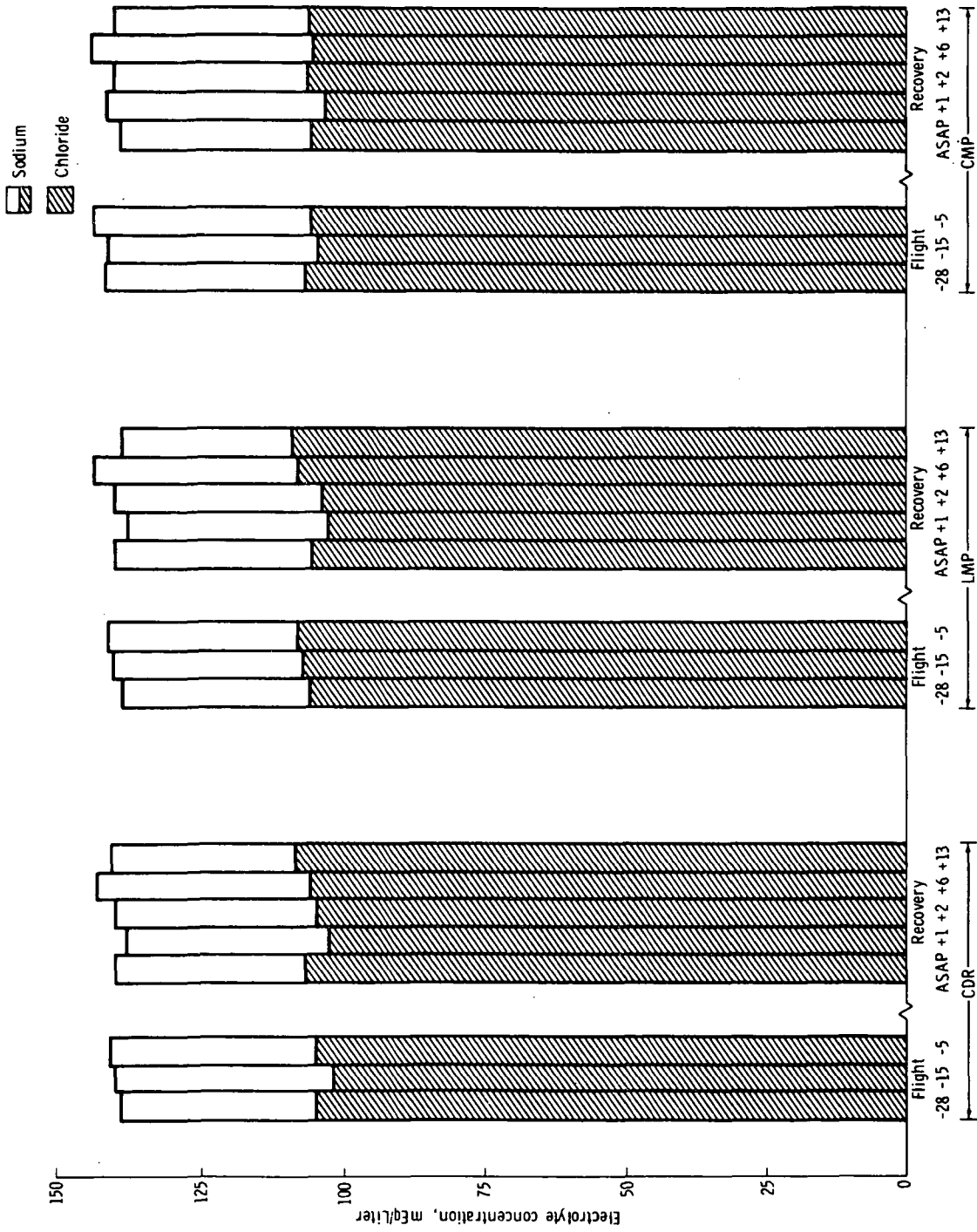
Figure 1-5.— Apollo 15 serum electrolytes.
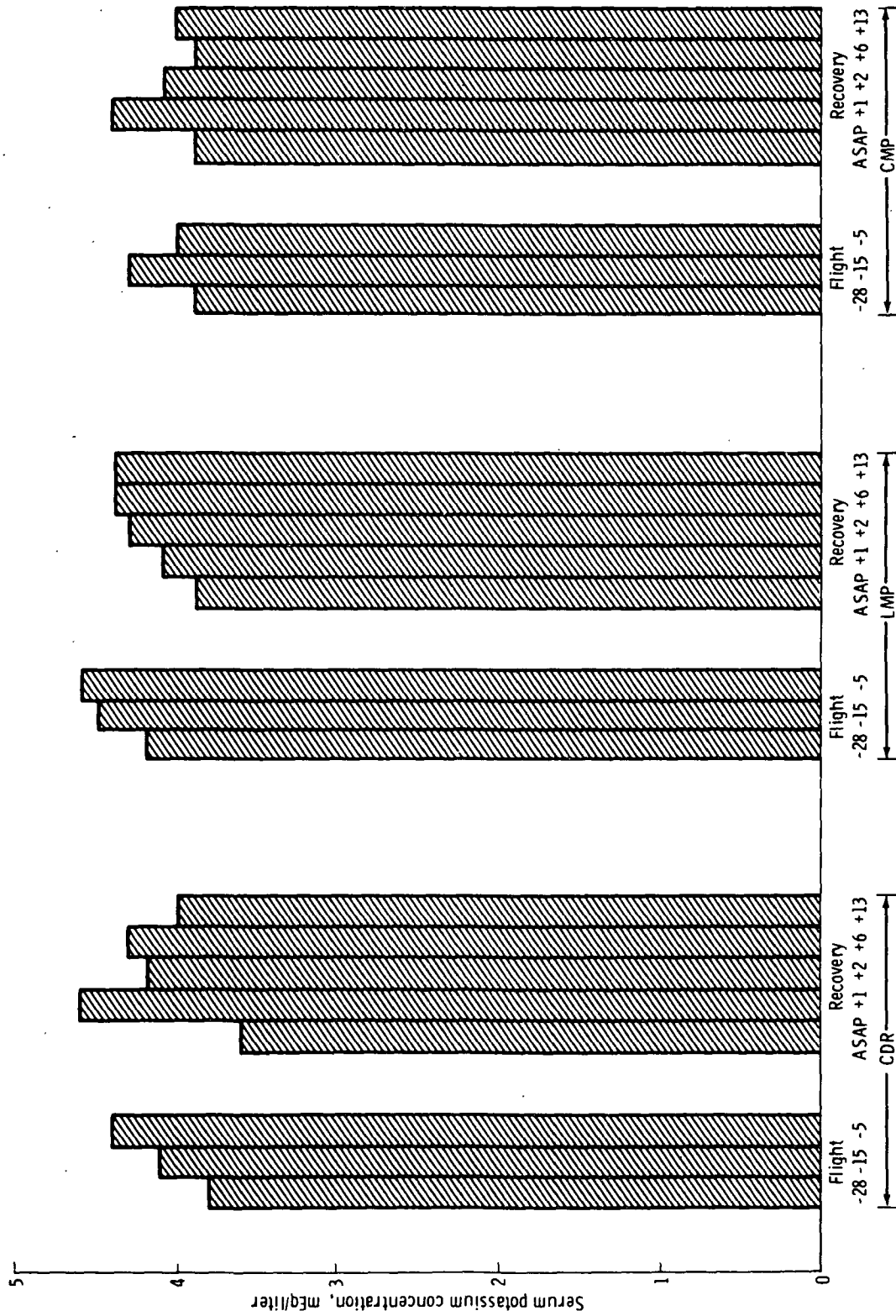
Figure 1-6.- Apollo 15 serum electrolytes: potassium.
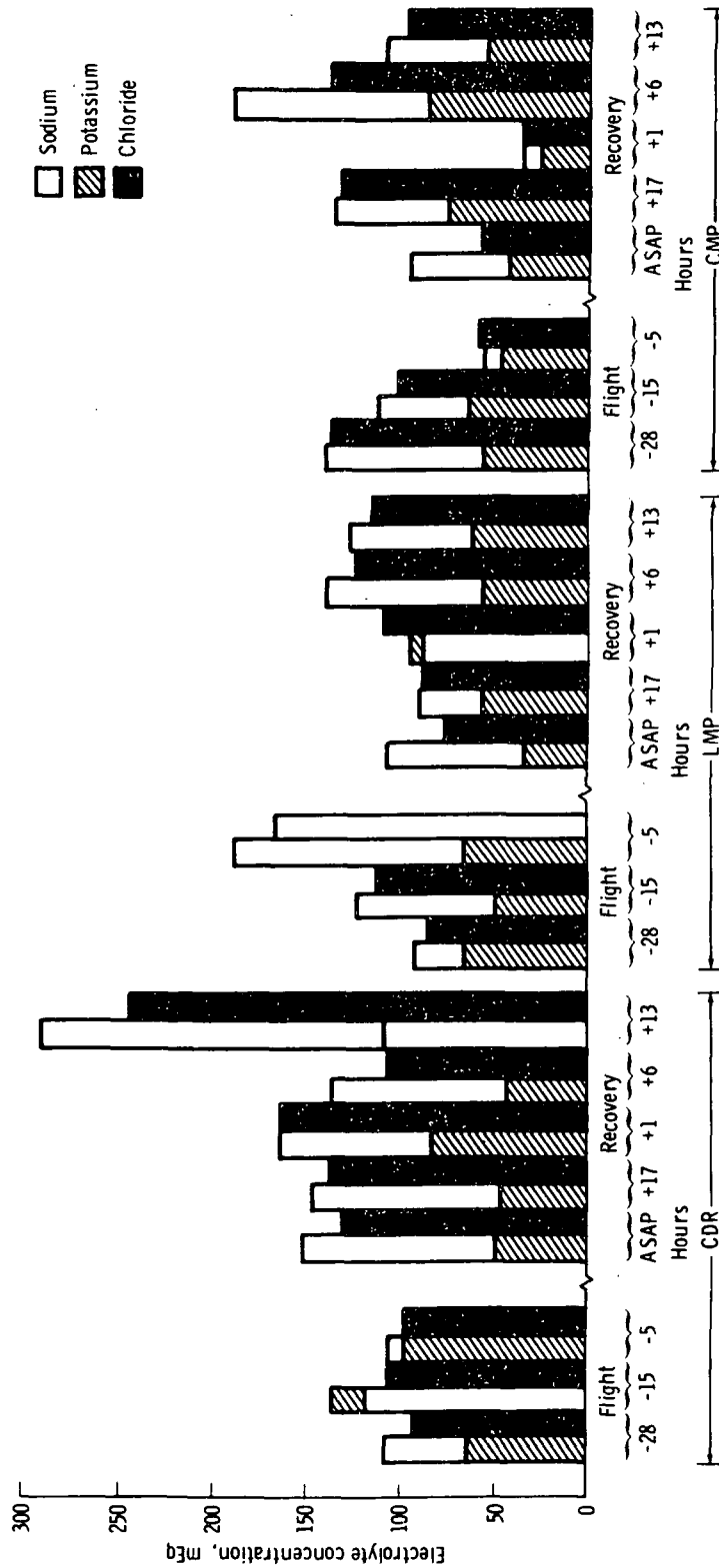
Figure 1-7.- Apollo 15 24-hour urinary electrolytes.
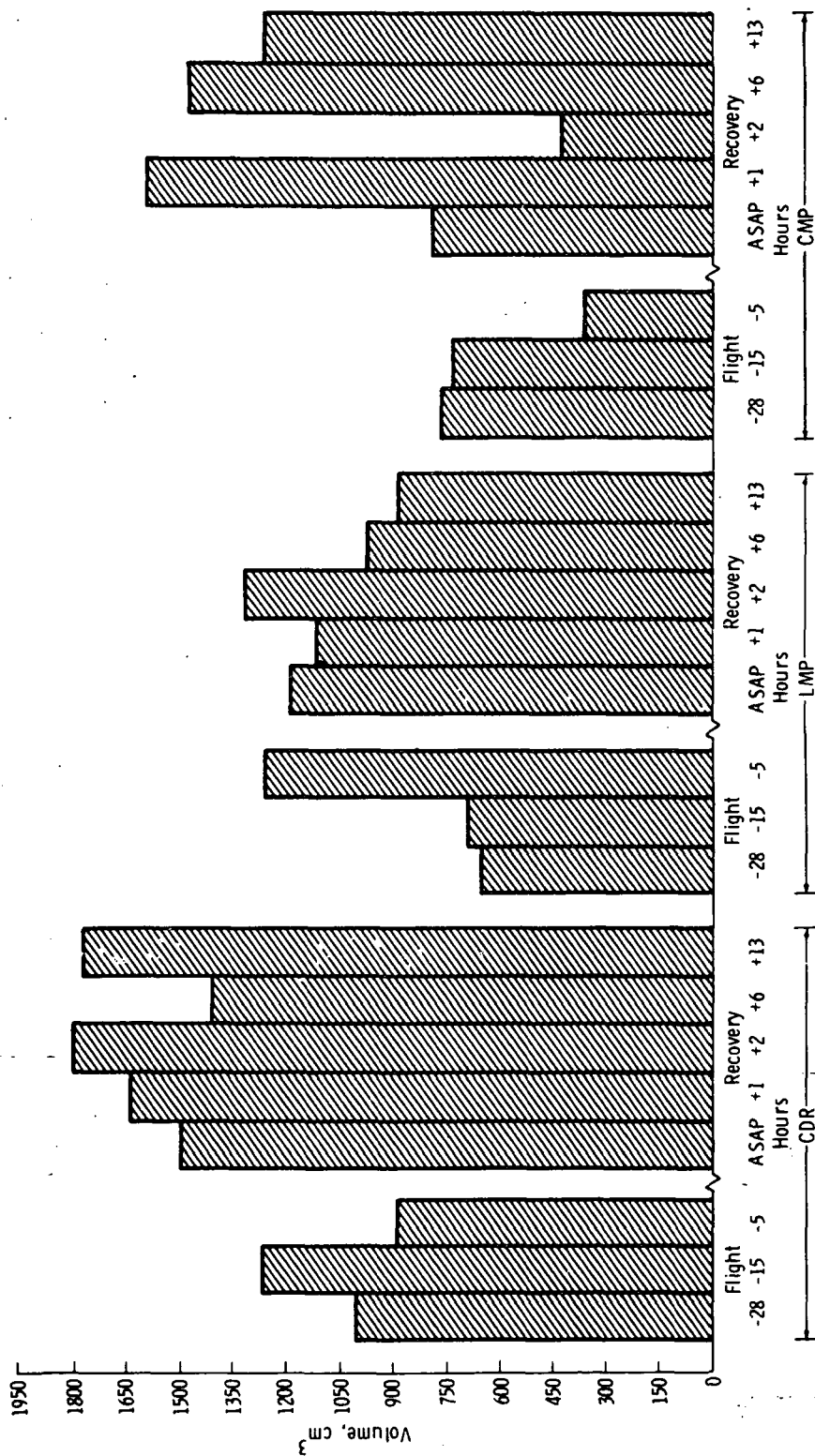
Figure 1-8.- Apollo 15 24-hour urine volume.
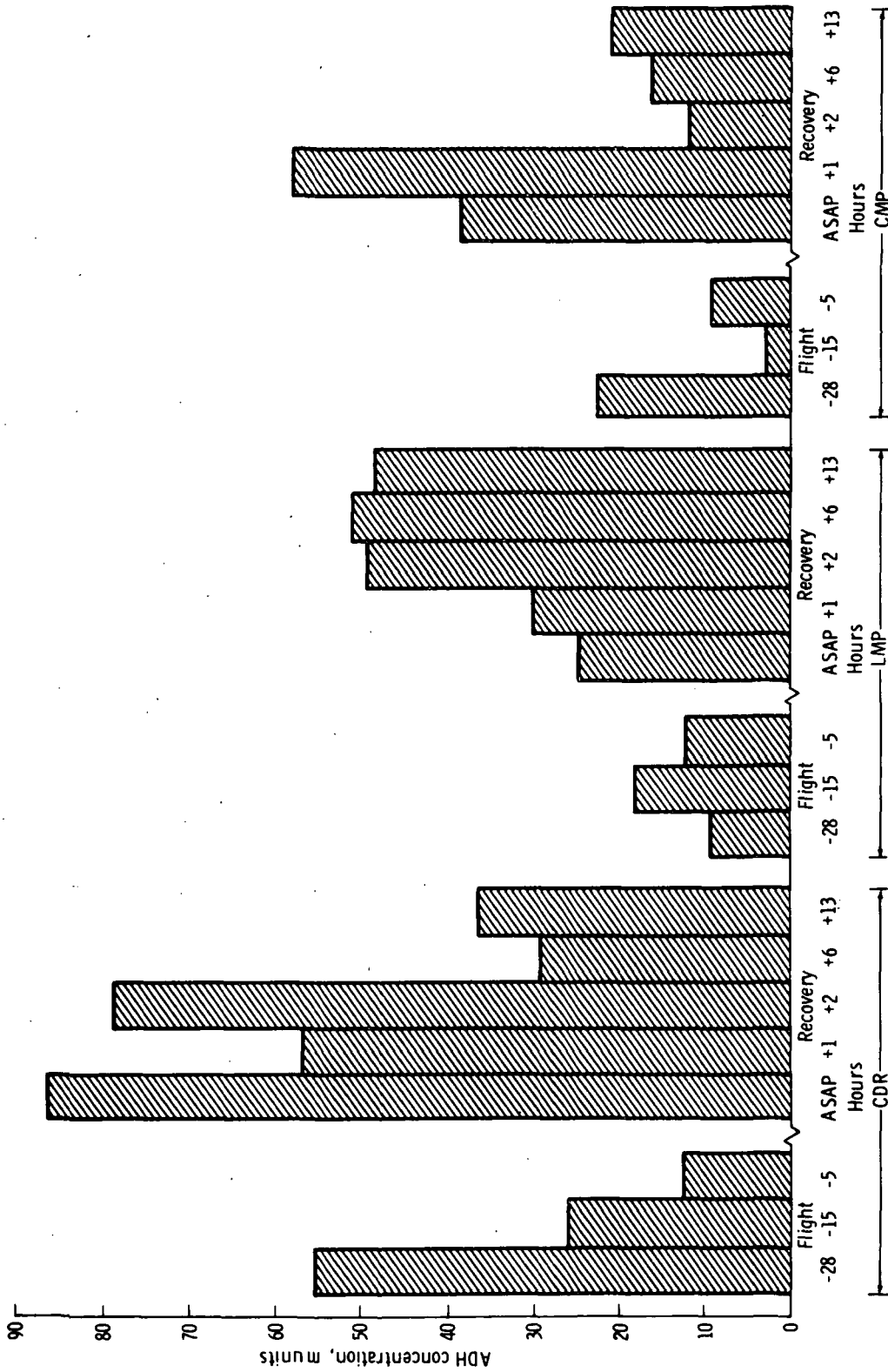
Figure 1-9.- Apollo 15 24-hour urinary ADH.
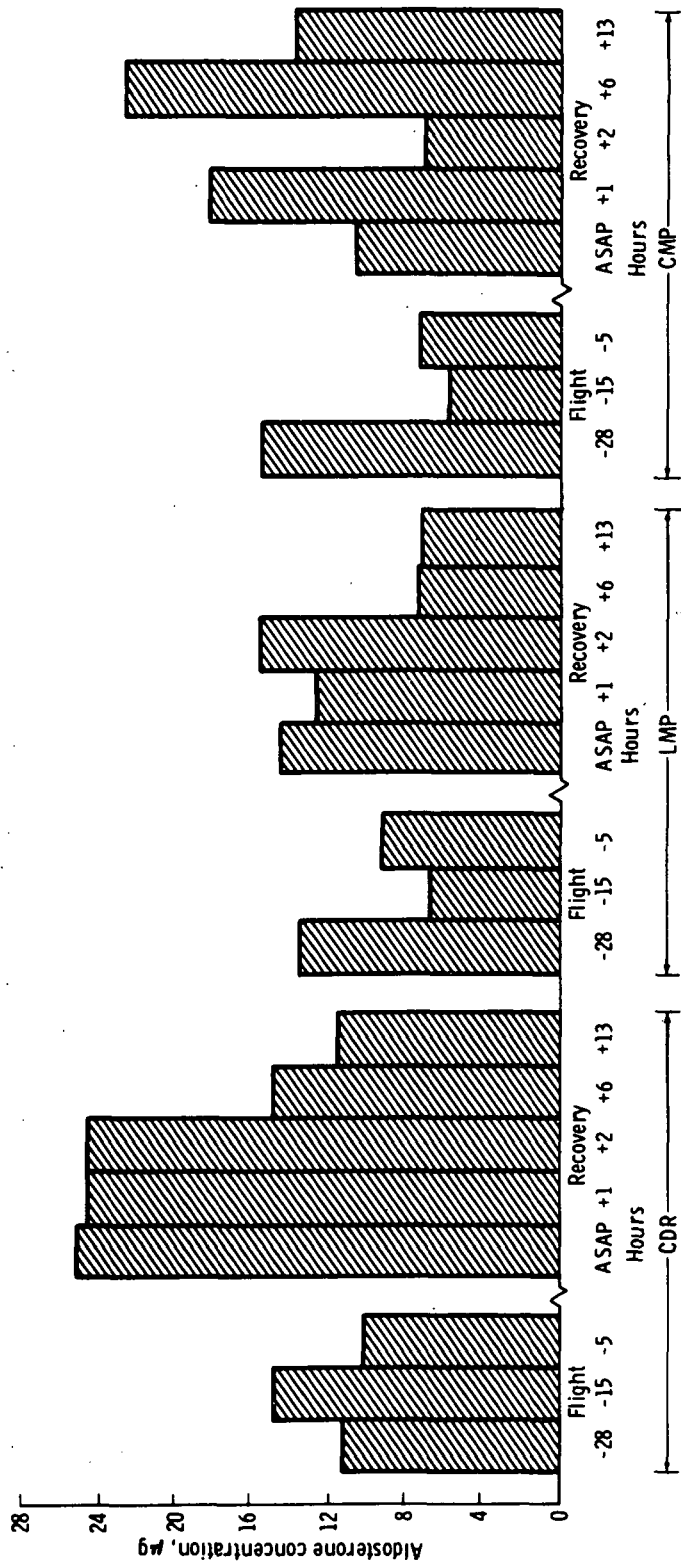
Figure 1-10.- Apollo 15 24-hour urinary aldosterone.

2. THE MEDICAL ASPECTS OF SPACE FLIGHT SEEN FROM

THE VIEWPOINT OF NUCLEAR MEDICINE

By Philip C. Johnson, M.D., and Theda B. Driscoll

INTRODUCTION

Before Project Mercury, considerable concern and discussion was evidenced about how the cardiovascular system would respond to prolonged weightlessness and about how this response would affect the crewmen when they returned to one-g. Cardiovascular studies were begun in Project Mercury and have been continued throughout the Apollo Program. In the recent Apollo missions, some cardiovascular deconditioning is invariably present. It is reassuring to note that this decrease generally lasts only 1 day, but some crewmembers have taken 7 days to return to normal; however, the crewmembers generally feel well during this time.

Shortly after Project Mercury began, a weight loss of several kilograms was a constant finding in anyone who returned from orbital flight. Surprisingly, the first survey publication on this finding did not come from the NASA, but rather from a NASA consultant who noted that the Russian crews had quantitatively similar weight losses. The Russian results were of particular interest because U.S. space suits, atmosphere, and small cabin size were eliminated as the cause of the weight loss.

Project Mercury also brought the first attempt at studying the endocrine changes associated with weightlessness. However, endocrine studies really began during Gemini VII, the longest mission to date. Very little attention was given to possible endocrine changes until after Dr. Leach came to the Manned Spacecraft Center (MSC) and began her comprehensive endocrine program. From a humble beginning at MSC, endocrinology has increased in stature to a point where I believe it to be a major thrust of the Apollo medical program.

DISCUSSION

Although endocrine studies had their beginnings in Project Mercury, radionuclide volume studies did not start until the second manned Gemini mission. Originally, plasma-volume determinations were initiated to study the possible relationship between cardiovascular deconditioning and decreases in blood volume. This study was performed under the first Atomic Energy Commission license to include U.S. carriers at sea.

The first radiopharmaceutical (^{125}I-labelled albumin) was injected into the two Gemini IV astronauts by personnel at MSC. Baylor College of Medicine furnished the injection dose, performed the necessary radioassays, and did the final calculations to determine the plasma volume of the two astronauts. A postflight drop in plasma volume was expected and found. In addition, we found something else which had not been predicted. This unsuspected finding was that the crewmembers' red cell masses had decreased. The red-cell mass was derived from the iodinated-human-albumin space and the peripheral hematocrit. While this is not an accurate way to determine red-cell mass, the findings were sufficiently great to suggest that a true change had occurred. The decrease in derived red-cell mass changed the thrust of the radionuclide volume studies; after Gemini IV, most of our efforts involved investigating the cause of the red-cell-mass decrease. Dr. Fischer and I believed that the hyperbaric-oxygen partial pressure in the Gemini atmosphere was the cause of red-cell-mass decrease, because the Gemini atmosphere was hyperbaric with respect to oxygen before launch and in orbit. As the studies progressed throughout two other Gemini missions, the red-cell-mass decrease was quantitated and an attempt was made to explain the mechanism responsible for the decrease. From the findings and from other studies, Dr. Fischer came to believe that perioxidation of red-cell-membrane lipids caused the circulating red cells to die prematurely.

The Apollo Program began with the disastrous fire of Apollo 1. This fire changed the situation for researchers interested in perioxidation of the red-cell membrane because it resulted in a change in the atmosphere used at launch. The atmosphere was changed from the Gemini type, pure oxygen, to one containing 60 percent oxygen and 40 percent nitrogen. Because of the design of the Apollo command module (CM) the atmosphere is gradually lost after launch. Inflight cabin pressure is maintained by the addition of pure oxygen. However, the gas-leak rate is slow enough that residual nitrogen will remain in the atmosphere unless the hatches are opened for extravehicular activity. Attaching the lunar module (LM) decreases the nitrogen partial pressure because only oxygen is available to compensate for the atmosphere vented into the LM. Oxygen and nitrogen partial pressures were measured on the Apollo 7 mission. At the end of the first day, the atmosphere was 80 percent oxygen, and after the second day, the atmosphere was 90 percent oxygen. The change in the launch atmosphere from 100 percent oxygen to a 40-percent-nitrogen/60-percent-oxygen atmosphere makes possible a decrease in the time needed for crew preoxygenation before launch. Even so, breathing 100 percent oxygen at 101080 N/m^2 (760 torr) for at least 2 hours is still required to prevent dysbarism when the pressure of the CM decreases to 34314 N/m^2 (258 torr) upon reaching orbit. Thus, Apollo crew exposure to oxygen includes 100 percent oxygen at 101080 and 34314 N/m^2 (760 and 258 torr).

The percent of change in red-cell mass when the postmission value is compared with the premission value is presented in table I. The red-cell mass has generally decreased (19 out of 21 astronauts) during the orbital missions. After the Gemini missions, the red-cell-mass changes generally were greater, that is, -8 to -22 percent. On the Apollo missions, the changes were smaller, but the red-cell-mass decrease was not caused by blood drawing; blood was drawn from control simultaneously and the mean values did not change.

The Apollo missions contrast with the Gemini missions in that the ^{51}Cr red-cell survivals are normal for Apollo crewmen. Originally, the red-cell-mass loss was explained as being a hemolytic event, but as depicted in the Apollo results, the hyperbaric atmosphere also may be suppressing red-cell production. Another possibility for the results is that the high oxygen partial pressures are causing loss of older red cells. These cells are tagged by the ^{51}Cr to a lesser extent than are the younger red cells. Thus, a selective loss of older cells might not affect the red-cell survival curves of Apollo. The somewhat larger red-cell-mass drops for Gemini crewmen may have involved more of the younger cells, thus showing more clearly in the red-cell survivals. The red-cell-survival results for Apollo missions are shown in table II. The ^{51}Cr red-cell-survival results of the missions utilizing the LM (Apollo 9, 14, and 15) are separated because crewmen showed greater red-cell-mass losses than did the Apollo missions not using the LM (7 and 8).

Plasma-volume decreases approximately 10 percent with bedrest within the first 3 or 4 days, and the plasma volume may decrease as much as 20 percent after prolonged bedrest. The initial 10-percent plasma-volume loss is caused by the loss of the labile portion of plasma volume. This portion is helpful in keeping the blood pressure normal as the human changes from a lying to a sitting position. Bedrest subjects lose this increment of the plasma volume when they stay recumbent for more than 24 hours. After the first change, a continuing but slower change in plasma volume occurs. This continuing loss of plasma volume may represent changes in the body composition caused by inactivity of the muscles used in standing. Because weight loss does not occur during bedrest, the muscles would be replaced by fat; fat contains less blood per kilogram than does muscle.

Urinary excretion of aldosterone is low during bedrest; the decrease in plasma volume may be one manifestation of this fact. However, aldosterone excretion has been reported to be elevated during weightlessness. Elevation of aldosterone should return plasma volume to normal or increase it to above normal. When arranged according to mission duration, the results show that the longer the mission, the less the plasma-volume drop (table III). Red-cell-mass changes were not related to mission duration; however, blood-volume changes followed the same pattern as the plasma-volume changes.

The extracellular-fluid-volume (ECF) measurements made on the Apollo 14 and 15 missions are shown in table IV. The 30-minute-distribution space of ^{35}S sulphate was determined. Only a slight decrease in ECF volume is shown. When this volume is converted to cm^3/kg body weight, the ECF values tend to increase postflight. This fact is evidence that tissue loss, not ECF loss, was the cause of the body-weight loss. The results are analogous to what would be expected during starvation, that is, as body tissue is lost, ECF increases to compensate for this tissue loss. This increase results when muscle mass is lost, decreasing the tension on the skin. The decrease in elastic tension of the skin no longer counteracts vascular hydrostatic pressures. To return to equilibrium, ECF accumulates and returns the skin tension to normal. In starvation, the collected fluid is great enough to be seen as edema. Edema was not found postflight in any of the crewmembers.

Also, the total-body-water results were consistent with a tissue loss because six out of six values decreased and the changes were generally greater than the ECF changes (table V). When the total-body water is corrected to units of cm^3/kg body weight, postmission increases are found. Because total-body water included ECF, part of the increase is from the increased ECF. This fact suggests that the weight loss included fat (a non-water-containing tissue).

Total-body potassium was determined on the Apollo 15 crewmembers (table VI). The crewmen ingested ^{42}K and collected a 24-hour urine. A spot urine was obtained to determine specific activity. In addition, the next 24-hour urine was collected and another spot urine was obtained to determine specific activity. Although ^{42}K does not completely equilibrate within 24 hours, only a slight change was found in specific activity between 24 and 48 hours, showing that distribution of the ingested radioactive potassium was nearly complete at 24 hours.

The premission total-body-potassium (K) values were higher than reported values for normal subjects. However, one astronaut was extremely well conditioned physically, and all three crewmembers did not have much fat as was shown by the percent of body water. A significant postmission drop in total-body K was evidenced in all three crewmembers. After 1 to 2 weeks postmission, total-body K had returned to the premission range. The postmission total-body-K decrease confirmed the Gemini VII results and other data, which indicate that aldosterone secretion is elevated during the mission.

CONCLUSIONS

The results of the radionuclide volume measurements performed on the crews who have taken part in selected Apollo missions indicate the following.

1. Invariably, there is a small drop in red-cell mass of the returning crewmembers. This drop is presumed to result from the hyperbaric-oxygen partial pressures.

2. Plasma-volume decreases similar to those experienced during bedrest were found in the crewmen of short Gemini missions. After longer missions, the plasma-volume decrease was no longer present. Increased aldosterone secretion during weightlessness may account for this difference between weightlessness and bedrest, the classical experimental analogue of weightlessness.

3. Extracellular- and total-body water changes prove that spaceflight weight loss includes actual tissue losses. As indicated by the data, the changes are not only fluid-electrolyte losses.

4. The loss of total-body exchangeable potassium found after the Apollo 15 mission was evidence of increased aldosterone secretion during that mission.

TABLE 2-I.- RED-CELL-MASS CHANGE ON GEMINI

AND APOLLO MISSIONS[a]

Mission	Red-cell-mass change, percent			Mean change, percent
	Astronaut 1	Astronaut 2	Astronaut 3	
Gemini				
VII	-19	-8	--	-14
V	-20	-22	--	-21
IV	-12	-13	--	-12
Apollo				
15	-14	-7	-10	-10
9	-4	-7	-10	-7
14	-2	-9	-4	-5
7	-1	0	-9	-3
8	+2	-2	-4	-1

[a]Fourteen Apollo control subjects were used; the mean for the control group was -0.4 ± 0.8.

TABLE 2-II.- ^{51}Cr-LABELLED RED-CELL SURVIVAL DATA[a]

Experimental situation	Specimen-acquisition time		
	Preflight	Inflight	Postflight
Without LM Red-cell half life, days	25.5 ± 0.8	28.3 ± 1.3	24.7 ± 1.0
With LM Red-cell half life, days	24.1 ± 1.1	25.1 ± 1.3	28.2 ± 1.5

[a]Controls = 23.4 ± 2.0 days.

TABLE 2-III.- VOLUME CHANGE IN RELATIONSHIP TO MISSION DURATION

Mean change	Short, 50 to 150 hrs	Medium, 150 to 250 hrs	Long, 250 to 350 hrs
Plasma volume	-11 ± 2	-6 ± 2	+1 ± 3
Red-cell mass	-6 ± 3	-10 ± 3	-8 ± 2
Blood volume	-9 ± 1	-8 ± 2	-3 ± 1

TABLE 2-IV.- EXTRACELLULAR FLUID VOLUME

Mission	Astronaut			Mean
	1	2	3	
Volume change, percent				
Apollo 14	-0.5	0.0	-0.5	-0.3
Apollo 15	-4.8	-3.1	0.0	-2.6
cm^3/kg change, percent				
Apollo 14	+1.6	+9.4	+2.5	+4.5
Apollo 15	-2.0	+1.4	+1.4	+0.3

TABLE 2-V.- TOTAL BODY WATER

Mission	Astronaut			Mean
	1	2	3	
Volume change, percent				
Apollo 14	-1.9	-17.7	-1.8	-7.1
Apollo 15	-2.8	-3.7	-0.8	-2.4
cm^3/kg change, percent				
Apollo 14	+0.4	-9.8	+1.6	-2.6
Apollo 15	+0.1	+0.6	+0.7	+0.5

TABLE 2-VI.- APOLLO 15 TOTAL BODY POTASSIUM

Time of sampling	Astronaut			Mean
	1	2	3	
Preflight, meq/kg	54	51	49	51
ASAP[a], percent change	-14	-16	-9	-13
R[b] + 6 days, percent change	+4	-3	-4	-1
R + 13 days, percent change	-2	-5	+2	-2

[a]As soon as possible after recovery.

[b]R = Recovery.

3. URINARY EXCRETION OF ANTIDIURETIC HORMONE IN MAN

By Myron Miller, M.D.

INTRODUCTION

For many years, attempts have been made to assess directly neuro-hypophyseal function in man by means of estimation of antidiuretic hormone (ADH) excretion in the urine. Early attempts were hindered by the lack of an efficient, simple procedure for extracting and concentrating ADH from urine and by the dependence on laborious bioassay procedures for the quantitation of ADH.

With the development of a sensitive and specific radioimmunoassay for ADH, along with the application of an efficient extraction procedure for ADH, it has been possible to assay the hormone in the urine, thus providing a suitable means for estimation of neurohypophyseal function. In this report, radioimmunoassay has been used to determine urinary ADH excretion in normal man, to study the influence of water loading and dehydration on the excretion of ADH, and to study ADH regulation in several clinical states of abnormal water balance.

The author wishes to express his appreciation to Mrs. Leelo Bertram who provided expert technical assistance in performing these studies.

MATERIAL AND METHODS

Twenty-four-hour urine samples were collected in containers to which 2 cubic centimeters of glacial acetic acid had been added. After the specimen had been collected, the pH was adjusted to 4.5 by means of the addition of further acetic acid whenever necessary. Specimens that were obtained during water-loading and dehydration tests first were analyzed for osmolality and then were adjusted to pH 4.5 with the addition of glacial acetic acid. Urine samples that were not further processed immediately after collection were stored frozen at 253° K (-20° C).

To extract and concentrate ADH from the urine, 25 cubic centimeter aliquots of acidified urine were passed through columns of the ion exchange resin CG-50 and the ADH was eluted with 75 percent ethyl alcohol adjusted to pH 2. The eluates were evaporated and redissolved in a

2 cubic centimeter volume and ADH was quantitated using a radioimmuno-
assay procedure. The lower limit of sensitivity of the assay was 25 µU
arginine vasopressin (AVP)/cm^3 of urine concentrate, thus allowing as
little as 2 µU AVP/cm^3 of unextracted urine to be detected.

RESULTS

On 14 different occasions, determination of the AVP content of a
standard preparation of beef posterior pituitary extract, as a means of
estimating the reproducibility of the immunoassay procedure, gave a
coefficient of variation of 18.3 percent. Analysis of 32 aliquots of a
single urine specimen, extracted and assayed over a period of 3 months,
gave a similar coefficient of variation of 21.8 percent, indicating that
little additional variability was introduced by the extraction procedure.

When different amounts of AVP were added to urine extracts from a
water-loaded normal subject, the immunoassay curve obtained was identical
to the curve resulting from the original AVP preparations (fig. 1).
Thus, no nonspecific effect of the urine extract on the AVP standard
curve was apparent. When varying amounts of AVP were added to 25 cubic
centimeter aliquots of urine from a water-loaded normal subject and then
were extracted and assayed, a linear relationship was found between the
amount of AVP added and the amount of AVP recovered. The percent re-
covery was constant over the range of doses tested (fig. 2).

The CG-50 columns extracted ADH quantitatively when either 25 or
50 cubic centimeter aliquots of urine were applied (table I). In both
volumes, recovery was complete and similar to the value of 98.5 percent
previously obtained.

Acidified urine, frozen for as long as 3 weeks before extraction and
assay, showed no loss of immunoreactivity of ADH (table II). Recovery
of the added AVP was complete. The variability present (16.8 percent) is
of the same magnitude as that found in the above determinations of beef
posterior pituitary extract and replicates of a single urine specimen.
In other experiments, frozen samples have been stored for as long as
2 months, and these samples have shown no loss of activity.

In 30 normal male subjects ranging in ages from 12 to 43 years,
urinary ADH excretion under conditions of random diet, fluid intake, and
physical activity ranged from 11.0 to 91.4 mU/24 hr, with a mean value of
28.9 ± 17.9 (S.D.) mU/24 hr or 1.20 ± 0.75 mU/24 hr (table III, fig. 3).
The distribution of values is skewed so that the median value is
22.8 mU/24 hr and 70 percent of the subjects excreted less than 30 mU
ADH/24 hr and only 13 percent excreted more than 50 mU/24 hr (fig. 3).

In five subjects, urine was collected from time of arising to time of bed and from time of bed to time of arising (table IV). Mean urinary ADH excretion during the daytime hours was 0.92 ± 0.20 (S.E.) mU/hr and during sleep was 0.68 ± 0.13 mU/hr. These values were not significantly different from each other. Mean urinary ADH excretion during sleep in 12 subjects was 0.96 ± 0.20 mU/hr, a value not different significantly from the value of 1.20 ± 0.14 mU/hr for the hourly excretion over the total 24 hours in the 30 normal subjects.

Water loading was performed by administering orally 20 cubic centimeters of water per kilogram of body weight and then replacing voided urine volumes with an equal volume of tapwater to maintain the water load. Voided urine specimens were collected at 15-minute intervals during the period of diuresis and analyzed for volume, osmolality, and ADH. The response in a normal subject is shown in figure 4. Following ingestion of the water load and the beginning of diuresis, urine osmolality fell from 925 to 70 mOsm/kg. In parallel with the fall in urine osmolality, urinary ADH excretion fell rapidly from 2.6 mU/hr to levels that were undetectable and remained at those levels during the period of diuresis. When water was withheld so that the urine osmolality began to rise, again ADH appeared in the urine, first being apparent when the urine osmolality reached 86 mOsm/kg.

Following the administration of a maintained oral-water load to 22 normal subjects, plasma osmolality fell from 284 to 293 mOsm/kg to 269 to 283 mOsm/kg with concomitant onset of diuresis of 600 to 1480 cm^3/hr and decrease in urine osmolality to 45 to 86 mOsm/kg. During the peak of the diuresis, ADH excretion was not detectable in 19 of the 22 subjects (fig. 5).

The response to 17 hours of fluid deprivation in a normal subject is shown in figure 6. By the twelfth to fifteenth hour of fluid deprivation, urine osmolality reached a maximum value of 1090 mOsm/kg and did not increase further with an additional 2 hours of dehydration. Urinary ADH excretion rose progressively from 0.42 mU/hr in the predehydration period to a maximum of 1.98 mU/hr by the sixteenth hour of fluid deprivation.

Urine was collected from the hours 13 to 14 after fluid deprivation in 13 normal subjects. Urinary ADH excretion increased in each subject from 1.00 ± 0.19 mU/hr in the predehydration period to 2.74 ± 0.53 mU/hr after 14 hours of dehydration (fig. 7). During the same time intervals, urine osmolality rose from 941 ± 48 to 1000 ± 47 mOsm/kg. Mean plasma osmolality at the end of the dehydration period was 290.6 ± 1.0 mOsm/kg.

Studies were conducted on six patients who had severe hypothalamic diabetes insipidus, with urine volumes that ranged from 4700 to 10 700 cm^3/24 hr. With the patients on ad libitum fluid intake, urine

osmolality ranged from 80 to 197 mOsm/kg. Plasma osmolality ranged from 292 to 300 mOsm/kg, and no ADH was detectable in the urine (fig. 8).

Three patients with congenital nephrogenic diabetes insipidus were studied. In contrast to the patients with hypothalamic diabetes insipidus, these patients on ad libitum fluid intake had urinary ADH excretion ranging from 1.15 to 4.30 mU/hr. After 5 hours of water deprivation, urinary ADH excretion rose significantly to 4.83 mU/hr in one subject and to 27.52 mU/hr in another subject. When the patients were given an oral water load of 20 cm^3/kg body weight, urinary ADH became undetectable (fig. 9). Therefore, patients with nephrogenic diabetes insipidus excrete large amounts of ADH under conditions of both random hydration and fluid deprivation.

Six patients with inappropriate ADH syndrome were studied, five of whom had oat cell carcinoma of the lung. Urine osmolality ranged from 450 to 960 mOsm/kg during ad libitum fluid intake. Only one of these patients, who excreted 13.2 mU/hr, had urinary ADH excretion in excess of that found in normal individuals (fig. 10). However, when the urinary ADH excretion was related to plasma osmolality, it was apparent that the ADH excretion was inappropriate (fig. 11). In normal subjects, ADH excretion generally is undetectable when the plasma osmolality is less than 281 mOsm/kg. In the patients in whom plasma osmolality ranged from 254 to 270 mOsm/kg, urinary ADH was always present.

The patients were given an oral water load, which further lowered plasma osmolality to a mean of 251 mOsm/kg, and the response was contrasted with the response seen in normal subjects (fig. 12). Urinary ADH excretion fell but was never undetectable, in contrast to what was seen in normal subjects where ADH could not be found in 19 of 22 subjects. Urine volume did not increase and urine osmolality fell only modestly from 667 to 522 mOsm/kg.

CONCLUSIONS

The studies shown here indicate that urinary excretion of ADH can be detected readily and quantitated accurately. The ADH excretion in normal subjects is seen to be inhibited following the administration of a water load and to be stimulated following water deprivation. It appears that measurement of ADH excretion in man can provide a means of quantitating alterations in neurohypophyseal ADH secretion. By determining not only the basal excretion of ADH but also the response to such physiological influences as water loading and dehydration, it becomes possible to study the dynamics of ADH release. Thus, the ability to extract ADH efficiently

from urine combined with a sensitive and specific technique for determination of ADH concentration allows the exploration of regulatory systems for ADH control in the normal state as well as the etiological role of altered ADH secretion in clinical disorders of water balance.

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TABLE 3-I.- RECOVERY OF ARGININE VASOPRESSIN FROM

25 AND 50 CUBIC CENTIMETER ALIQUOTS OF URINE

25 cubic centimeter aliquot, 0.33 mU/25 cm^3		50 cubic centimeter aliquot, 0.66 mU/50 cm^3	
mU/25 cm^3	Percent recovery	mU/50 cm^3	Percent recovery
0.38	115	0.68	103
.34	103	.74	112
.39	118	.69	104
.36	109	.74	112
.37	112	.70	106
.34	103	.52	78
.40	121	.70	106
.28	84	.60	91
.37	112	.58	88
.36	109	.48	72
Mean			
0.36	109	0.64	97
Standard error of the mean			
0.01	3.3	0.03	4.5

TABLE 3-II.– RECOVERY OF ARGININE VASOPRESSIN ADDED

TO URINE AND FROZEN FOR UP TO 22 DAYS

BEFORE EXTRACTION AND ASSAY

Days frozen	AVP, 0.33 mU/25 cm^3	
	mU/25 cm^3	Percent recovery
1	0.38	115
	.34	103
	.39	118
	.36	109
	.37	112
	.32	97
	.31	94
	[a].35 ± 0.01	[a]107 ± 3.4
8	0.34	103
	.40	121
	.28	84
	.37	112
	.36	109
	.25	76
	.27	82
	[a].32 ± 0.02	[a]98 ± 6.6
14	0.24	73
	.25	76
	[b].24	[b]74
22	0.42	127
	.39	118
	[b].40	[b]122

[a]Mean ± standard error of the mean.

[b]Mean.

TABLE 3-III.- TWENTY-FOUR HOUR URINARY ANTIDIURETIC HORMONE DATA

[Thirty normal male subjects, ranging in age from 12 to 43 years]

Subject	Age, yr	ADH excretion, mU/24 hr	Urine volume, cm^3/24 hr	Urine osmolality, mOsm/kg	Creatinine excretion, g/24 hr
J. L.	43	11.0	1000	1036	1.8
D. M.	23	11.6	700	755	--
R. B.	27	12.5	820	995	2.0
R. C.	26	13.3	950	1044	1.6
A. P.	24	13.3	1040	756	1.3
M. M.	36	14.7	800	893	1.4
J. C.	12	15.9	620	1120	--
J. G.	26	16.5	1020	850	--
R. Co.	27	16.8	2040	456	2.1
K. T.	26	17.1	2840	467	1.3
D. H.	25	17.6	1000	958	1.9
A. S.	26	18.8	2180	577	2.2
P. H.	25	20.2	720	1098	1.7
J. V.	26	21.9	1440	915	2.6
D. L.	23	22.8	1190	884	2.1
D. C.	22	25.6	955	1040	--
M. Mi.	37	25.6	780	935	1.8
D. Ha.	26	26.3	3130	377	2.3
A. M.	41	27.6	1150	988	2.0
R. Cou.	28	28.5	990	1041	1.8
P. Ho.	26	29.6	475	1226	1.4
N. E.	23	34.7	985	862	2.0
M. B.	24	39.6	990	1062	--
R. F.	23	41.1	870	1026	1.3
K. Tu.	25	44.9	1220	819	2.3
J. Cr.	26	49.6	1070	814	1.8
A. K.	25	51.1	760	1131	1.7
T. W.	25	52.5	925	994	1.9
M. W.	24	56.2	990	978	2.0
J. W.	28	91.4	1360	988	2.0
Mean					
--	--	28.9	1167	903	1.9
Standard deviation					
--	--	17.9	610	206	0.3

TABLE 3-IV.- ANTIDIURETIC HORMONE

EXCRETION DURING WAKING HOURS AND

DURING SLEEP IN NORMAL SUBJECTS

Subject	ADH excretion, mU/hr	
	Awake	Asleep
1	1.59	0.44
2	.35	.78
3	.99	1.14
4	.71	.62
5	.95	.42
Mean ± standard error of the mean		
--	0.92 ± 0.20	[a]0.68 ± 0.13

[a]P = Not significant compared to awake.
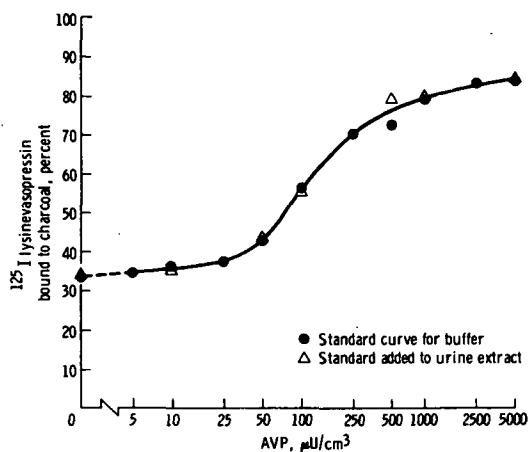
Figure 3-1.- Standard curve prepared in the usual fashion (solid dots) compared with standard curve prepared by adding AVP to dried extract of urine from a water-loaded normal subject (open triangles). The two curves are identical, indicating the absence of a nonspecific effect of the urine extract on the curve.
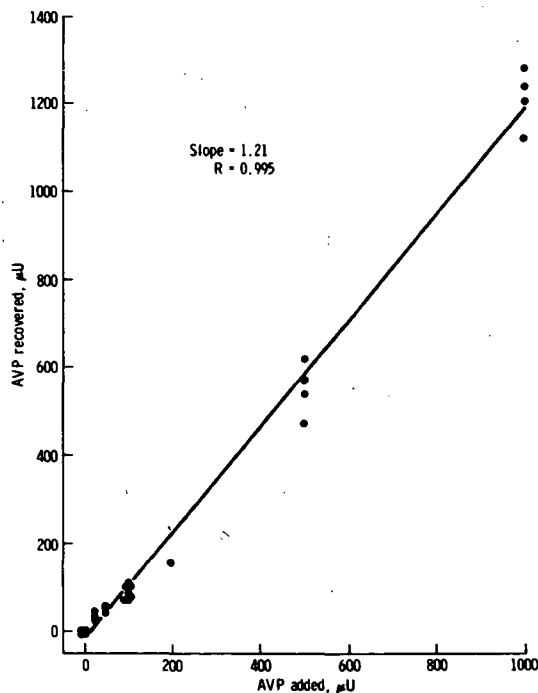
Figure 3-2.- Recovery of varying amounts of AVP added to 25 milliliters of urine aliquots obtained from a water-loaded normal subject. The amount of AVP added is plotted on the X axis while the amount of AVP recovered is plotted on the Y axis.
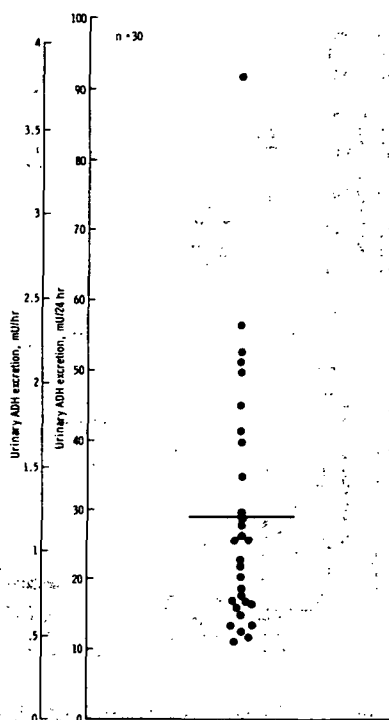
Figure 3-3.- Urinary excretion of ADH in 24-hour urine collections from
30 normal male subjects, expressed as mU/24 hr on the left and as mU/hr
on the right. Seventy percent of the values are less than 30 mU/24 hr.
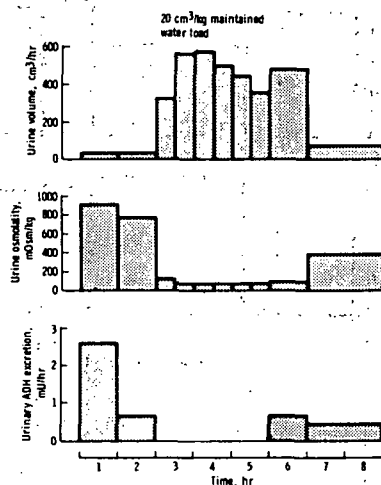
Figure 3-4.- Response to a maintained oral-water load of 20 mg/kg in a nor-
mal subject. After maximum diuresis was achieved, further water inges-
tion was discontinued and the subject was allowed to excrete the water
load. No ADH was detectable in urine collected from hours 3 to 5.
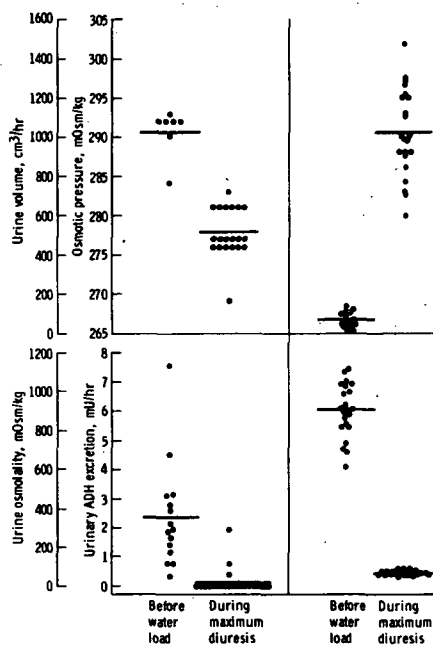
Figure 3-5.- Response to maintained oral-water load of 20 ml/kg in 22 normal subjects. Values just before the water load and during the period of maximum diuresis for each of the parameters measured are plotted for each subject. Mean values are indicated by the horizontal lines.
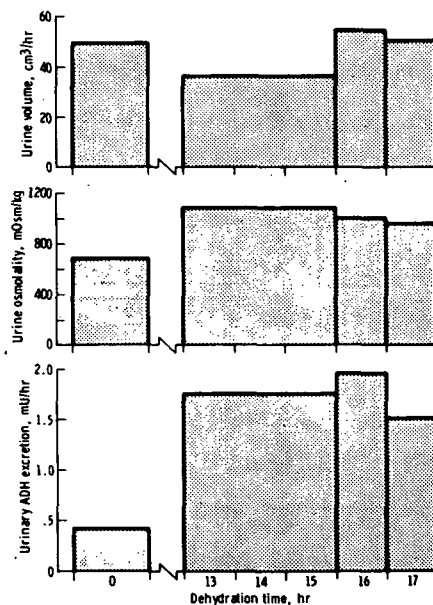
Figure 3-6.- Response to 17 hours of fluid deprivation in a normal subject.
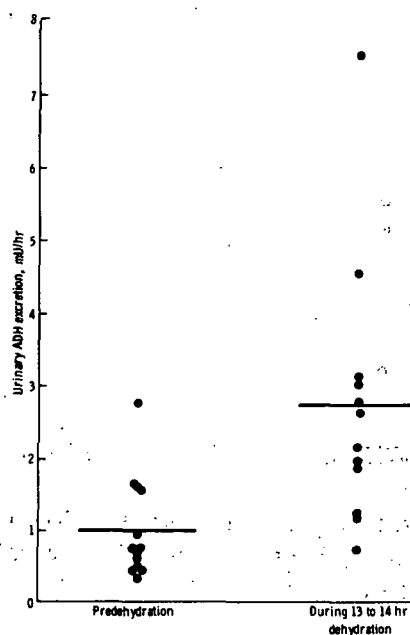
Figure 3-7.- Urinary ADH excretion following 14 hours of fluid deprivation in 13 normal subjects. Values just before fluid deprivation and during the hours 13 and 14 of fluid deprivation are plotted for each subject. Mean values are indicated by the horizontal lines. In response to fluid deprivation, urinary ADH excretion increased in each subject.
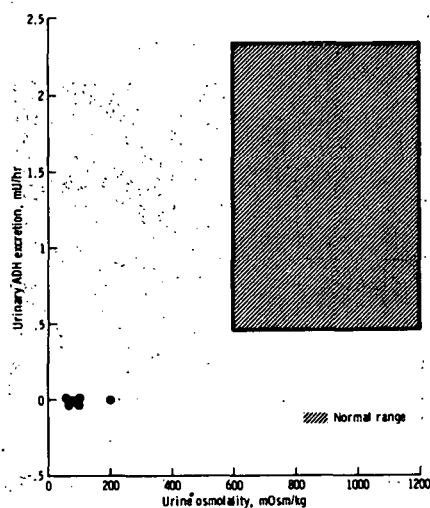
Figure 3-8.- Urinary ADH excretion and osmolality in 24 hour urine collections from six patients with severe hypothalamic diabetes insipidus (solid dots). The normal range for ADH excretion and urine osmolality is indicated by the shaded area.
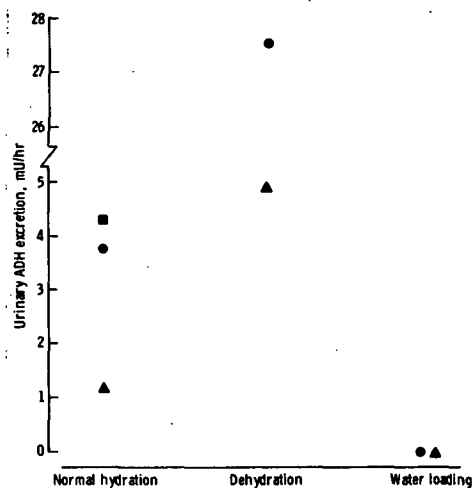
Figure 3-9.- Urinary ADH excretion in three patients with nephrogenic dia-
betes insipidus under conditions of ad libitum fluid intake and after
dehydration and water loading.
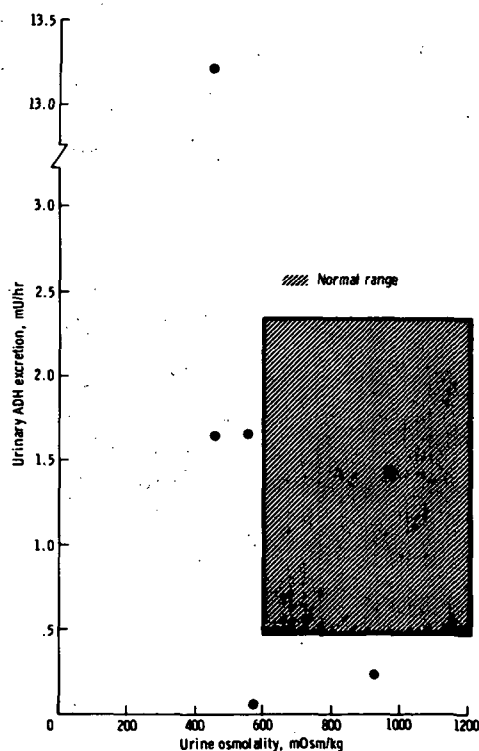
Figure 3-10.- Urinary ADH excretion and osmolality in 24-hour urine collec-
tions from six patients with the inappropriate ADH syndrome (solid
dots). The normal range is indicated by the shaded area.
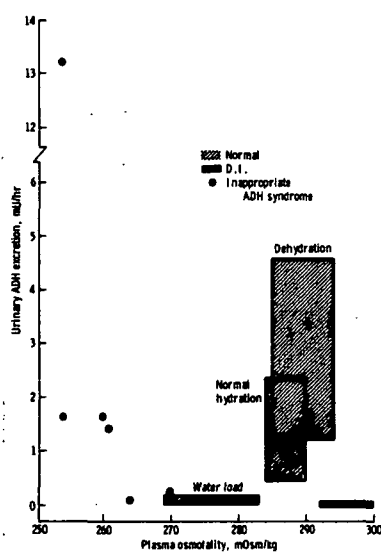
Figure 3-11.- Correlation of urinary ADH excretion with plasma osmolality in normal subjects during various states of hydration and in patients with the inappropriate ADH syndrome.
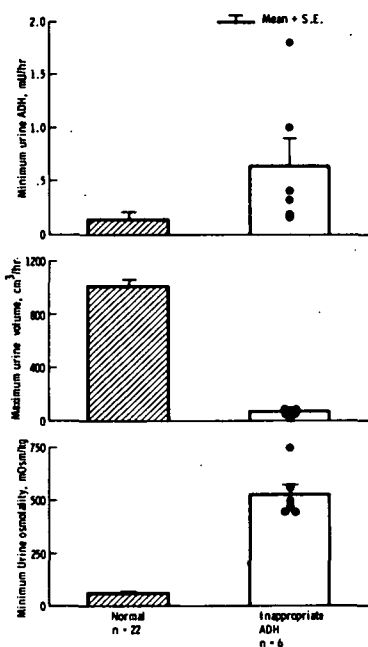
Figure 3-12.- Response of urinary ADH excretion, volume, and osmolality to oral water loading. The response in patients with the inappropriate ADH syndrome (right) is compared with that in normal subjects (shaded area on the left).

4. ESTIMATION OF VASOPRESSIN EXCRETION IN THE URINE AS A METHOD OF

MONITORING VASOPRESSIN SECRETION DURING SPACE FLIGHT

By Walter H. Moran, Jr., M.D.

INTRODUCTION

The monitoring of the urinary excretion of antidiuretic hormone (ADH, arginine vasopressin) as a measure of ADH neurosecretory activity during space flight might be considered to be a step backward or, at best, a temporary expedient until better methods for the estimation of plasma ADH levels are available. In the discussion that follows, it will be demonstrated that, under the circumstances of space flight, the measurement of plasma ADH levels might be misleading and that only the urinary ADH levels would provide reliable information. The results of a partially completed survey of ADH levels in urine samples from human subjects in which simultaneous plasma ADH levels were available also are included.

DISCUSSION

The monitoring of urinary ADH levels during space flight was not considered seriously until 1969, when a change in plans was caused by technical necessity rather than by theoretical considerations. Until that time, considerable effort had been spent in developing and testing techniques for the preservation of ADH in plasma samples during long space flights. To determine the magnitude of the ADH levels that might be encountered, plasma samples were obtained from the Apollo 9 crewmen before lift-off and at various intervals after recovery. These determinations were unsatisfactory technically because the volume of plasma remaining after aliquots for other important tests had been removed was insufficient for the number of replicates required for the biological assay used to determine significant changes at the ADH levels encountered. A second factor making the determinations technically unsatisfactory was the presence of a substance that produced an osmotic diuresis of short duration in the rats used for the bioassay and made a quantitative interpretation of the antidiuretic responses difficult. Furthermore, the range of plasma ADH levels encountered fell along that portion of the biological dose-response curve where very small changes in ADH levels are

associated with large changes in urine flow, making it doubtful that even an immunoassay requiring only 1 cubic centimeter of plasma would be sufficiently precise without at least four or five replicates. Because of considerations, the use of plasma samples was abandoned and attention was focused on sampling urine.

The use of such indirect parameters as the classical change in "free-water clearance" (CHOH) was considered briefly because of the possibility of inflight measurement by electronic means. Before adequate assays for vasopressin were available, changes in CHOH frequently were used to monitor ADH secretion. At that time, investigators fully understood that the results obtained were valid only if the subject were artifically maintained in a state of diuresis; in other words, the urinary solute concentration was less than or equal to that of the plasma solute concentration. Investigators also recognized that a maximum urinary concentration existed that could not be exceeded, no matter how much ADH was administered. The osmolal clearance associated with this maximum urinary concentration was called the "osmotic floor" of urine excretion. Because urine is almost always hypertonic in normal individuals and might be expected to be hypertonic during some periods of space flight, the concept of monitoring CHOH was not considered further. The only remaining possibility was to monitor the urinary excretion of ADH.

The estimation of urinary ADH levels was used extensively during the 1950's, before satisfactory plasma methods had been developed. However, investigators were skeptical of the values obtained by the estimation. Because only 10 percent of injected ADH appeared in the urine, investigators felt that the urinary ADH levels would be very susceptible to changes in rates of hepatic detoxification and renal clearance and might not accurately mirror changes in ADH secretion. These criticisms are valid and must be kept in mind when interpreting urinary ADH levels, but, as will be mentioned later, these same criticisms also apply equally well to plasma ADH levels.

By the early 1960's, adequate methods had been developed to allow measurement of ADH in reasonably large samples of human plasma. In these methods, an initial step of concentration and desalting on an ion-exchange resin was followed by an intravenous antidiuretic bioassay in a rat. Within the next few years, a great deal of information was gained using this type of assay; however, no new receptors or control systems were discovered as a result of these studies. Most of the new information concerned the quantitative aspects of ADH secretion, which had been greatly underestimated when the renal tubule was the only indicator. This new information led to new concepts concerning the methods by which the ADH control systems integrate their functions.

The first important concept to emerge was that of a hierarchy of closed-loop control systems based on the biological effectiveness of ADH on the target organ affecting the controlling sensor. It was found that purely osmotic stimuli could modulate blood ADH levels over a range from 0 to 6 $\mu U/cm^3$. This range also corresponded to the range in which the renal tubule could respond to blood ADH levels (by reducing urinary output) by conserving water alone. The response was exponential, with the largest changes in urinary volume occurring in conjunction with the smallest changes in plasma ADH levels at the lower limits of plasma concentration. As plasma levels rose and approached the tubular limit, larger and larger changes produced smaller and smaller changes in urinary volume until the "osmotic floor" was reached. This reaction implied that the osmoreceptor control system is a closed-loop feedback system that depends on the biological effectiveness of ADH on the renal tubule to close the loop. The stiffest point of control was about the point where urine was isotonic with plasma and at a point where plasma ADH levels were the most difficult to determine. If the renal tubules could not respond to ADH, as in the case of nephrogenic diabetes insipidus, ADH secretion was not inhibited and control passed to the next system in the hierarchy, the left-atrial low-pressure volume-control system. This system can modulate blood ADH levels from 0 to 60 $\mu U/cm^3$ and, therefore, can control renal tubular water reabsorption as well as control the stretch of the left atrium by means of rerouting the visceral blood flow. The third closed-loop control system of the hierarchy consists of the baroreceptor system, which can modulate ADH blood levels over a range of 0 to 600 $\mu U/cm^3$ or greater and which feeds back through the vasoconstrictor effect of ADH on large vessel walls. Unfortunately, urinary ADH levels were not checked during these investigations, so it is impossible to tell if urinary ADH excretion approached a limit as visceral blood flow was altered. Usually, the urinary output was very low during these periods.

The second concept to emerge from studies involving the use of plasma ADH levels to monitor ADH secretion was the discovery of the extremely transient nature of blood-level elevations and the speed of the neurosecretory response or release to stimuli. The maximum ADH blood level obtainable from a single 1-minute step input had occurred before the stimulus was completed. The biological half life of ADH in the blood stream was approximately 3 minutes, indicating that sampling at least once per minute would be required in order to adequately monitor a secretory-response pattern. The effect of changes in the rates of hepatic detoxification, the volume of distribution caused by changes in the state of hydration, and the changes in renal clearance could have a significant effect on plasma ADH without necessarily mirroring changes in ADH release from the neurohypophysis. A significant rise in the plasma ADH of a crewman during the entry of a space vehicle might be dissipated before a blood sample could be drawn, but the increased amount of ADH cleared by the kidney would still be in the urinary bladder.

The last point in the previous paragraph is illustrative of one of the most important advantages of measuring urinary ADH levels, that of cumulative integration. The best example of the usefulness of this technique as far as vasopressin is concerned occurred in 1953, when Ames (ref. 1) reported that she had observed extremely high levels of ADH in the urine of infants shortly after birth but was unable to detect any ADH thereafter until the third or fourth month of life. Because these results had been obtained from urinary determinations and could not be reconciled logically with any current point of view, the high urinary ADH levels after birth generally were disregarded. This point of view was reaffirmed further when early attempts to measure plasma ADH levels in infants revealed that ADH could not be detected in the plasma until the third or fourth month of life (ref. 2). However, when more sensitive methods for measuring plasma ADH levels were applied, the transitory increase at birth of the ADH secretion, which had been integrated in the urine, was confirmed (ref. 3).

Another advantage of monitoring urinary ADH levels is that the concentration of ADH in the urine is at least 1000 times that found in plasma, allowing for precise determinations in small aliquots using currently available immunoassay techniques, such as the one described by Dr. Myron Miller in the previous paper. Finally, none of the information gained by using urinary ADH determinations has been subsequently disproven by use of the plasma ADH assay.

The principal problems to be faced in applying the urinary ADH assay are the degradation of ADH in the bladder before excretion, the presence of interfering substances, and the effect of reduced renal clearance on the excretory limit of ADH.

RESULTS

Since 1962, aliquots of urine from patients undergoing studies of their plasma ADH levels have been collected, frozen, and stored. Recently, a program of determining the ADH content of the samples was undertaken to ascertain the range of urinary ADH levels that might be encountered in pathological conditions and to see if any relationship exists between urinary ADH excretion and plasma ADH levels. There were no normal subjects in this group.

The urinary assay of ADH consisted of a modification of the plasma ADH bioassay procedure. The urine samples, which had been frozen without the addition of any preservatives, were thawed rapidly and extracted with 2 volumes of ether. Then, the washed urine was adjusted to a pH of 4.2 and was added to a column of CG-50 resin. From this point on, the

procedure was identical to the plasma bioassay. The extracts of urine produced antidiuretic responses that were similar to those produced by the arginine vasopressin reference standards. The high levels in some of these samples were indicative that very little (if any) ADH had been lost during prolonged storage. At present, 90 urine samples have been assayed, 57 of which had plasma ADH levels timed so that ADH clearances could be calculated.

Five patients might be classified as having diabetes insipidus (DI) (table I). The DI-1 and DI-2 patients had pulmonary carcinoma and metastases to the brain. The DI-3 patient had a carcinoma of the thyroid and cerebral metastases as a cause of her DI. The DI-4 patient was a boy with DI, and DI-5 was a diabetic who had severe retinopathy and was undergoing a pituitary-stalk section. The urinary ADH levels were very low in this group, ranging from not detectable to 9.4 $\mu U/cm^3$. Plasma samples were taken infrequently because of the difficulty in detecting ADH levels in DI patients. The high plasma ADH level in DI-2 occurred after a dose of vasopressin, and the high plasma levels in DI-5 occurred during the surgical procedure. The mean urinary ADH excretion rate in DI without an open loop stimulus was 9.93 ± 0.1 $\mu U/min$ (N = 12). The mean urinary ADH excretion, when injected pitressin had achieved a clinically satisfactory response (DI-2), was 11.6 ± 0.9 $\mu U/min$ (N = 7).

Two surgical patients (SURG-1 and SURG-2) had samples taken while they were undergoing surgical procedures (table II). A partial gastrectomy was performed on SURG-1; a partial colectomy was performed on SURG-2. Their mean urinary ADH excretions were 339 ± 58 (N = 6) and 241 ± 29 (N = 6) $\mu U/min$, respectively.

Four patients had the inappropriate antidiuretic hormone secretory (IADHS) syndrome (table III). The cause of the inappropriately high ADH levels in IADHS-1 was severe pulmonary emphysema. Although his urinary excretion rate (8.6 ± 3 $\mu U/min$ (N = 4)) and plasma levels (2.6 ± 0.4 $\mu U/cm^3$ (N = 4)) might be considered to be within the normal range, they were definitely too high for his plasma osmolality of 240 mOsm/μg and were unresponsive to water loading. The IADHS-2, IASHA-3, and IADHS-4 patients had oat-cell pulmonary carcinomas. Their urinary ADH excretion rates were quite high (411, 50.1, and 224 $\mu U/min$) and were unresponsive to water loading.

The average values for urinary ADH excretion, plasma ADH concentration, and renal clearance of ADH are listed in table IV. The ADH clearance data of the DI patients were insufficient to warrant any conclusion. The ADH clearance values of all of the patients except IADHS-2 varied between 3.7 and 11.5 cm^3/min but were not really significantly different. The 43.5-cm^3/min clearance of IADHS-2 cannot be explained at present.

Careful examination of the data in tables I, II, and III revealed that small changes in urinary flow in patients with low urinary outputs were associated with small changes in urinary ADH excretion that were directly proportional and were probably related to small changes in renal clearance. If the urinary output was high and not completely osmotic, the urinary flow was inversely proportional to ADH excretion (DI-2 and DI-5).

A plot of all 57 urinary ADH excretion rate/plasma ADH concentration value pairs (fig. 1) demonstrated a fairly linear relationship between them. Five of the points from SURG-1 fell far above the line and represented a period when both renal and hepatic clearance could have been reduced.because of events surrounding the surgical procedure. The other point for this patient.fell on the plotted line and represented the highest urinary ADH excretion rate observed (616 μU/min). The points from IADHS-2 fell below this line, as was mentioned earlier. In general, the urinary ADH excretion rates paralleled plasma ADH concentrations, giving an average renal clearance of ADH of approximately 10 cm^3/min.

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TABLE 4-I.- DIABETES INSIPIDUS

Patient	Urine tests				Plasma tests	Renal clearance rate, ADH
	Duration[a], min of test	Rate[b], cm^3/min V	ADH, $\mu U/cm^3$ U	ADH, $\mu U/min$ UV	ADH, $\mu U/min$ P	cm^3/min UV/P
DI-1	720	0.67	1.4	1.0	0.0	--
	720	1.39	1.2	1.6	--	--
DI-2	60	18.92	.6	11.0	--	--
	60	10.83	.3	3.2	6.3	0.5
	60	6.33	1.1	7.1	--	--
	60	4.25	3.3	14.2	--	--
	60	3.00	4.6	13.6	--	--
	60	2.67	4.0	10.6	--	--
	60	2.33	5.5	12.8	--	--
	60	1.65	7.3	12.0	--	--
DI-3	60	.93	.6	.6	--	--
	15	1.53	.3	.5	--	--
	15	1.40	.3	.3	.7	.5
	15	2.67	N.D.[c]	--	--	--
	15	4.33	N.D.	--	--	--
	15	4.80	N.D.	--	--	--
	15	4.80	N.D.	--	--	--
	15	5.40	N.D.	--	--	--
	15	4.67	N.D.	--	--	--
DI-4	60	1.50	1.4	2.1	--	--
	60	1.80	.3	.6	--	--
	60	1.08	.7	.8	--	--
	60	.50	1.4	.7	--	--
	60	.83	1.2	1.0	--	--
	60	1.25	.9	1.1	--	--
	60	1.00	.8	.8	--	--
DI-5	240	.73	1.9	1.4	12.8	.1
	120	.82	9.4	7.7	6.8	1.1
	120	.53	7.5	4.0	--	--
	120	.53	3.8	2.0	3.1	.7
	360	.90	5.3	4.8	2.7	1.8
	360	3.32	.8	2.8	--	--

[a]Length of time of pool or interval of sampling.

[b]Amount of urine excreted in 720 minutes.

[c]N.D. = Not detectable.

TABLE 4-II.- SURGICAL PROCEDURE

| Patient | Urine tests | | | | Plasma tests | Renal clearance rate, ADH |
	Duration[a], min	Rate[b], cm^3/min V	ADH, $\mu U/cm^3$ U	ADH, $\mu U/min$ UV	ADH, $\mu U/cm^3$ P	cm^3/min UV/P
Surg-1	30	1.30	474.	616.	41.2	15.0
	30	2.47	118.	291.	44.2	6.6
	30	4.17	81.3	339.	41.0	8.3
	30	1.90	117.	222.	54.2	4.1
	30	1.83	135.	248.	54.5	4.5
	30	1.30	243.	315.	45.7	6.9
Surg-2	30	1.83	149.	273.	21.8	12.5
	30	5.03	59.0	297.	24.7	12.0
	30	8.63	29.1	251.	17.5	14.4
	30	7.83	29.2	229.	23.7	9.7
	30	6.23	47.0	293.	20.8	14.1
	30	--	--	--	--	--
	30	1.12	96.9	108.	16.7	6.5

[a]Length of time of pool or interval of sampling.

[b]Amount of urine excreted in 720 minutes.

TABLE 4-III.- INAPPROPRIATE ADH SECRETORY SYNDROME

| Patient | Urine tests | | | | Plasma tests | Renal clearance rate, ADH |
	Duration[a], min	Rate[b], cm^3/min V	ADH, $\mu U/cm^3$ U	ADH, $\mu U/min$ UV	ADH, $\mu U/cm^3$ P	cm^3/min UV/P
IADHS-1	1440	0.44	17.5	7.6	1.5	5.0
	1440	.29	48.9	14.3	2.4	6.0
	1440	.34	33.3	11.3	3.6	3.2
	1440	.10	12.5	1.3	2.7	0.5
IADHS-2	720	.93	359.	334.	11.7	28.5
	720	.85	340.	288.	9.7	29.7
	720	1.21	445.	537.	9.1	58.9
	720	1.46	341.	497.	13.8	36.1
	720	1.85	283.	522.	8.5	61.8
	720	1.95	245.	475.	--	--
	720	1.31	339.	442.	9.9	44.7
	720	1.33	342.	455.	--	--
	720	.95	295.	278.	6.3	44.8
	720	.93	307.	285.	--	--
IADHS-3	720	.57	73.5	41.8	4.7	8.9
	720	1.04	58.4	60.4	5.8	10.5
	720	.74	71.0	52.3	5.5	9.6
	720	1.00	45.4	45.4	6.5	7.0
	720	.61	70.2	42.9	5.9	7.3
	720	.88	46.7	41.2	4.2	9.8
	720	.73	63.6	46.4	5.0	9.3
	720	1.18	44.5	52.5	5.9	8.9
	720	.46	56.4	25.8	4.7	5.5
	720	1.15	54.6	62.9	5.1	12.3
	720	.57	58.4	33.2	6.3	5.3

[a]Length of time of pool or interval of sampling.

[b]Amount of urine excreted in 720 minutes.

TABLE 4-III.- INAPPROPRIATE ADH SECRETORY SYNDROME - Concluded

Patient	Duration[a], min	Rate[b], cm^3/min V	ADH, $\mu U/cm^3$ U	ADH, $\mu U/min$ UV	ADH, $\mu U/cm^3$ P	cm^3/min UV/P
			Urine tests		Plasma tests	Renal clearance rate, ADH
IADHS-3	720	1.13	47.7	53.6	5.3	10.2
	720	1.29	39.7	51.2	6.9	7.4
	720	1.25	43.6	54.4	8.7	6.3
	720	2.08	30.3	63.0	8.3	7.6
	720	1.46	51.0	74.4	9.7	7.7
	720	.82	68.6	56.2	6.5	8.7
	720	.96	64.3	61.6	6.5	9.5
	720	.46	104.	47.6	7.0	6.8
	720	.54	64.3	34.8	--	--
IADHS-4	720	.80	230.	184.	13.7	13.4
	720	1.29	177.	228.	16.1	14.2
	720	1.04	181.	189.	20.1	9.4
	720	1.53	174.	265.	17.3	15.4
	720	2.64	125.	329.	18.7	17.7
	720	3.11	116.	361.	--	--
	720	.78	231.	180.	21.0	8.6
	720	1.10	226.	248.	--	--
	720	.61	198.	119.	21.1	5.7
	720	.83	257.	214.	--	--
	720	.60	244.	146.	21.2	6.9
	720	.78	283.	220.	--	--

[a]Length of time of pool or interval of sampling.

[b]Amount of urine excreted in 720 minutes.

TABLE 4-IV.- AVERAGES FROM TABLES I, II, AND III

Patient	Urine ADH, μU/min UV	Plasma ADH, μU/cm^3 P	Renal clearance rate, cm^3/min UV/P
DI-1	1.3 + 0.3(N=2)	0.0	--
DI-2	12.6 + .63(N=5)	6.3	0.5
DI-3	.5 + .1(N=3)	0.7	0.5
DI-4	1.0 + .2(N=7)	--	--
DI-5	3.8 + .9(N=6)	6.4 + 2.(N=4)	0.9 + 0.6(N=4)
SURG-1	339. + 58(N=6)	46.8 + 2.5(N=6)	7.6 + 1.6(N=6)
SURG-2	241. + 29(N=6)	20.9 + 1.3(N=6)	11.5 + 1.2(N=6)
IADHS-1	8.6 + 3.(N=4)	2.6 + 0.4(N=4)	3.7 + 1.2(N=4)
IADHS-2	411. + 33(N=10)	9.9 + .9(N=7)	43.5 + 5.0(N=7)
IADHS-3	50.1 + 2.6(N=20)	6.2 + .3(N=19)	8.4 + 0.4(N=19)
IADHS-4	224. + 20(N=12)	18.7 + .97(N=8)	11.4 + 1.5(N=8)
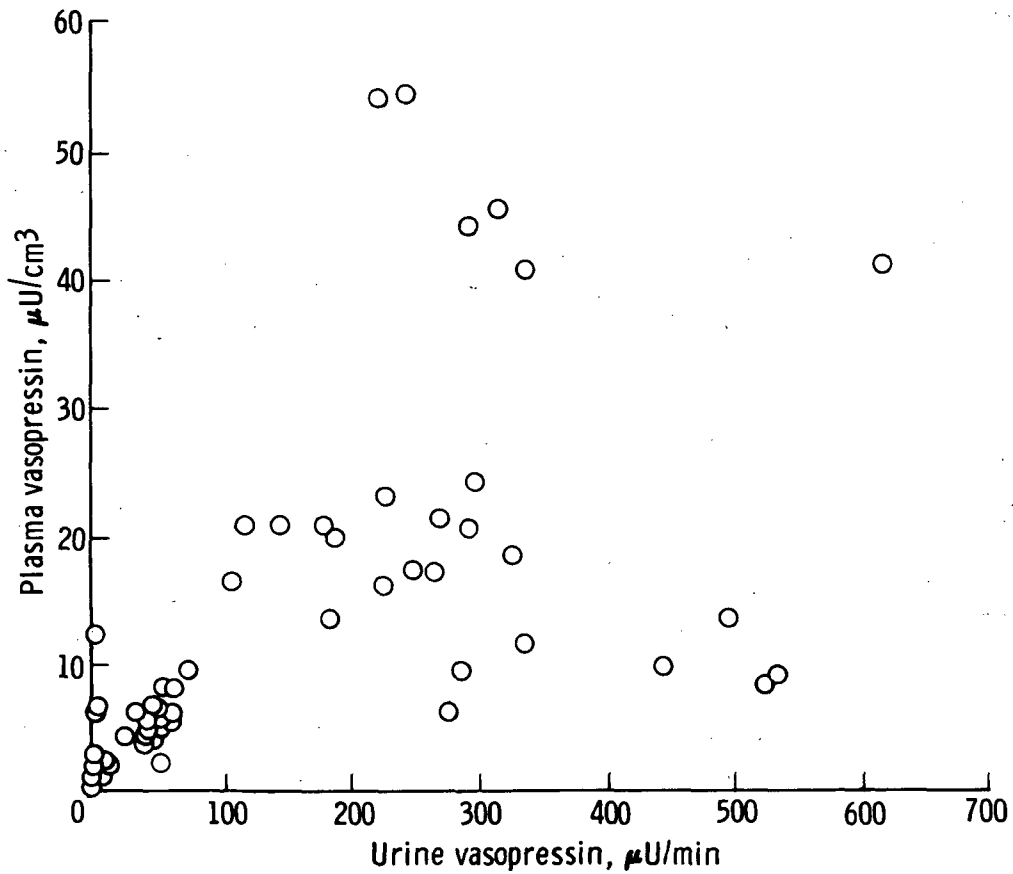
Figure 4-1.- A plot of the 57 urinary ADH excretion rate/plasma ADH con-
centration pairs. The cluster of five points between 200 to 350 µU/min
and above 40 µU/ml represents SURG-1. The cluster of seven points be-
tween 250 to 550 µU/min and below 15 µU/ml represents IADHS-2.

5. ADRENOCORTICOTROPHIC HORMONE LEVELS

IN GROUND BASED STUDIES

By Bonnalie O. Campbell, Ph. D.

INTRODUCTION

Beginning with Project Mercury, the function of the adrenal gland has been considered an important factor in the adaptation of man to space flight. A study of the pituitary-adrenal axis has offered the potential of assessing human metabolic response to the possible mental/physical stresses of prolonged space flight.

An investigation of the role of adrenocorticotrophic hormone (ACTH) in the pituitary-adrenal axis has established normal circulating values for this hormone in both plasma and serum samples. Levels of ACTH have been determined for a series of ground-based studies of imposed work/rest cycles.

METHODS

Immunoreactive ACTH was measured directly using human venous plasma and serum. Duplicate samples of standards (Lerner human ACTH) and plasma-EDTA or serum were extracted and assayed using the method described by Donald (ref. 1). Rabbit antihuman serum was used at final dilutions of 1:20 000 and 1:30 000. The labeled ^{125}I human ACTH was repurified each time before use. The total incubation time was 4 to 5 days at 277° K (4° C). A dextran-coated charcoal preparation was used for separation. Both supernatant and charcoal fractions were counted for 10 minutes. Calculations were based on precent binding compared with B/F ratios (fig. 1).

DISCUSSION

To establish normal baseline levels of ACTH secretion for healthy human adults, only blood samples collected under similar conditions were analyzed. Blood was drawn during the morning and afternoon hours under

conditions normally not considered conducive to stress. A study of circulating plasma levels of ACTH in 105 healthy employed adults is summarized in table I. The mean level of plasma ACTH for all subjects was 23.1 pg/cm^3. The mean value of ACTH was 25.0 pg/cm^3 for 76 males ranging in ages from 24 to 61 years. The mean value for 29 females, ranging in ages from 16 to 45 years, was 18.1 pg ACTH/cm^3. When the data were separated into morning and afternoon values, there was an apparent elevation of levels of ACTH secretion in the afternoon for both sexes and higher values during both periods were recorded for males (table II).

When the data were separated on the basis of sex, the level difference was shown to be nonexistent when the samples that were collected under identical conditions and that were closely related in time were analyzed (table III). Seventy-five employed individuals of both sexes were tested in a routine situation (an annual physical examination). The samples were obtained between 0745 and 0855 and all samples were obtained by the same trained laboratory personnel. The mean level of plasma ACTH for 62 males was 20.74 pg/cm^3. The mean level of the hormone for 13 females was 20.46 pg/cm^3 (ref. 2).

A study was initiated to investigate the feasibility of the use of serum in addition to plasma-EDTA. The values for human serum were obtained under essentially the same conditions as for those cited in table III. The mean level of serum ACTH for 51 male subjects was 30.1 pg/cm^3, and there was less than 1 picogram difference between morning and afternoon values. The mean afternoon serum value for 10 female subjects was 26.4 pg/cm^3 (table IV). No morning serum samples from women were analyzed. The average for all 61 subjects was 29.5 pg/cm^3.

When plasma and serum values were compared, there was no great difference in radioimmunoreactive levels of ACTH. The mean value for 105 subjects was 23.1 pg/cm^3 plasma, and the mean serum value for 61 subjects was 29.5 pg/cm^3. There was no detectable difference in the afternoon plasma and serum levels. The mean for both afternoon values was 28.4 pg/cm^3 (table V). There was a difference in the morning values. The lowered plasma levels may be a reflection of time or method of collection for the female-subject samples not referred to in table III.

It is of interest that the mean afternoon value for ACTH did not follow the traditionally described pattern of ACTH secretion (for example, high morning levels followed by a gradual decline during the rest of the 24-hour period). If a downward trend was constant, then the afternoon values should have been significantly lower than early morning

values. ·The occurrence of additional peaks of ACTH secretion throughout a 24-hour study of four normal subjects is shown in reference 3. The relative consistency of values reported here could reflect such peaks of hormone secretion during the daytime hours.

The anticipated circadian rhythm of ACTH secretion appears to be altered when certain activities are imposed on the individual studied. In one chamber study of an abnormal work/rest cycle, the expected 24-hour rhythm of plasma ACTH was not shown (table VI). During this 3-day simulation of an Apollo lunar mission, a slight decrease in ACTH levels was noted for each succeeding day. Although the 23.9-hour rhythm of hydrocortisone secretion persisted during the study (ref. 4), the highest daily levels of ACTH were recorded after the imposed sleep period. These findings are in agreement with those of Curtis and Fogel, showing that · individuals subjected to random sleep schedules have secondary peaks of adrenal steroid secretion after sleep and also have high early morning levels.

CONCLUSIONS

Baseline values of immunoreactive ACTH were established in the normal healthy adult. Normal levels of ACTH secretion were determined for both the male and the female in circulating plasma and serum. The data obtained in these studies are particularly significant in that the sampling was carefully controlled; only healthy employed individuals of both sexes were tested in a routine work situation that would not be considered conducive to stress. These data are of additional importance because of the uniformity in time of collection and because the samples were obtained under similar conditions by the same trained personnel.

It has been found that alterations in the classically described circadian rhythm of ACTH secretion can occur when activities (such as work/rest cycles) are imposed on the individual studied. These changes can be demonstrated even when there is no appreciable change noted in the rhythm of hydrocortisone secretion.

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TABLE 5-I.— RADIOIMMUNOASSAY OF NORMAL HUMAN PLASMA ACTH

Subjects	Number	Age	Plasma ACTH range, pg/cm^3	Mean ACTH level, pg/cm^3
Males	76	24 to 61	0.19 to 127.4	25.0
Females	29	16 to 45	4.6 to 55.4	18.1

[a]Mean level of plasma ACTH for 105 subjects = 23.1 pg/cm^3.

TABLE 5-II.— MORNING AND AFTERNOON NORMAL HUMAN PLASMA ACTH LEVELS

Subjects	Number of subjects	Plasma ACTH range, pg/cm^3	Mean ACTH level, pg/cm^3
Males			
Morning	65	0.19 to 127.4	23.9
Afternoon	11	13.2 to 78.2	31.7
Females			
Morning	24	4.6 to 39.2	17.4
Afternoon	5	6.8 to 55.4	21.3

TABLE 5-III.- ACTH VALUES FOR MORNING SPECIMENS[a]

Subjects	Number of subjects	Collection times, a.m.	Age	Plasma ACTH range, pg	Mean ACTH level, pg
Males	62	7:45 to 8:40	24 to 61	0 to 120	20.74 ± 2.31
Females	13	8:10 to 8:55	16 to 45	10 to 40	20.46 ± 2.71

[a]Specimens were collected during the 7:45 to 8:55 a.m. period.

TABLE 5-IV.- RADIOIMMUNOASSAY OF SERUM ACTH[a]

Subjects	Number of subjects	Serum ACTH range, pg/cm^3	Mean ACTH level, pg/cm^3
Males			
Morning	34	11.6 to 62.2	30.3
Afternoon	17	13.4 to 45.5	29.6
Females			
Morning	0	--	--
Afternoon	10	16.9 to 50.4	26.4

[a]Mean level of serum ACTH for 61 subjects = 29.5 pg/cm^3.

TABLE 5-V.- COMPARISON OF PLASMA AND SERUM

LEVELS OF IMMUNOREACTIVE ACTH

Source of specimen	Time of specimen collection	
	Morning	Afternoon
Plasma		
Concentration, pg/cm^3	22.1	28.4
Number of specimens	89	16
Serum		
Concentration, pg/cm^3	30.3[a]	28.4
Number of specimens	34	27

[a]No values for female subjects.

TABLE 5-VI.- LEVELS OF ACTH SECRETION FOR ONE INDIVIDUAL

DURING AN IMPOSED WORK/REST STUDY

Day	Time	Preceding event	ACTH, pg/cm^3
1	0630	Sleep	40.4
	0845	2 hr 100 percent oxygen	27.8
	2020	7 hr EVA[a]	15.2
2	0505	Sleep	28.0
	0655	2 hr 100 percent oxygen	19.9
	1730	7 hr EVA	22.8
3	0230	Sleep	23.0
	0425	2 hr 100 percent oxygen	16.4
	1415	6 hr EVA	16.4
	2230	Sleep	16.2

[a]Extravehicular activity.
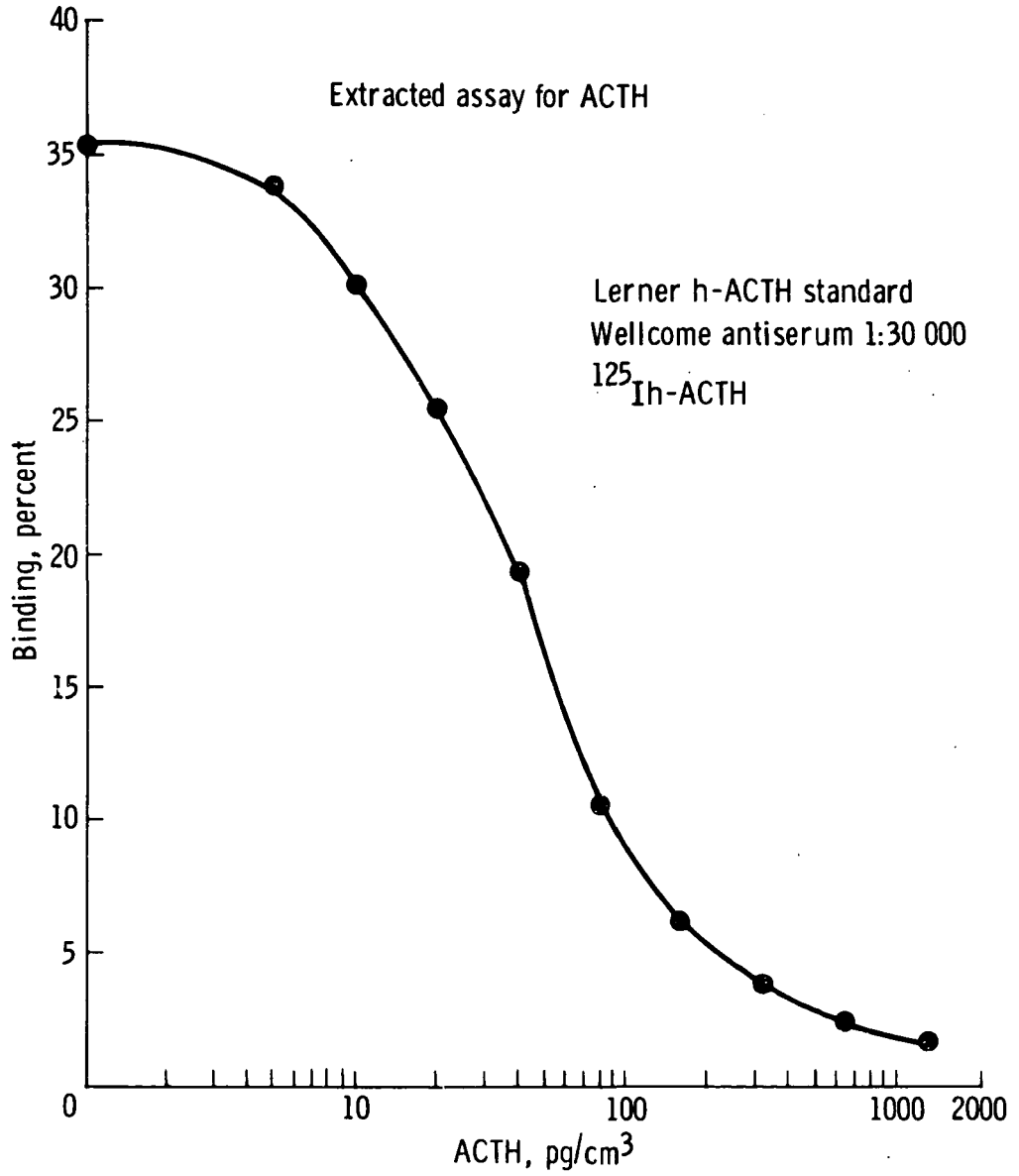
Figure 5-1.- Typical percent binding standard curve for radioimmunoassay of ACTH.

6. STUDIES OF THE RENIN-ALDOSTERONE SYSTEM AND SODIUM

HOMEOSTASIS DURING SIMULATED WEIGHTLESSNESS:

APPLICATION OF THE WATER IMMERSION MODEL TO MAN

By Murray Epstein, M.D.

INTRODUCTION

Theoretical considerations have suggested that the exposure of astronauts to prolonged periods of gravity-free environment causes abnormalities of sodium and volume homeostasis (refs. 1 and 2). Recent reports on the Gemini VII (ref. 3) and Biosatellite III orbital flights (ref. 4) have indeed borne out this concept, demonstrating a significant natriuresis and weight loss following manned space flight. If a contemplated space shuttle system with orbiting space stations necessitating prolonged human habitation is to become feasible, the heretofore unexplained severe volume depletion associated with weightlessness must be defined clearly, and specific countermeasures must be applied toward its management.

Because of the inherent difficulties in performing inflight physiological investigations, investigators have resorted to the use of the models of absolute bedrest (refs. 5 and 6) and water immersion (refs. 1 and 2) are used as analogs of the zero-g state. Both analogs are thought to produce a diuresis and natriuresis by producing a redistribution of blood volume with relative engorgement of the heart and intrathoracic vessels; these responses are similar to those that occur during weightlessness. However, the relative degree of redistribution induced by immersion is thought to exceed that induced by absolute bedrest (ref. 7). Thus, despite its limitation, the tendency of water immersion to exaggerate the anticipated hemodynamic effects of zero-g renders it an excellent investigative model for studying the circulatory mechanisms of plasma-volume control during weightlessness.

Recently, the effects of water immersion on plasma-renin activity, aldosterone excretion, and renal-sodium handling in normal man have been assessed under conditions carefully controlled for sodium and potassium balance, posture, and time of day (refs. 8 to 10).

DISCUSSION

Studies of plasma-renin activity, urinary-aldosterone excretion, and renal sodium and potassium excretion were conducted on eight subjects on the fifth, sixth, and tenth days of dietary-sodium deprivation, when all subjects achieved sodium balance (ref. 8). Control studies were conducted on day 5 of the low-salt diet and immersion studies were done on day 6 and 10. Four subjects were immersed to the level of the umbilicus (waist immersion) on day 6 and to the level of the sternoclavicular notch (neck immersion) on day 10. The remaining four subjects were immersed to the neck on day 6 and to the waist on day 10. Immersion was done in a water-tank 2.13 meters long, 1.22 meters high, and 0.71 meters wide (7 feet long, 4 feet high, and 2 feet 4 inches wide). A constant temperature of $307 \pm 0.5°$ K $(34 \pm 0.5°$ C) was maintained by two heat exchangers that had a combined output of 14 233 725 joules/hr (13 500 Btu/hr) and that was controlled by an adjustable temperature-calibrated control meter, the input to which was derived from two thermistors immersed at different water levels.

The effect of water immersion on the rate of sodium excretion $(U_{Na}V)$ is shown in figure 1. During control studies with the subjects seated but not immersed, $U_{Na}V$ was remarkably stable over the 6-hour period of the study. Waist immersion did not alter $U_{Na}V$. By contrast, neck immersion produced a progressive increase in $U_{Na}V$, beginning with hour 4 of study. At 6 hours, $U_{Na}V$ was 20-times greater than that which was documented during either control or waist immersion.

The effect of water immersion on plasma-renin activity (PRA) is shown in figure 2. Plasma-renin activity was measured in blood samples obtained at 2-hour intervals during each of the studies described. Waist immersion produced a decrease of 1/3 in PRA at 2 hours (compared with the preimmersion value), and this decrease persisted at 4 and 6 hours. Neck immersion produced an even greater suppression of PRA, and values were significantly lower than control and waist immersion both at 4 and 6 hours.

Because the observed decrease in PRA could have been the result of an immersion-mediated decrease in renin-substrate concentration, renin-substrate levels were measured. Values of renin substrate did not change significantly in subjects immersed to the neck (fig. 3), showing that suppression of PRA observed was not attributable to a decrease in substrate concentration.

The effect of water immersion on the aldosterone excretion rate (AER) was determined on pooled 2-hour urine specimens during each of the study periods; the double-derivative isotope technique of Kliman and Peterson was used. Aldosterone excretion during waist immersion was not

significantly different from the control (fig. 4). By contrast, neck immersion resulted in a 2/3 decrease in AER during hours 3 to 4, and the decrease persisted for the remainder of the study.

An examination of changes in renal-potassium handling during immersion contributes to further elucidation of the mechanism of the encountered natriuresis. During the control run, the rate of potassium excretion ($U_K V$) remained relatively stable (fig. 5). By contrast, waist immersion was associated with an increase in $U_K V$ beginning with the hour 3 and persisting thereafter. Neck immersion produced an even more profound increase in $U_K V$, exceeding both control and waist immersion during periods two through four. The documentation of an increase in $U_K V$ at a time when AER was significantly suppressed suggests that the suppression of renin and aldosterone are not likely to be the only etiologic factors in the natriuresis that was observed.

Because several lines of evidence had suggested that adrenocortico-trophic hormone (ACTH) plays a role in the physiological control of aldosterone release (ref. 11), it is not clear whether the suppression of the renin-aldosterone system during immersion was selective or represented a generalized suppression of adrenocortical secretion mediated by way of the pituitary-adrenal axis. Furthermore, because Lipman et al. (ref. 12) recently showed a generalized decrease in pituitary-adrenal reserve after 2 weeks of absolute bedrest, it was possible that adrenocortical secretion may be affected similarly during water immersion. Thus, further studies were conducted to assess the effects of water immersion on both aldosterone and 17-hydroxycorticosteroid (17-OHCS) release in normal man under identical experimental conditions of sodium and potassium balance, posture, and time of day (ref. 9).

The effect of neck-level water immersion on AER is shown in figure 6. During quiet sitting, AER was relatively stable over the 8-hour period of study, with values ranging from 4.3 to 5.0 µg/hr. By contrast, AER was significantly lower throughout the 4-hour period of neck immersion and the initial 2 hours of recovery.

Changes in PRA during neck immersion (fig. 7) paralleled those changes occurring in urinary aldosterone and showed a 30 percent decrease after 2 and 4 hours of immersion compared with the preimmersion values ($p < 0.05$). By the second and fourth hours of recovery, the level increased slightly above control levels. By contrast, immersion did not significantly alter plasma 17-OHCS levels. The mean plasma 17-OHCS levels during control ranged from an initial value of 18.7 µg percent to 14 µg percent after 8 hours, reflecting the anticipated decrease occurring during the day as a result of the diurnal rhythm in adrenal-steroid

secretion. At hours 2 and 4 of immersion, when PRA was clearly suppressed, plasma 17-OHCS was not significantly different from the control ($p > 0.3$) and, again, reflected the expected diurnal fall.

These data show that the suppression of the renin-aldosterone system occurring during neck immersion appears to be selective and not a manifestation of a generalized decrease in adrenocortical activity. Furthermore, because plasma 17-OHCS levels closely parallel changes in plasma ACTH (ref. 13), the constancy of plasma and urinary 17-OHCS observed during control and immersion periods strongly suggests that immersion under the conditions studied is not attended by significant alterations in circulating ACTH. Therefore, this study further supports the concept that the changes observed during immersion are specifically related to alterations in the renin-aldosterone system.

Although the precise mechanisms remain to be elucidated, the ability of water immersion to reproducibly suppress renin and aldosterone and to produce a significant natriuresis suggests that the water-immersion model may constitute a useful investigative tool for elucidating the mechanism of the natriuresis occurring during manned space flight and the specific countermeasure for use in its management.

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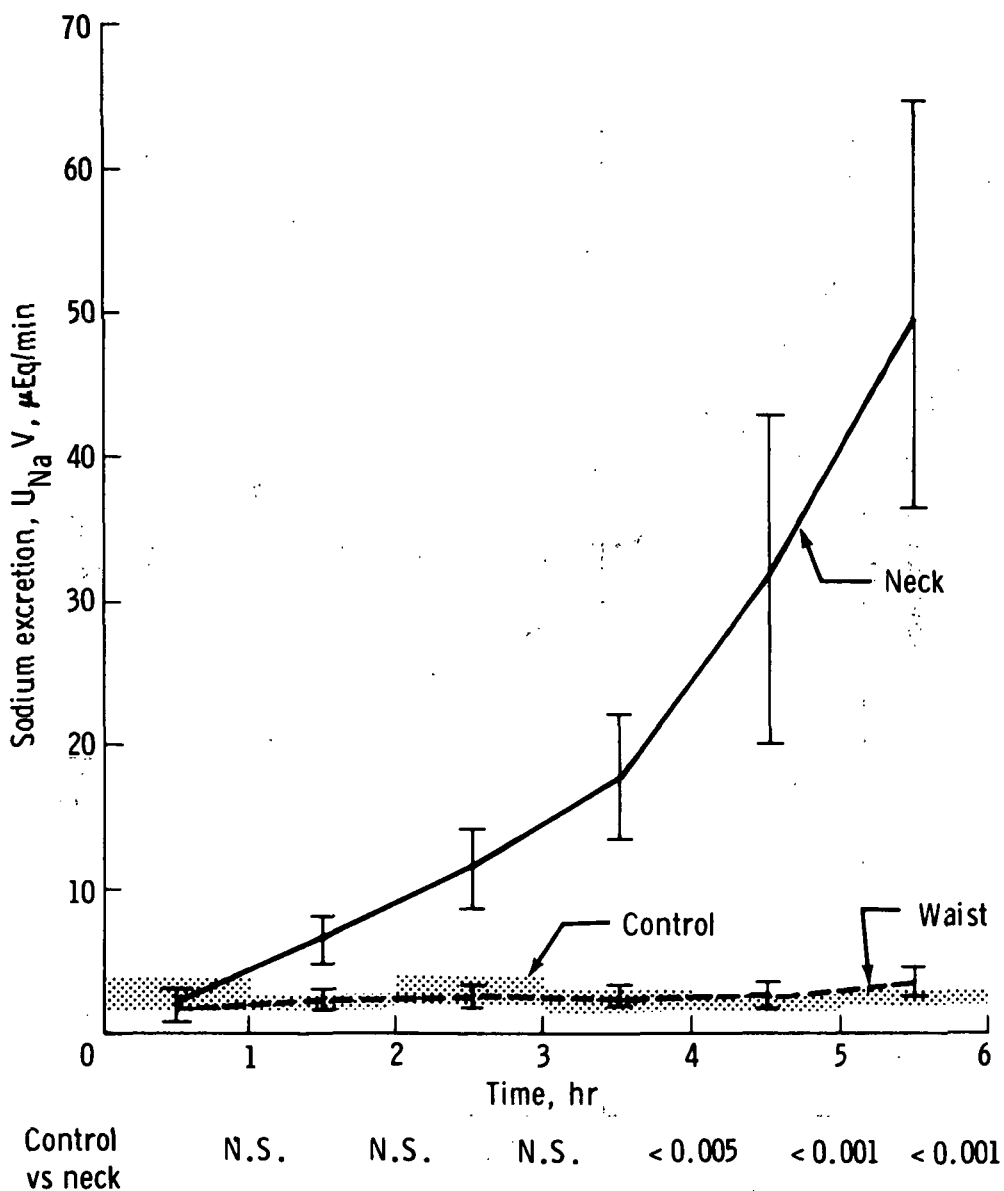
Figure 6-1.- Comparison of rates of sodium excretion during control and 6-hr water immersion for eight subjects. Note that despite identical sodium balances, $U_{Na}V$ increased significantly during neck immersion, in contrast to the stable levels of $U_{Na}V$ occurring during control and waist immersion. Brackets represent mean ± standard error (ref. 9).
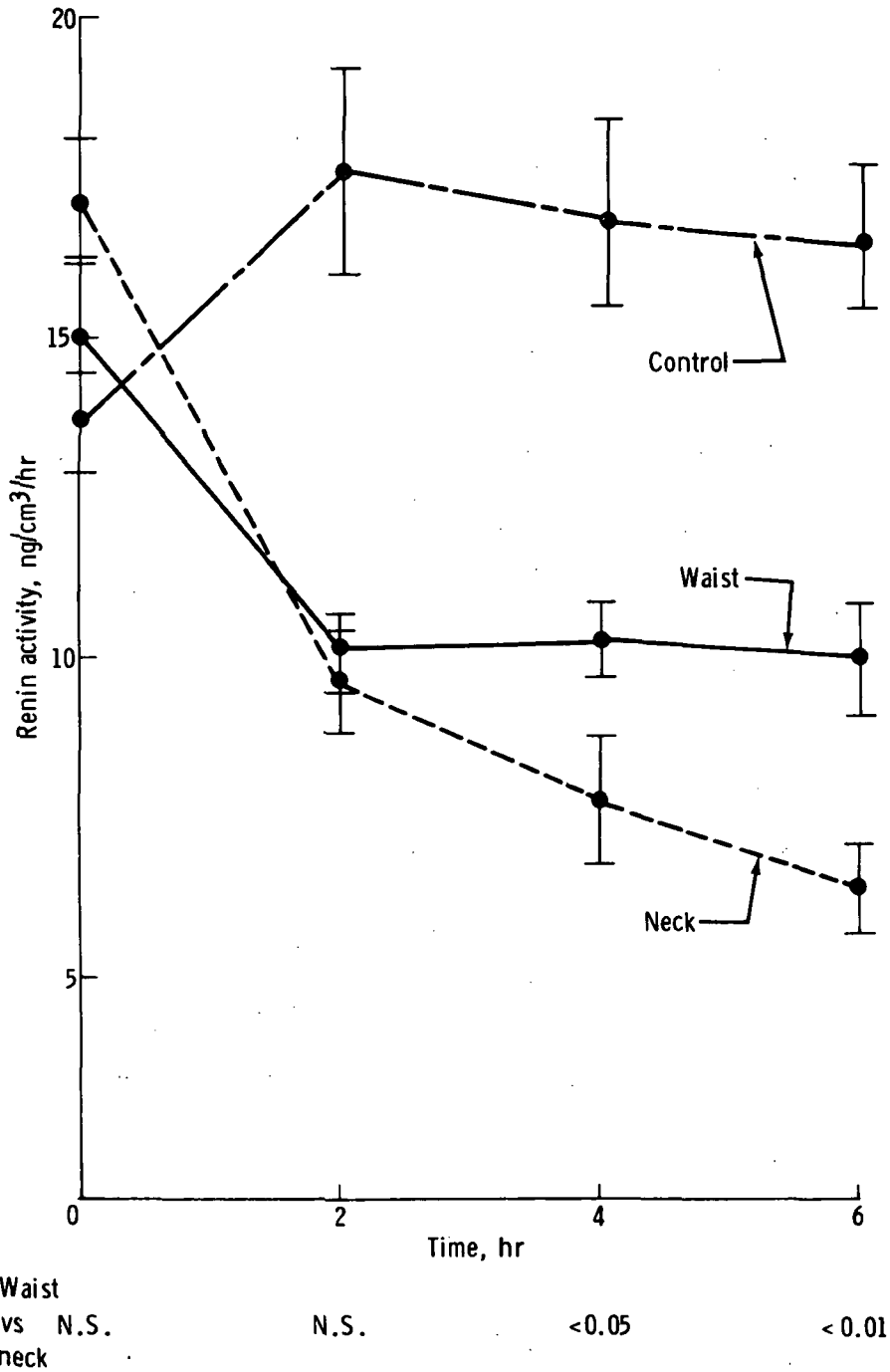
Figure 6-2.- Serial changes in PRA in seven normal subjects during control and in eight additional subjects undergoing both waist and neck immersion (6 hr). All studies were done using identical water loads, sodium and potassium balances, seated body positions, and at identical times of day. Brackets represent mean ± standard error (ref. 9).
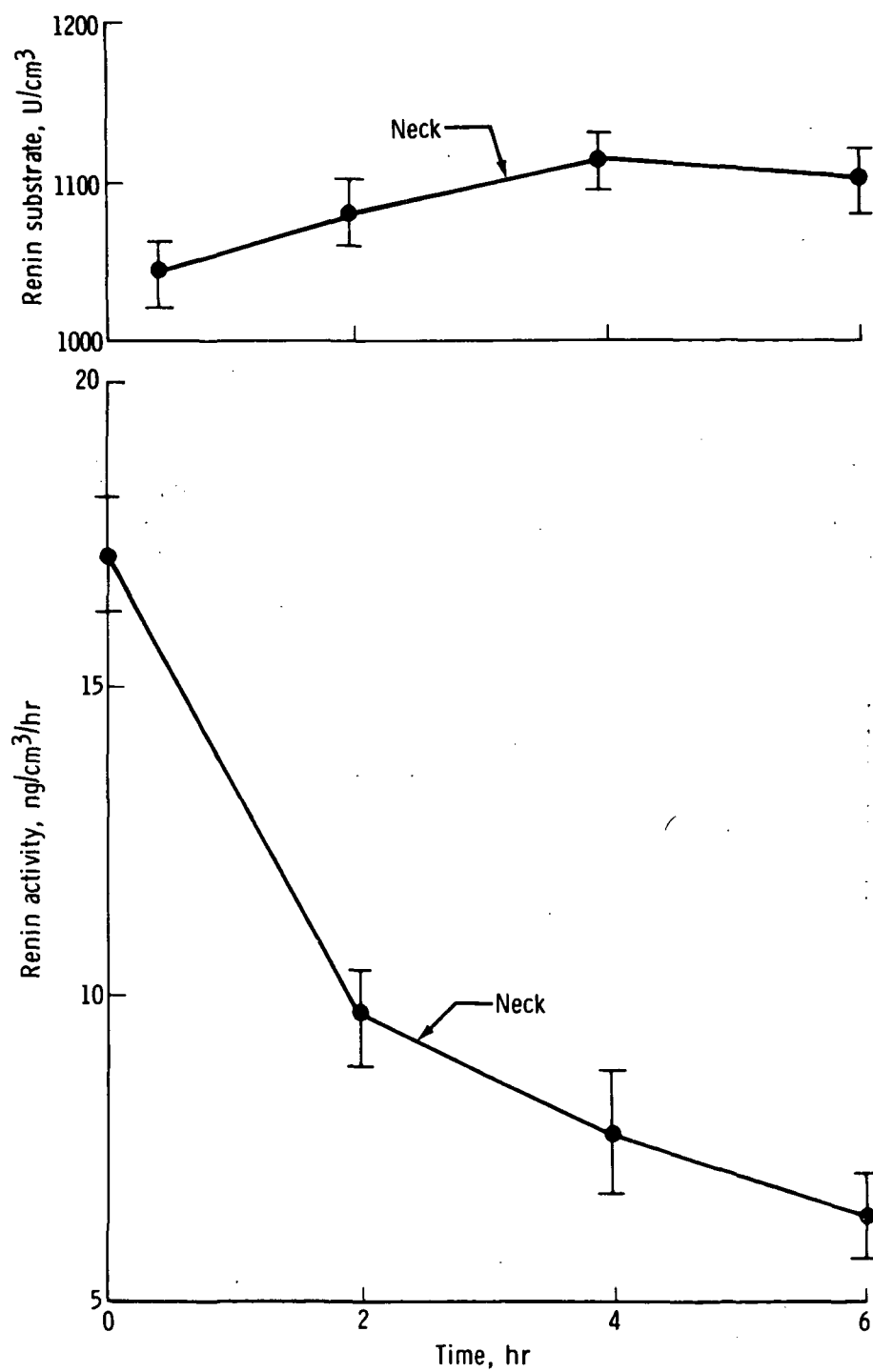
Figure 6-3.- Relationship of PRA and renin substrate during neck immersion. Note the constancy of renin substrate at a time when PRA is suppressed. Brackets represent mean ± standard error.
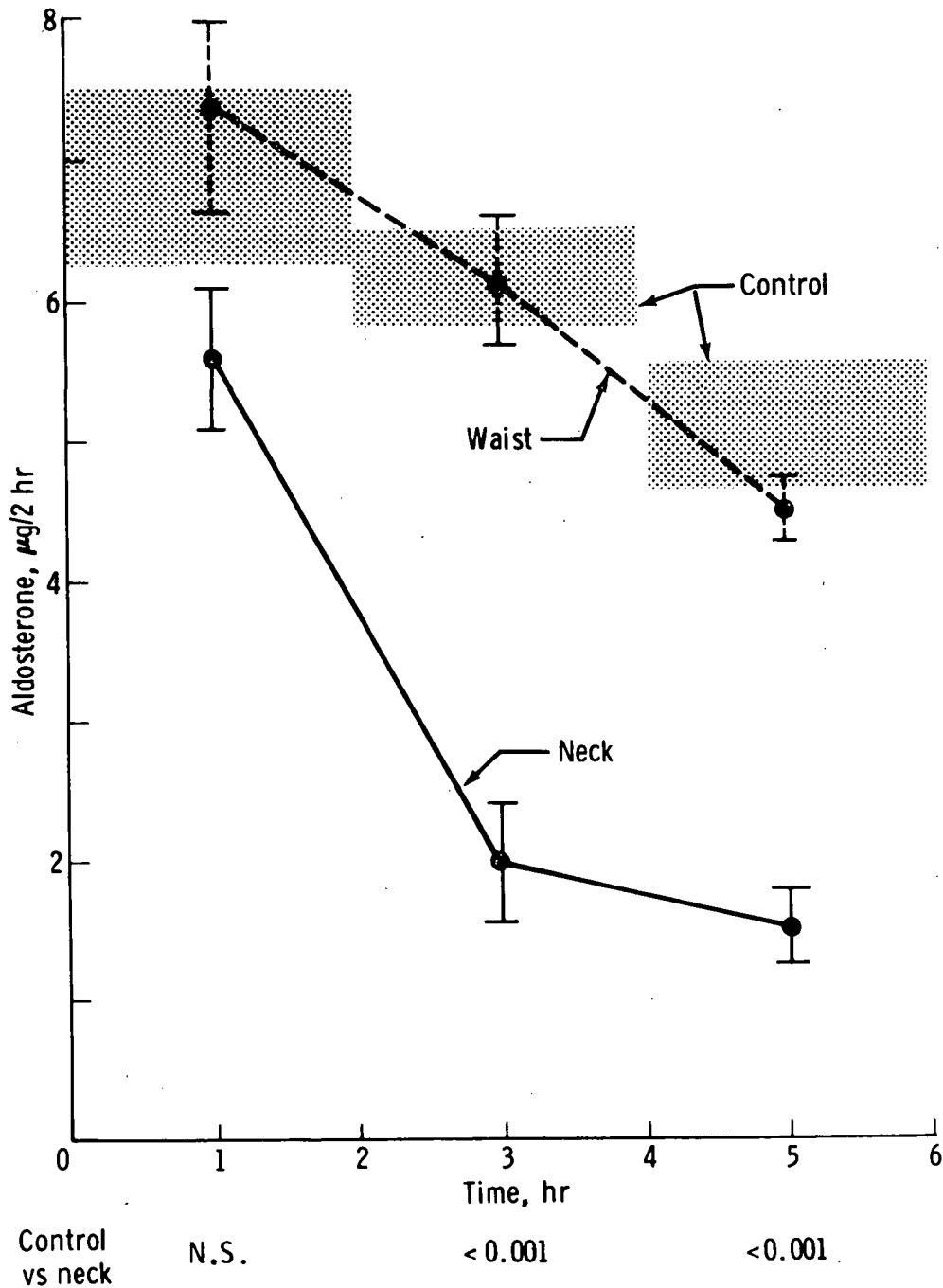
Figure 6-4.— Comparison of changes in urinary-aldosterone excretion during control and water immersion for eight subjects. Note the significant suppression of aldosterone excretion during the last 4 hours, compared with control and waist immersion (ref. 9). Brackets represent mean ± standard error.
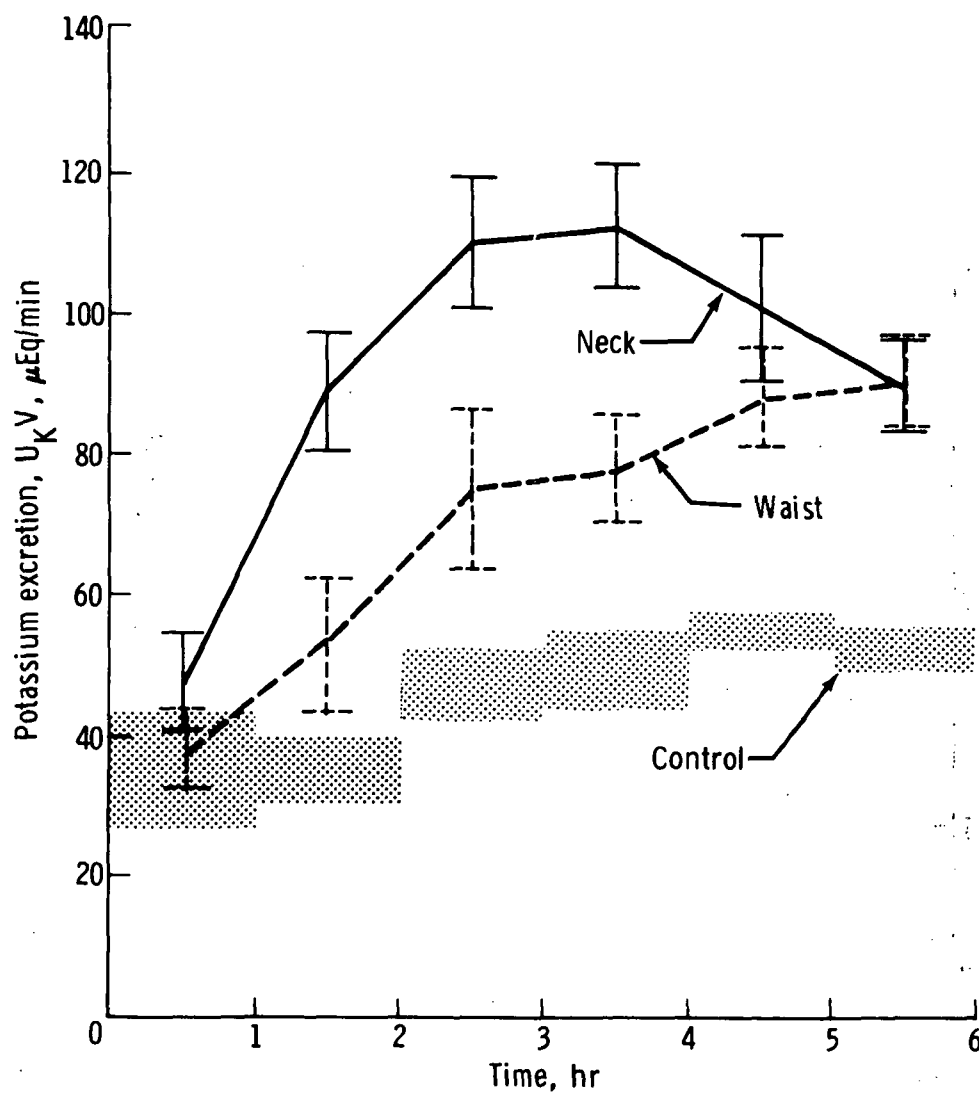
Figure 6-5.— Comparison of rates of potassium excretion during control and water immersion. Note the increases in U_KV during waist and neck immersion contrasted with control values. Brackets represent mean ± standard error.
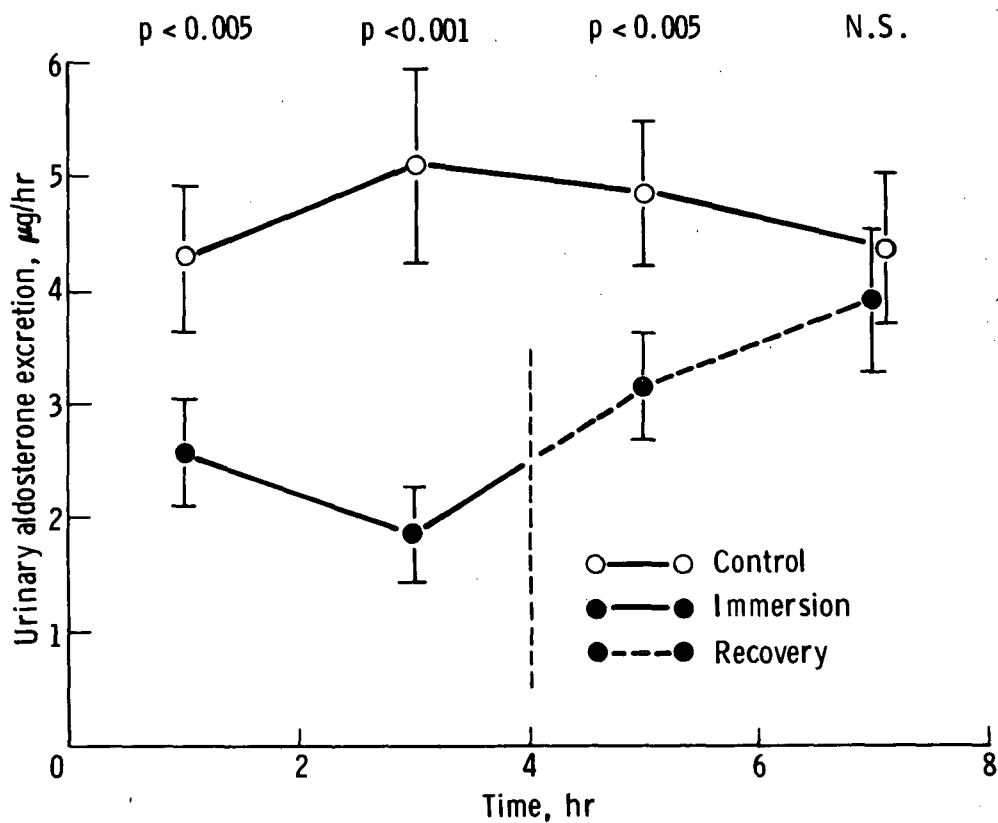
Figure 6-6.- Comparison of changes in urinary-aldosterone excretion during control and during neck-level water immersion. Aldosterone excretion was decreased significantly during immersion and during the initial 2 hours of recovery (ref. 10). Brackets represent mean ± standard error.
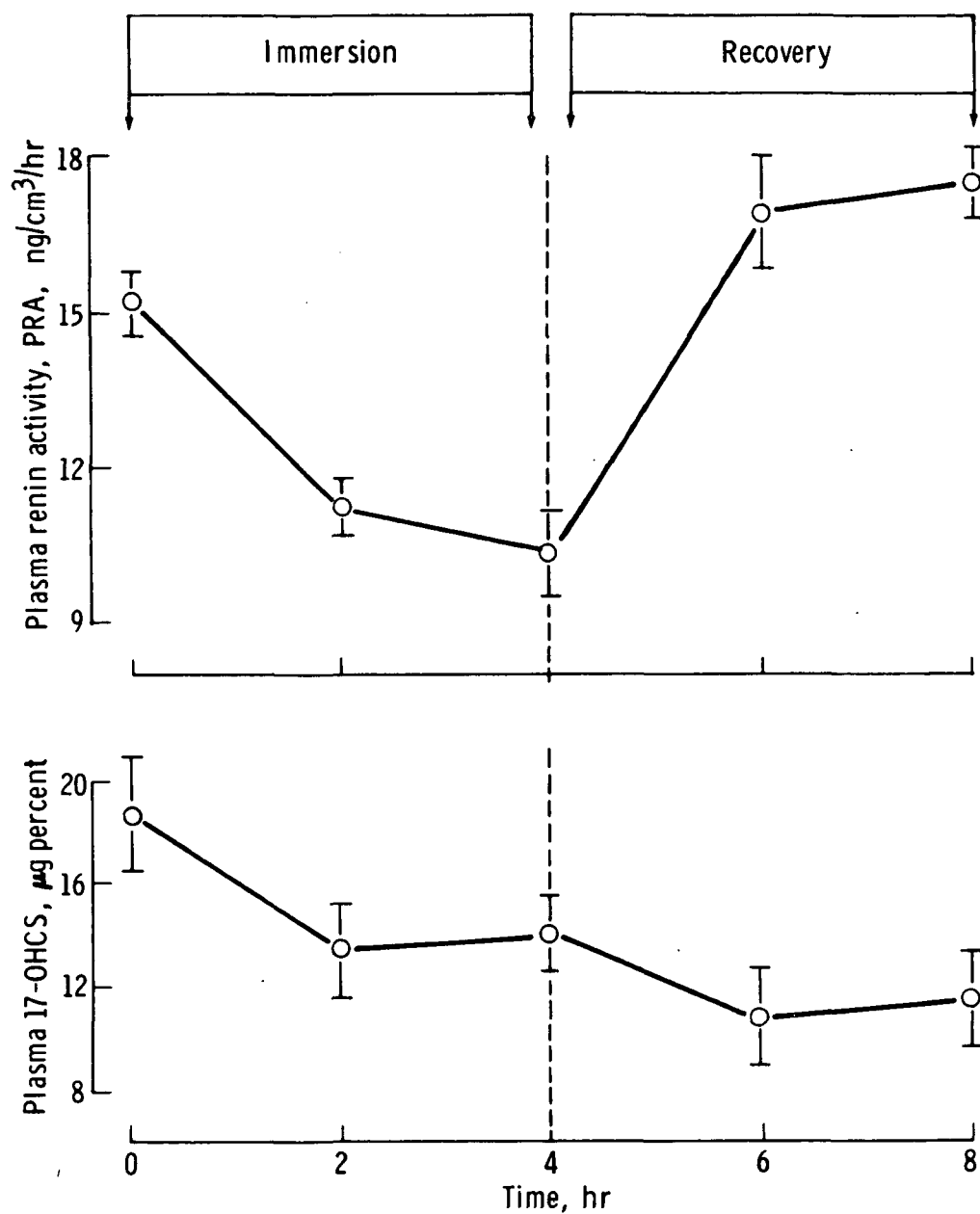
Figure 6-7.- Relationship between PRA plasma 17-OHCS in six normal sub-
jects undergoing water immersion to the neck level. The significant
suppression of PRA at hours 2 and 4 occurred independently of changes
in plasma 17-OHCS (ref. 10). Brackets represent mean ± standard
error.

7. DEVELOPMENT OF SENSITIVE AND DIRECT METHODS FOR

MEASURING PLASMA ALDOSTERONE AND CATECHOLAMINE

CONCENTRATIONS

By Edgar Haber, M.D.

INTRODUCTION

The human circulation is regulated via two major pathways: vasomotor reflexes and hormonal control. The principal hormones involved are angiotensin, aldosterone, and the catecholamines. To date, the role in homeostasis of these substances is but incompletely understood. A major obstacle has been the inability to measure blood levels conveniently and repetitively in man under a variety of physical stresses. The advent of a sensitive radioimmunoassay for renin activity, angiotensin I (ref. 1), and angiotensin II (ref. 2) has given new insight into the role of the renin system in maintaining postural homeostasis (ref. 3). This was the first demonstration that renin concentrations are changed within minutes in response to alterations in effective blood volume. Unfortunately, similar studies with aldosterone and catecholamines have not been possible because of the large volumes of blood needed for these measurements, which exclude repetitive sampling without risk of the experiment itself inducing a hemodynamic change. This report describes laboratory work contributing to the development of simple and very specific assay procedures for aldosterone and catecholamines that may be conducted on small volumes of blood.

ALDOSTERONE

Background

A number of methods are presently available that depend either on double-isotope-derivative techniques or radioimmunoassay. The former methods are relatively insensitive because of the large losses incurred, generally requiring 25-50 cubic centimeters of blood. After extraction of aldosterone, a series of chromatographic procedures is carried out which serves to separate aldosterone from other steroids. Low productivity is a consequence of the many manipulations because a single

technician generally is capable of only 20 determinations per week. Radioimmunoassay procedures have offered some improvement in sensitivity, though the specificity of the antisera is low, with the same chromatographic procedures being required before measurement.

Antibody Specificity

In figure 1, the structure of aldosterone together with two steroids of closely related structure (cortisol and corticosterone) is shown. Although it is possible to conceive of an antibody of sufficient specificity to differentiate among these compounds, the problem is magnified because the concentration of aldosterone in plasma is approximately 50 pg/cm^3, while the concentrations of corticosterone and cortisol are 10 000 and 100 000 pg/cm^3, respectively. Consequently, the measurement of aldosterone in the presence of these other compounds without prior separation requires an antibody of extreme selectivity.

The displacement of labeled aldosterone from an antibody by the unlabeled compound as well as by several other steroids is shown in figure 2 (ref. 4). This is clearly a very sensitive and selective antibody, capable of quantifying aldosterone at picogram levels and discriminating between this steroid and others by a factor of 100. Unfortunately, this degree of discrimination is insufficient when the other steroids exceed the concentration of aldosterone by factors of many thousands. Consequently, a detailed examination of three methods of coupling aldosterone to protein carriers was undertaken, and the antibodies raised to these antigens were critically studied.

Synthesis of Antigens

Aldosterone was reacted with p-hydrazinobenzoic acid, which previously had been shown to react exclusively at the Δ3-4 position (ref. 4). The carboxyl group of the hydrazone then was allowed to react with bovine γ-globulin by the use of a carbodiimide reaction.

Aldosterone was allowed to react with carboxymethyl hydroxyl oxime, under mild conditions, which formed an oxime at positions 3 and 20. The carboxyl group of the oxime then was allowed to react with bovine γ-globulin by the use of a carbodiimide condensation.

Aldosterone 21 hemisuccinate was purchased from Yeda, Inc. and subjected to a carbodiimide condensation with bovine γ-globulin. This procedure resulted in specific binding of the steroid to the protein at position 21. The site of substitution of each of these derivatives is indicated in figure 3.

Immunization

Each antigen was emulsified with complete Freund adjuvant and injected into the footpads of rabbits. Booster injections were given at two weekly intervals, and blood samples were collected before each injection

Radioimmunoassay

^{3}H-aldosterone (specific activity 20-50 Ci/mmole) was purchased from New England Nuclear Company. In a typical assay procedure, diluted anti-serum, ^{3}H-aldosterone (specific activity 20 to 50 Ci/mmole), and varying amounts of unlabeled aldosterone were incubated at 277° K (4° C) for 18 hours in a final volume of 1 cubic centimeter. At the end of this time, charcoal (Norit A) that was suspended in a dextran solution was added, and the tubes were centrifuged at 3000-g. The supernatant solution was added to Bray solution and counted in a liquid scintillation counter.

Comparison of Antisera

Typical experiments for each of the three types of antisera elicited are shown in figures 4, 5, and 6. In these plots, the displacement by aldosterone and several other steroids of ^{3}H-aldosterone from antibody is compared. Antisera elicited by either the antigen linked at position 21 (succinate) or by the antigen linked at position 3 (hydrazone) are relatively nonspecific. Although they possess adequate sensitivity (50 picograms of aldosterone will achieve approximately 50 percent displacement of label in each instance), there is sufficiently marked cross-reactivity with other steroids so that use in a direct radio-immunoassay is not feasible. However, an examination of figure 6 indicates that the antiserum raised to the dioxime antigen (aldosterone bound to protein at positions 3 and 20) yields a displacement curve characterized by both adequate sensitivity and extreme specificity. Whereas 50 picograms of aldosterone are sufficient to achieve 50 percent displacement of label from antibody, there is no significant cross-reactivity with other steroids tested, even up to concentrations of 10^6 pg/cm^3. Cross-reactivity is substantially greater than one part in 10^5.

Of six rabbits immunized with the dioxime antigen, three yielded antisera of similar specificity, indicating that the experiment shown in figure 6 does not reflect a rare or unusual response.

Plasma Aldosterone Determination

The dioxime antisera may be used in a direct plasma radioimmunoassay. One-tenth cubic centimeter of an aldosterone-free human serum or plasma may be added to a final incubation volume of 1 cubic centimeter containing diluted antiserum and ^{3}H-aldosterone without significant interference of displacement of labeled by unlabeled aldosterone. Plasma from adrenalectomized humans results in no displacement of label.

A critical comparison was made between the direct assay procedure and a procedure dependent on chromatography, isotope dilution, and a final radioimmunoassay step. A wide variety of patient samples having both high and low circulating levels of aldosterone was studied. There was remarkable agreement between the two methods. A correlation coefficient, r = 0.92, was achieved.

A substantial simplification in the determination of aldosterone by radioimmunoassay has been achieved, allowing accurate measurement of small blood samples (0.1-cubic centimeter of plasma) with speed and increase in productivity. It is anticipated that this will promote kinetic investigations of the role of this hormone in human vasoregulation.

CATECHOLAMINES

Background

As with aldosterone, currently available methods for the determination of plasma concentrations of catecholamines lack sensitivity, are cumbersome, and often are nonspecific, particularly in methods in which fluorescence is used. A radioimmunoassay is unlikely to provide antibody of sufficient resolution because of the small size and relative simplicity of the norepinephrine molecule. Consequently, efforts were directed at identification of the physiological receptor because it was clearly capable of differentiating between norepinephrine and its inactive metabolites.

Isolation of the Receptor

The heart is an organ of substantial mass in which all of the constituent muscle may be stimulated by norepinephrine. Consequently, this should provide a rich source of catecholamine receptors.

The left ventricle of the canine heart was homogenized with a Teflon pestle and larger particles sedimented at 30 000-g for 5 minutes. The supernatant then was centrifuged at 100 000-g for 1 hour, and the pellet was collected. After resuspension in saline and a second centrifugation, particles could be stored at 253° K (-20° C) indefinitely (ref. 5).

Radiodisplacement Assay

Particles were suspended in phosphate-buffered saline and incubated with ^{3}H-norepinephrine (370 disintegrations/second/mmole (10 Ci/mmole, New England Nuclear)) for 1 hour. Following incubation, particles were collected on a 0.22μ millipore filter. The filter was placed in a scintillation vial containing Bray solution, and particles were counted. Particles effected a thirtyfold concentration of ^{3}H-epinephrine from the medium. Unlabeled norepinephrine competed with ^{3}H-norepinephrine for binding sites.

Specificity

The particle binding site appears to have the specificity of the β-adrenergic receptor of the heart. In figure 7, the relative efficacy of five β-active agonists in displacing ^{3}H-norepinephrine from particles is shown. Isoproterenol is most effective; whereas, epinephrine and norepinephrine are less effective. This is in accord with the relative potency of these compounds in affecting the rate and force of contraction of the heart. The information in figure 8 indicates that the α-active or indirectly β-active catecholamines are much less effective or are ineffective in competing with ^{3}H-norepinephrine. In figure 9, it can be seen that the β-blocking agent, propranolol, can displace ^{3}H-norepinephrine from particles; the α-blocking agent, phentolamine, cannot perform this function. In addition, it can be shown that metabolites of catecholamines, such as metanephrine and nor-metanephrine are ineffective in competing with norepinephrine, even at very high concentrations. The information in figure 10 shows the specificity of the receptor as deduced from studies of interaction with a variety of analogs of catecholamines.

Development of a Radioreceptor Assay

It is possible to measure 1 to 2 ng/cm^3 of either epinephrine or norepinephrine in aqueous solution with reproducibility and specificity.

A direct plasma assay has not been possible because of nonspecific inter-
ference by plasma constituents. Procedures are being developed for
rapidly extracting catecholamines from plasma so that they may be
measured in the radioreceptor assay.

SUMMARY

A new tool has become available in the study of vasomotor regulation,
and another is soon to become available. It now should be possible to
answer important questions concerning the relative roles of the nervous
system and circulating hormones in maintaining vascular volume and
pressure homeostasis.

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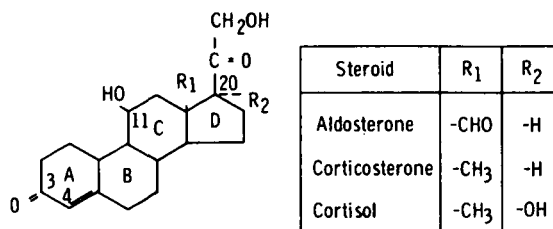
Figure 7-1.- Structures of aldosterone, corticosterone, and cortisol. The C_{18} (R_1) aldehyde of aldosterone exists in solution in a hemiacetal link with the C_{11} (ref. 4).
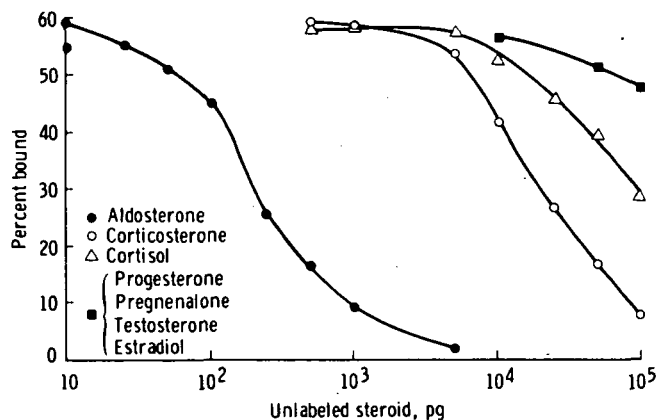
Figure 7-2.- Displacement of ^{3}H-aldosterone by [1]H-aldosterone and by other steroids. Incubation mixtures contained 21 picograms of ^{3}H-aldosterone and a 1:150 dilution of serum from rabbit number 810, obtained 10 weeks after initial immunization. In the absence of nonlabeled steroids, 61 percent of the ^{3}H-aldosterone was bound by the antiserum.
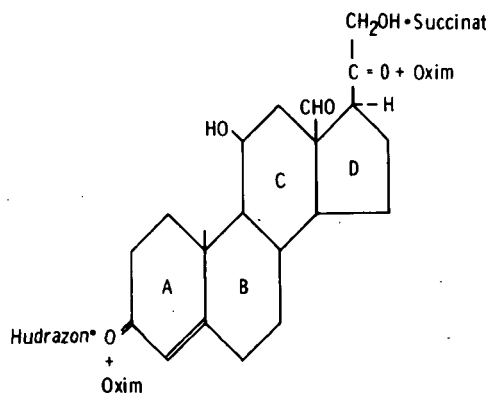
Figure 7-3.- Structure of aldosterone with schematic indication of the site of substitution of the reagents studied. Oxim is carboxymethyl hydroxyloxime; succinat is succinate; hydrazon is p-hydrazinobenzoic acid.
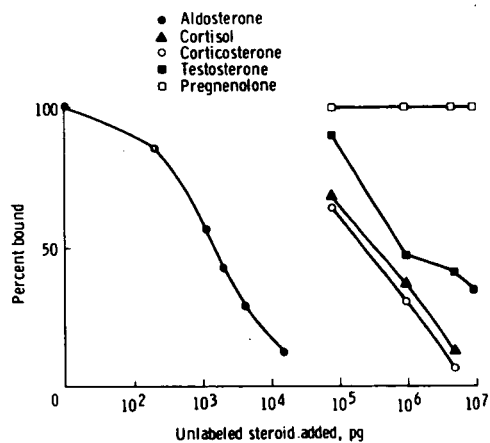
Figure 7-4.- Displacement of ^{3}H-aldosterone from a typical antiserum raised with the 21-aldosterone hemisuccinate conjugate.
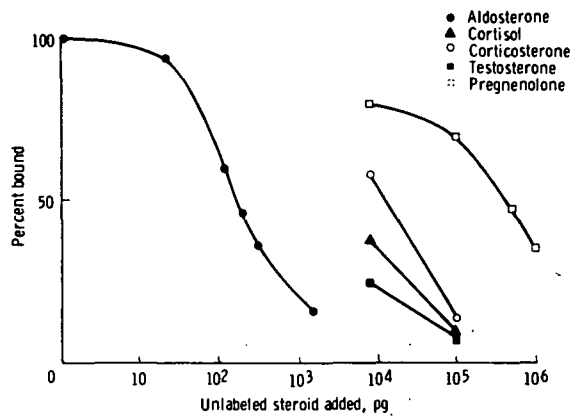
Figure 7-5.- Displacement of ^{3}H-aldosterone from a typical antiserum raised with the Δ3-4 hydrazone conjugate.
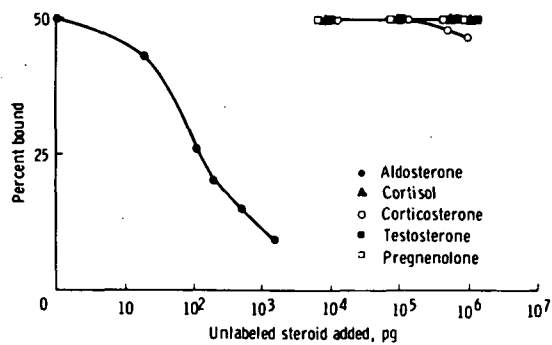
Figure 7-6.- Displacement of ^{3}H-aldosterone from a typical antiserum raised with the Δ3-4, 20 dioxime conjugate.
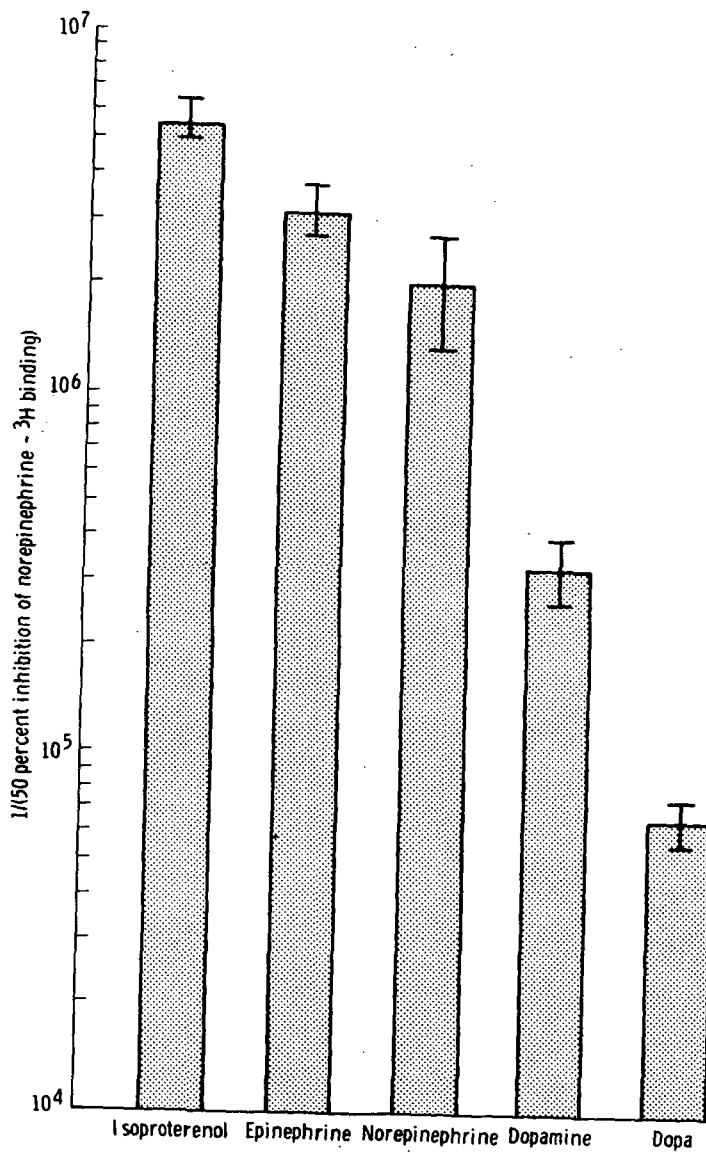
Figure 7-7.- Inhibition of [³H] norepinephrine binding to cardiac microsomes by β-adrenergic agents (ref. 5).
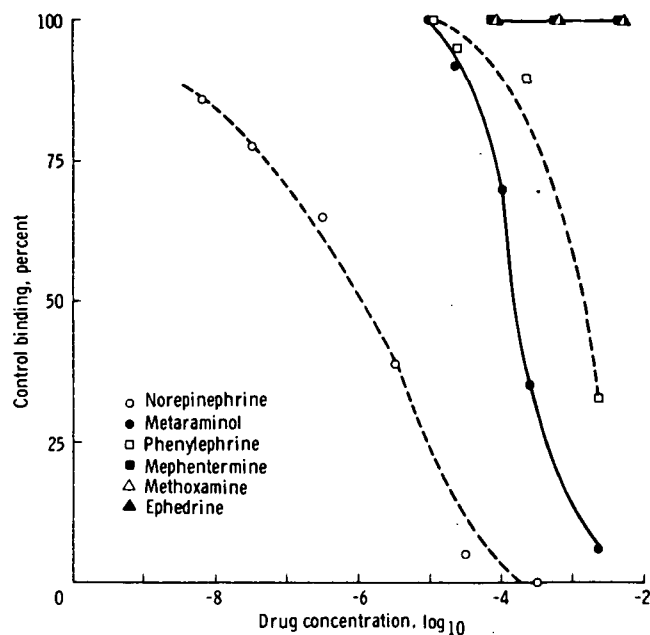
Figure 7-8.- Inhibition of [^{3}H] norepinephrine binding to cardiac microsomes by α-adrenergic agents and indirectly active β-adrenergic agents (ref. 5).
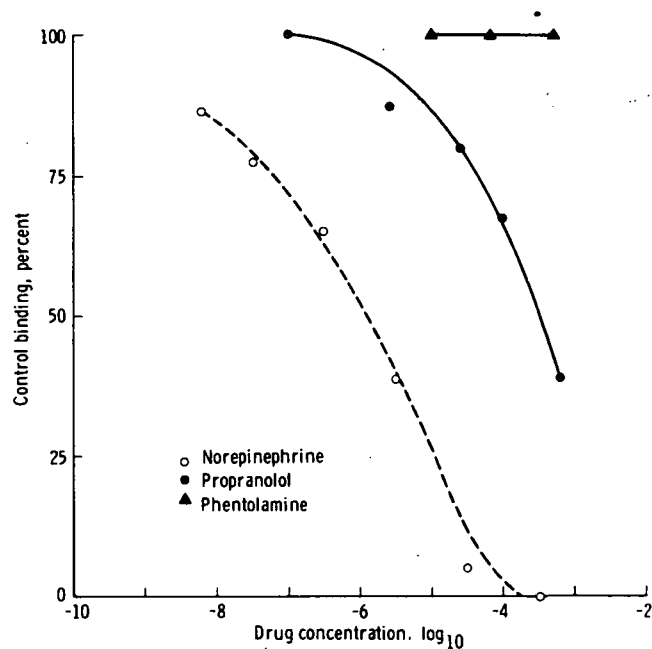
Figure 7-9.- Inhibition of [^{3}H] norepinephrine binding to cardiac microsomes by adrenergic blocking agents. Each point is the mean of duplicates (ref. 5).
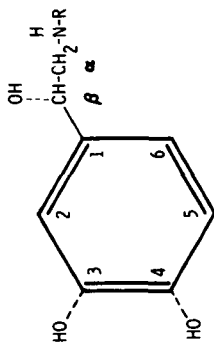
Action	Substituents		
	Required for activity	Not required, but enhance activity	Decrease activity
β-adrenergic inotropy and chronotropy (6)	3-OH, 4-OH	β-OH, R=CH$_3$, CH(CH$_3$)$_2$	---
Inhibition of NE[a] binding to cardiac microsomes	3-OH, 4-OH	β-OH, R=CH$_3$, CH(CH$_3$)$_2$	---
Inhibition of NE uptake by neural vesicles (4, 5)	None	β-OH	R=CH$_3$, CH(CH$_3$)2
Inhibition of NE uptake by whole heart (2)	None	3-OH, 4-OH	β-OH, R=CH$_3$, CH(CH$_3$)$_2$

[a] NE = norepinephrine

Figure 7-10.- Structure-activity relationships for inhibition of [^{3}H] norepinephrine binding to cardiac microsomes by adrenergic agents (ref. 5).

8. PARATHYROID HORMONE, CALCITONIN, AND VITAMIN D

By John T. Potts, Jr., M.D.

PARATHYROID HORMONE

Determination of the complete amino-acid sequence of the major form of the bovine parathyroid hormone (BPTH) (refs. 1 and 2) (fig. 1) and the development of a new technique have permitted preparation of numerous synthetic peptides related to parathyroid hormone (PTH) (refs. 3 and 4) as part of systematic studies of structure-activity relations. The successful synthesis (ref. 3) of a biologically active amino-terminal 1-34 fragment of the bovine parathyroid-hormone sequence confirmed the earlier observation (ref. 5) that the entire 84 amino-acid sequence was not required for biological activity. The synthetic tetratriacontapeptide was found to possess qualitatively all the specific physiological and biochemical properties associated with native parathyroid hormone (ref. 3).

Studies done with fragments of the native molecule indicated that the sequence 1-29 is sufficient for biological activity (ref. 6). Also, synthetic peptides were prepared corresponding to the sequences 1-30, 1-28, and 1-27 of the bovine molecule and to the sequence 1-30 of the porcine molecule (ref. 4). All sequences were active (fig. 2). However, the fragment 1-20 was completely inactive in the adenyl cyclase assay (ref. 6). Whether tested separately or together, the synthetic fragments 1-13, 14-34, 19-34, and 25-34 also were biologically inactive (refs. 3 and 4). Deletion of the amino-terminal residue alanine (as in the synthetic fragment 2-34) led to a complete loss of biological activity (refs. 3 and 6). In addition, carboxyl-terminal fragments 26-45, 46-52, and 53-84, derived from tryptic digestion of ϵ-aminolysine-blocked native parathyroid hormone, were completely void of biological activity (ref. 7). These findings define the minimum requirements of the biological effects of bovine parathyroid hormone as being present in a continuous sequence that extends at least 20 residues from the amino-terminal alanine (residue 1) to a point somewhere between arginine (residue 20) and lysine (residue 27). Detection of significant biological activity for the peptide norlue$_8$ (1-30 porcine), a peptide devoid of methionine, has established that methionine is not essential for biological activity (ref. 6).

The immunoassay for parathyroid hormone (refs. 8 to 11) has been refined and improved considerably. This work resulted from improvement

in the techniques used to purify the radioactive parathyroid hormone used in the immunoassay and by the development of a series of valuable antisera of sufficient sensitivity for use in detection of parathyroid hormone in human blood but with the added advantage of reaction with different areas of the linear sequence of parathyroid hormone (ref. 12) (figs. 3 and 4). With the addition of these antisera to those used previously in radioimmunoassay studies, a battery of selective reagents exists with which the recently recognized problem (refs. 13 to 15) of different circulating fragments of parathyroid hormone in blood can be analyzed (figs. 5 and 6). Recently, greatest interest has centered on findings that the biosynthesis, secretion, and metabolism of parathyroid hormone is much more complex than believed previously (refs. 12 and 13). The existence of a biosynthetic precursor to parathyroid hormone in human and bovine glands has been established (refs. 16 and 17) (fig. 7). Intracellular cleavage and storage of the prohormone appears to be in analogy with the conversion of proinsulin to insulin. A second cleavage of the 1-84 intact hormonal peptide in one or more peripheral organs occurs after release (refs. 12 and 14). Contrary to earlier reports, the hormone is released intact from the parathyroids; the cleavage to apparently inactive fragments represents a peripheral mechanism concerned with hormone destruction (refs. 12 to 14) (figs. 6 and 8). These findings explain much of the present confusion concerning clinical immunoassay studies. However, even at present, many useful clinical studies can be undertaken using radioimmunoassay (refs. 18 to 24).

Analyses of secretion of parathyroid hormone during tests of stimulation and suppression of hormone-secretory activity using infusions of EDTA[a] and calcium, respectively, have established that, in contrast to previous views, secretion of the hormone is not autonomous in many patients that have adenomatous hyperparathyroidism, but is responsive to changes in blood-calcium concentration (ref. 19). These findings have led to a new understanding of the pathophysiology of hormone production in hyperparathyroidism.

A related application of the diagnostic use of the radioimmunoassay is the preoperative localization of parathyroid tumors and the distinction between adenomas and chief-cell hyperplasia (refs. 18 and 23). Work involving catheterization and radioimmunoassay of blood samples obtained from the subclavin and innominate veins and the venae cavae, led to localization in a high percentage of patients (fig. 9). However, this procedure has been adopted recently to detect hormone concentration in the small veins directly draining the parathyroid glands. This detection has resulted in a great increase in sensitivity and in general utility of the technique (table I).

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[a]Ethylenediaminetetraacetic acid.

CALCITONIN

Multiple immunoassay systems, suitable for specific detection of salmon, ovine, porcine, and human calcitonin, have been developed successfully (fig. 10). By the use of these techniques, the control of calcitonin by blood calcium has been analyzed, and the fact has been established that calcitonin production is under directly proportional control by blood-calcium concentration (ref. 25). Studies in numerous animal species (refs. 20 and 26) have established many important physiological aspects of calcitonin secretion, including the quantitative response to hypercalcemia, the role of new hormone biosynthesis, and the metabolic clearance rate of the peptide.

In the porcine species, the secretion of calcitonin is directly proportional to the blood-calcium concentration (ref. 26). This species also has served as a model to study the effect of other factors on calcitonin secretion. Gastrointestinal hormones, such as glucagon and gastrin, have been shown to stimulate the secretion of this peptide (ref. 20).

The secretion of calcitonin also has been studied in the bovine species by means of radioimmunoassay (ref. 27). Preliminary studies have indicated that the concentration of calcitonin in the blood of bulls is significantly higher than that in the blood of cows (fig. 11). This fact may be related to the high dietary calcium that is required in bovines to meet the needs of cows that incur losses of calcium through pregnancy and lactation. However, bulls, that ingest the same calcium load as cows, must compensate for this large dietary-calcium challenge. Presumably, the increased plasma calcitonin in bulls represents a compensatory response of the calcitonin-secreting cells to maintain blood calcium within normal limits despite the dietary-calcium challenge. These observations take on more importance when considering the increased incidence of carcinomas of the calcitonin-secreting cells in bulls. As many as 30 percent of the bulls older than 6 years have such malignancies. The incidence of these malignancies is related to the prolonged dietary-calcium challenge in these animals.

Studies also have been conducted to determine the importance of calcitonin in salmon (ref. 28). The hormone plays some role in the migration of salmon from fresh to salt water and vice versa. In general, calcitonin concentrations are increased in salmon in salt water compared with salmon in fresh water; this fact may represent a homeostatic response to prevent the blood calcium from rising to dangerously high levels when the fish migrate into salt water. An anticipated finding in the salmon is the difference in calcitonin secretion between the sexes; the concentration of peptide is significantly higher in females than in males. In

the female, a progressive rise in calcitonin secretion is evidenced from the time of entry into fresh water to the time of spawning. After spawning, a precipitous drop in calcitonin concentration occurs. The significance of these findings is under further investigation.

The rate of calcitonin secretion in man has been clarified, correcting earlier overestimates (refs. 29 and 30). If secreted at all, calcitonin is produced in normal man at concentrations much below that in other mammals (fig. 12). The human-calcitonin assay has been applied to the measurement of calcitonin production in patients that have medullary carcinoma of the thyroid (fig. 13); the assay has been proven to be extremely valuable in the early detection of this malignancy. Because patients with medullary carcinoma of the thyroid have increased concentrations of calcitonin, the assay is an accurate procedure for the establishment of the diagnosis of this tumor in affected patients (ref. 31). This procedure allows for early diagnosis of the malignancy and for potential patient cure by means of surgery. Although not a common tumor, medullary carcinoma of the thyroid often has a familial distribution; therefore, the relatives of affected members can be screened so that early diagnosis may be made.

The secretion of calcitonin also has been studied in hypocalcemic patients (fig. 14). These subjects have increased stores of calcitonin in their thyroid gland, presumably promoted by the hypocalcemia (ref. 20). Upon appropriate stimulation, these calcitonin stores are released in the peripheral circulation where they can be detected by the use of immuno-assay. These patients have been very important for studying the control of peptide secretion in subjects other than those with medullary thyroid carcinoma. With the exception of patients with medullary thyroid carcinoma, it is only in hypocalcemic human subjects that calcitonin can be detected readily in the peripheral circulation of humans.

Because the concentration of this peptide presumably is so low, special means will be necessary to study the secretion of this peptide in man (other than in patients with medullary thyroid carcinoma). One of the proposed approaches is the use of immunoabsorbant chromatography to concentrate the calcitonin in plasma samples (ref. 32). (For example, calcitonin-specific antibody is attached to a solid phase-supporting medium such as Sepharose). Then, plasma can be passed through this column, and any calcitonin in the plasma will be trapped by the antibody on the column. Subsequent to the trapping of calcitonin, this peptide can be eluted from the column and concentrated into a small volume. This procedure should increase the effective sensitivity of human calcitonin assay by several orders of magnitude. Associated with this procedure should be the use of similar techniques to improve the actual sensitivity of the calcitonin assay. By coupling calcitonin to a solid phase, it will be possible to isolate from antiserum those species of calcitonin

antibody that have the highest affinity for the hormone. These high-affinity antibodies then can be used directly in the immunoassay (ref. 33). Such procedures will allow the measurement of calcitonin concentration in normal human subjects and the evaluation of the effect of variables such as prolonged immobilization and weightlessness on calcitonin secretion.

VITAMIN D

Vitamin D is necessary for normal bone formation and remodeling and for normal intestinal absorption of calcium. The availability of radioactive vitamin D of high specific activity made possible the discovery of the complex metabolism of vitamin D that occurs before the expression of the physiologic action at the tissue level. Normally, the vitamin, stored in the body after formation in the skin or absorption from the diet, is converted to 25-OH vitamin D hydroxiecholecalciferal (25 HCC) by a specific hydroxylase in the liver (ref. 34). Although this compound is active in vitro (whereas vitamin D is inactive), the discovery has been made that, in vivo, further hydroxylation of the vitamin occurs before it acts on target tissues. For example, 25-OH vitamin D is metabolized in the kidney to $1,25-(OH)_2$ vitamin D dihydroxycholecalciferol (1,25 DHCC) and to other dihydroxy metabolites that have not yet been identified fully (ref. 35).

In recently reported work, the suggestion has been made that 1,25 DHCC is the form of the vitamin that acts to stimulate calcium transport by the intestine (ref. 36). The characteristic timelag before increased calcium transport is manifest in the gut after the administration of vitamin D or 25 HCC is reduced significantly after vitamin D repletion with 1,25 DHCC. On a weight basis, this dihydroxy metabolite also is more potent than either D or 25-OH D (ref. 37). Furthermore, although pretreatment with actinomycin D abolishes the intestinal action of both vitamin D and 25 HCC, it does not interfere with the intestinal effect of 1,25 DHCC (ref. 38). Thus, the antibiotic probably interferes with the metabolism of 25 HCC to the dihydroxy metabolites in the kidney (ref. 39). Presently, 1,25 DHCC is believed to be the intestinally active form of the vitamin.

This compound also can act to mobilize bone calcium, and has a time course somewhat faster than, but with a total effect no greater than, 25 HCC (ref. 40). In mobilizing calcium from fetal-rat calvaria (D is inactive in this system) (ref. 41), 1,25 DHCC is more active than is 25 HCC. The dihydroxy metabolite is effective in mobilizing bone calcium in anephric rats. The skeletal actions of vitamin D, 25 HCC, and 1,25 DHCC are blocked by actinomycin D (ref. 40) treatment.

A second dihydroxy metabolite, $21,25 (OH)_2 D$, also facilitates the mobilization of skeletal calcium in the vitamin D-deficient animal but has only limited potency on intestinal calcium transport (ref. 42). The physiologic significance of this metabolite has yet to be explained.

A third dihydroxy metabolite has been identified as $25,26 (OH)_2$ vitamin D. Although this compound stimulates active transport of calcium in the intestine, it does not mobilize bone calcium and is of unknown physiologic significance (ref. 43). The data are suggestive that the action of $1,25 (OH)_2 D_3$ in bone requires further metabolism of the vitamin or deoxyribonucleic acid (DNA) transcription to messenger ribonucleic acid (mRNA) or protein synthesis; however, the action of 1,25 DHCC in the gut apparently involves stimulation of a preformed transport mechanism.

The metabolic activation of vitamin D appears to be a regulated process in both the liver and the kidney. In vitro experiments imply product inhibition of the 25-hydroxylase in the liver (ref. 36); however, in vivo formation of both 1,25 DHCC and an unidentified metabolite (peak Vo) varies with the serum-calcium level. The formation of 1,25 DHCC by the kidney occurs when the serum-calcium level is less than 9.2 percent; however, formation of the peak Va metabolite occurs when the serum calcium exceeds 9.5 percent (ref. 44). The formation of one metabolite seems to preclude formation of the other, indicating close metabolic regulation based on the serum-calcium level or some other metabolic determinant that varies with the serum calcium. The formation of 1,25 DHCC, with subsequent stimulation of intestinal calcium transport, should be associated with a lowered serum calcium. An intrinsic renal action has been postulated for the peak Va metabolite but, as yet, no direct evidence bearing on this issue is available.

Indirect evidence also suggests regulation of vitamin D activation. The body adapts to increased calcium requirements (growth, pregnancy, and lactation) or to a low-calcium diet by increased efficiency in the absorption of dietary calcium (ref. 45). This facultative increase in absorptive efficiency is not dependent on the presence of parathyroid hormone, calcitonin, growth hormone, or adrenal steroids, but is absolutely dependent on the presence of vitamin D (ref. 46). As proved in recent experiments, the regulation of vitamin D activation (specifically the regulation of $1,25 (OH)_2$ vitamin D by the kidney) plays an important role in this adaptation (ref. 47). Further indirect evidence supporting regulation of vitamin D activation in man is the rarity of hypervitaminosis D despite wide variations in exposure to ultraviolet light and in dietary vitamin D intake. In fact, induction of hypervitaminosis D (as in the treatment of hypoparathyroidism) requires 50-100 times the minimum

daily dose of the vitamin that is needed to prevent rickets (ref. 21). In animals, a similar margin exists between the dose necessary to evoke the maximum physiologic response and the dose necessary to produce the manifestations of hypervitaminosis D on the skeleton or in the intestines (ref. 21). Therefore, a reasonable hypothesis is that vitamin D metabolism is regulated, that this regulation occurs at one or more of the points where metabolic conversion occurs, and that regulation of vitamin D activation plays a role in homeostatic regulation of skeletal calcium.

Using radioactive vitamin D and a specific D-binding plasma protein (refs. 48 and 49), the saturation-analysis assay is sufficiently sensitive to detect physiological concentrations of endogenous vitamin D and its active metabolites (fig. 15). This detection represents a potential advance in studies of normal vitamin D physiology and disorders of vitamin D metabolism in man. Already, the assay has been applied to the detection of vitamin D_3 and 25-OH vitamin D_3 in man and in rats made D deficient then repleted with various amounts of the vitamin. Many important aspects of the complex metabolic role of vitamin D, tissue-specific products, and the vitamin D-binding protein can now be explored. Vitamin D_2 (derived from diet supplements in foodstuffs) and vitamin D_3 (formed by the action of light on precursor compounds in the skin) are not of equivalent potency for displacement of radioactive vitamin D_3 from the binding protein (fig. 16). The assay is approximately four times more sensitive for the detection of D_3 than for D_2. This phenomenon prevents immediate application of the saturation-analysis assay in its present form to detection of physiologic concentrations of the vitamins in normal human subjects wherein both vitamin D_2 and D_3 must be assumed to be present in the circulation. Thus, efforts are necessary to modify assay conditions to measure vitamin D_2 and vitamin D_3 separately.

One approach to the separate estimation of vitamin D_2 or D_3 independent of the presence of the other compound is the use of a binding protein that is extremely insensitive for the detection of one form of the vitamin. Initial studies have been suggestive that such a binding protein may be present in the chicken blood, and the binding of vitamin D_2 by protein is extremely poor. Efforts will be made to investigate further the stability and general applicability of the binding-protein preparation prepared from chicken blood for specific assay of vitamin D_3. Another approach would be the development of antibodies selective to vitamin D_2 and D_3 and the use of these antibodies in a radioimmunoassay system to measure these vitamins individually.

Because the vitamin is derived from the diet and from the action of light on a precursor in the skin, the estimation of the normal vitamin D load (endogenous and exogenous) required to maintain normal skeletal metabolism has not been possible. With normal environment, a normal diet, and solar irradiation, this problem is not present except for certain disease states in which excess exogenous vitamin D can cause symptomatic hypercalcemia. In a completely closed environment and with an artifical diet, it becomes very important to define the vitamin D requirements necessary for normal skeletal homeostasis and to define any differences between "natural" vitamin D (endogenous vitamin D_3) and dietary vitamin D. The newer assay techniques should facilitate better definition of these important problems.

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TABLE 8-I.- VENOUS-CATHETERIZATION RESULTS

Peripheral blood			Thyroid veins, PTH ng/cm^3						Diagnosis
			Right			Left			
Case	Calcium[a], mg/100 cm^3	PTH[b], ng/cm^3	Superior	Middle	Inferior	Superior	Middle	Inferior	
[c]1	11.1	1.2	[d]3.2	--	--	1.0	--	--	Right upper, adenoma
2	13.2	.85	--	[e]1.2	--	--	--	27.5	Left upper, adenoma
[c,f]3	11.6	.77	--	--	(g)	[h].81	--	19.4	Left upper, adenoma
4	11.5	.98	--	--	.84	--	--	7.0	Left upper, adenoma
[c]5	10.5	1.6	--	16.6	--	--	--	--	Right lower, adenoma
6	11.8	2.9	2.5	--	--	2.8	--	166.0	Left lower, adenoma
7	11.0	1.8	5.0	--	29.1	--	--	[e]2.1	Right upper, adenoma
8	11.5	1.5	1.6	--	33.3	--	--	1.5	Right lower, adenoma
[f]9	14.0	7.2	--	--	300.0	7.2	--	(g)	Right upper, adenoma
10	10.4	.8	--	--	.9	--	--	19.1	Left lower, adenoma
[f]11	11.2	1.4	--	--	1.2	--	4.6	25.0	Left lower, adenoma
12	11.0	1.1	--	--	1.2	--	--	.85	Mediastinal adenoma
13	12.0	.7	0.65	--	[d]1.6	--	--	9.8	Double adenomas (left lower; right lower)
[i]14	14.2	3.4	5.5	--	22.9	[h]3.4	--	(g)	Hyperplasia (left thyroidectomy previously)
[i,j]15	11.5	.8	--	3.9	--	2.3	--	--	Hyperplasia
[i,j]16	11.2	.7	(g)	--	--	(g)	--	2.2	Hyperplasia (previous surgery; both upper glands removed)
[j]17	11.4	.6	0.5	--	28.7	--	--	4.1	Hyperplasia
[j]18	11.5	.8	--	--	5.0	--	--	3.2	Hyperplasia
[j]19	11.6	1.3	--	10.5	7.3	1.6	--	21.0	Hyperplasia
20	11.2	1.9	--	2.4	--	--	--	5.9	Hyperplasia
21	11.2	1.7	--	--	9.7	--	--	[k]2.2	Hyperplasia

[a]Normal range: 9.0 to 10.5 mg.

[b]Normal range: 0.3 to 0.8 ng/cm^3.

[c]Members of a kindred with familial hyperparathyroidism.

[d]All underlined values are significantly (P < 0.001) elevated above mean-peripheral level.

[e]P < 0.05.

[f]Hypothyroid; thyroid veins tiny.

[g]Ligated.

[h]Stump.

[i]Had previous surgery that caused distortion and interruption of venous drainage.

[j]Prior arteriogram outlined the lesion and indicated the appropriate vein to catherize.

[k]P < 0.1.
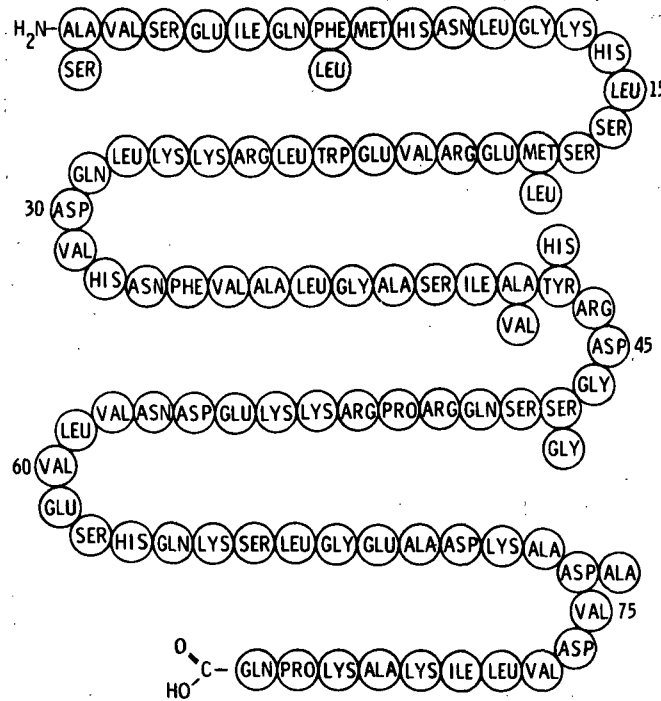
Figure 8-1.- Amino-acid sequence of BPTH with amino-terminal alanine. Amino-acid residues that differ between BPTH and porcine PTH are indicated by the porcine amino acids in apposition.

Figure 8-2.- Biological activity of natural and synthetic PTH fragments (fragments 1 to 28 and 1 to 27 not shown).
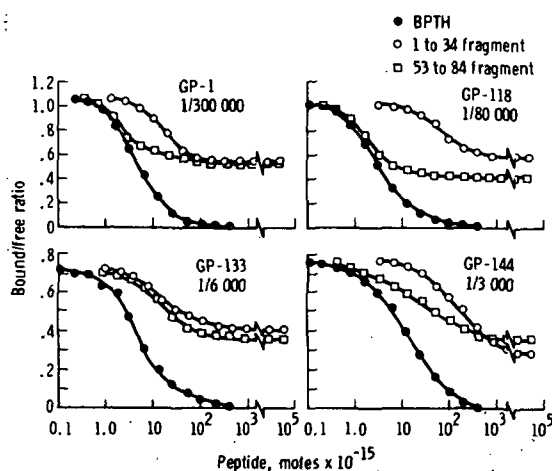
Figure 8-3.- Comparison of the fall of bound antibody to free ^{125}I-BPTH tracer from antibody control (bound/free ratio in the absence of unlabelled hormone) in assays using four antisera as a function of increasing concentrations of BPTH and BPTH fragments.
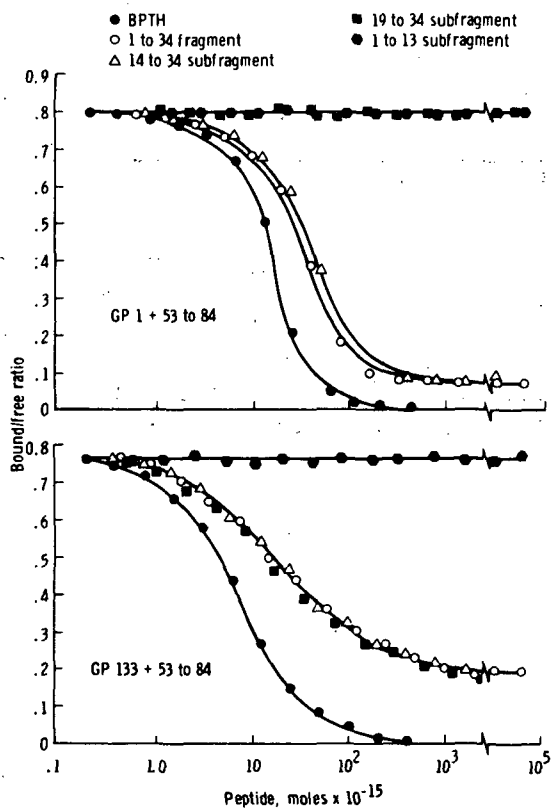
Figure 8-4.- Inhibition of binding of ^{125}I-BPTH tracer by increasing concentrations of BPTH, and BPTH fragment and subfragments.
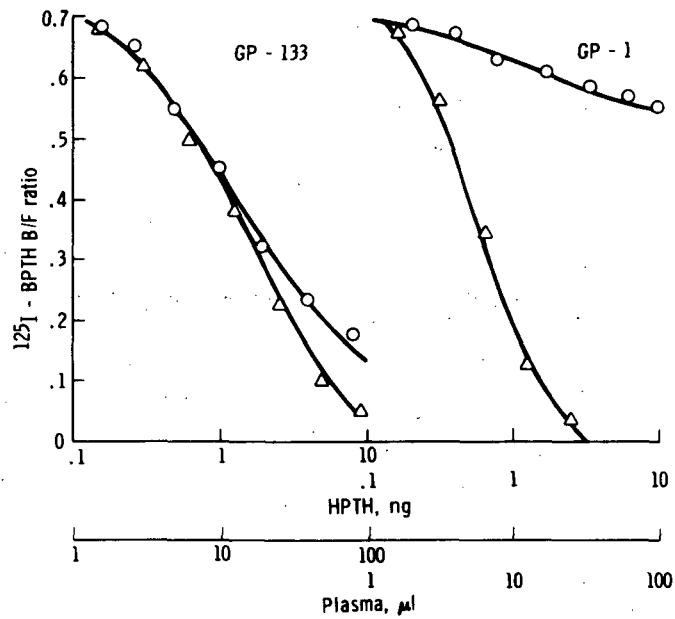
Figure 8-5.- Inhibition of binding by increasing volumes of the same plasma sample in assays using GP-133 and GP-1.
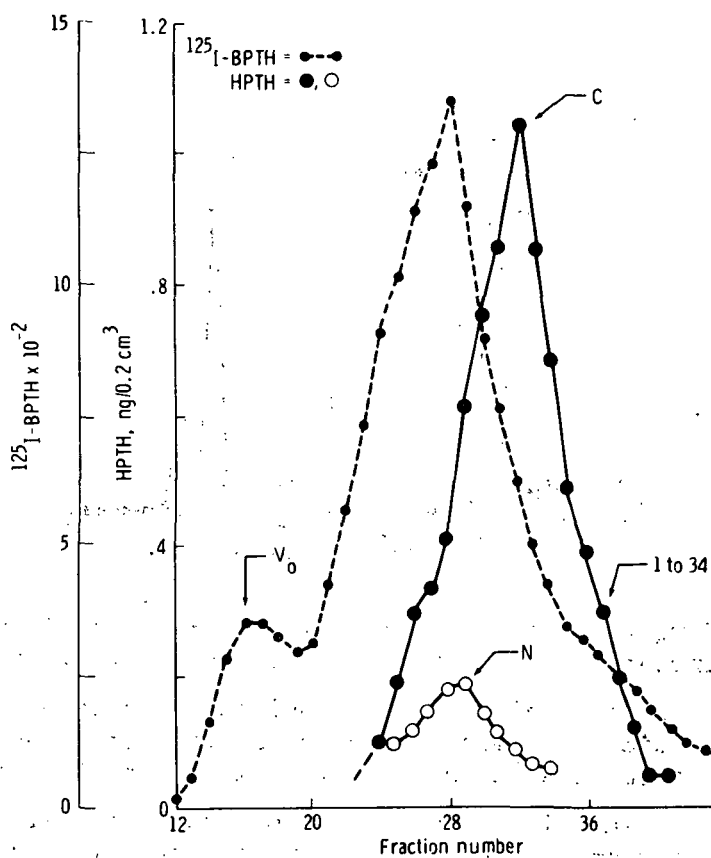
Figure 8-6.- Radioimmunoassay results for immunoreactivity in fractions
after gel filtration of a peripheral plasma sample on Biogel P10 using
GP-1 preabsorbed with excess concentrations of fragment 53 to 84 (N)
and 1 to 34 (C). It is demonstrated that the dominant form of immuno-
reactive hormone in human circulation consists of a hormonal fragment
that is totally void of an antigenic determinant requiring the 14 to 19
region of the hormone.
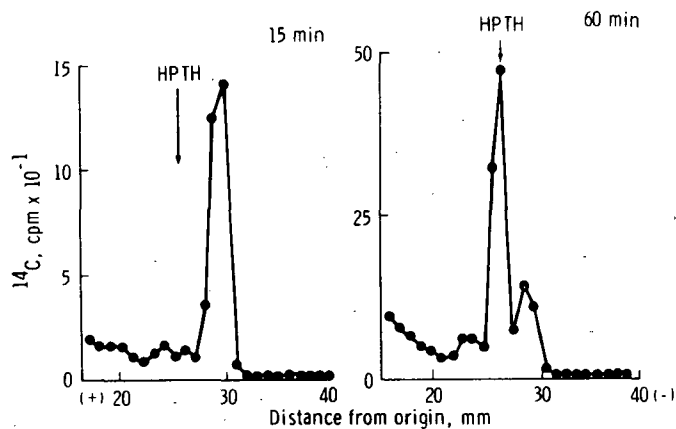
Figure 8-7.- Comparison of the gel-electrophoretic position of hormone 15 and 60 minutes after exposure of human parathyroid adenoma tissue slices in incubation media to ^{14}C-labelled amino acids. The arrow marks the position of human hormone extracted from glands. The electrophoresis medium was 8M urea pH 4.4 gel. Note that at 15 minutes the peak of ^{14}C-labelled material has migrated farther than the hormone extracted from glands, indicating that this material is more basic. At 60 minutes, the peak of ^{14}C-labelled material cannot be distinguished from the marker.
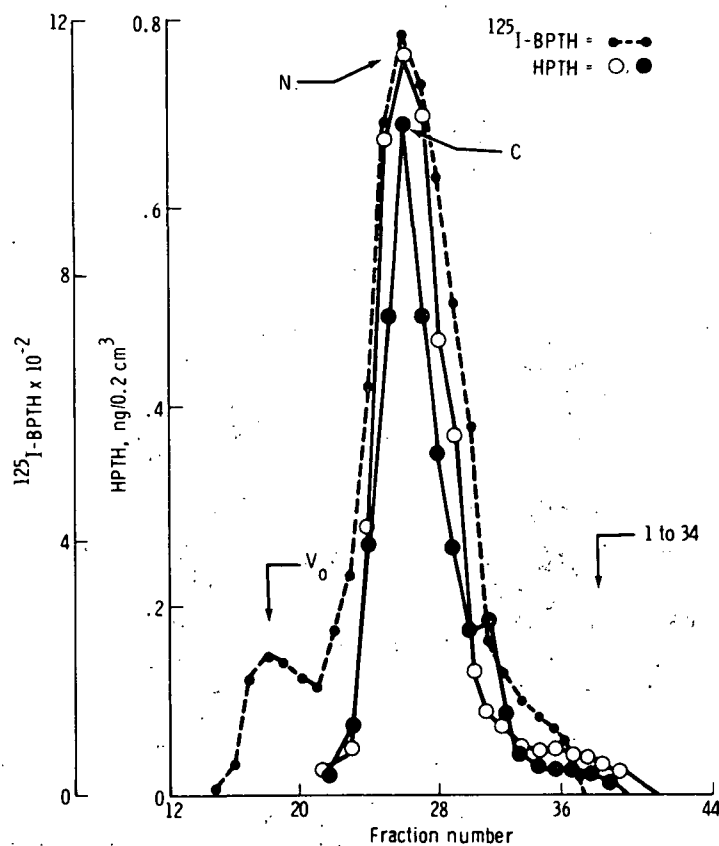
Figure 8-8.- Radioimmunoassay results of the immunoreactivity in fractions after gel filtration of a plasma sample obtained from the parathyroid venous effluent at the time of diagnostic venous catheterization. The filtration was done on Biogel P-10, and the fractions were assayed with GP-1 preabsorbed with 53 to 84 (N) and 1 to 34 (C). The hormone elutes as a single peak coincident with the marker and has equivalent concentrations of amino- and carboxy-terminal reactivity, indicating that the secreted hormone is identical to the hormone extracted from the glands.

I = innominate vein
Common IT = common inferior thyroid vein
J = jugular vein
LIT = left inferior thyroid vein
MT = middle thyroid vein
RIT = right inferior thyroid vein
ST = superior thyroid vein
SVC = superior vena cava

Case 4
(left upper adenoma)

Case 19
(hyperplasia)

0.98 (J)
10.5 (MT)
7.7 (MT)

1.6 (ST)

1.0 (J)

2.5 (J)
7.3 (IT)

2.1 (J)

0.92 (I)

1.8 (I)

2L0
(IT)

0.84 (IT)

7.0 (IT)

8.2 (I)

2.6 (I)

1.2 (SVC)

2.1 (SVC)

9.2
(Common IT)
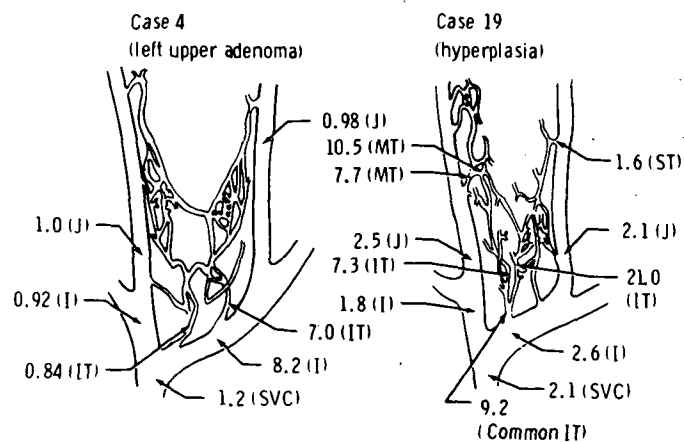
Figure 8-9.- Tracings from thyroid venograms in patients 4 and 19; the hormone results are shown on samples from the sites shown. In patient 19, both inferior thyroid veins join to form a common terminal trunk before entry into the left innominate vein, showing that, in case 4, there is a step-up in the LIT but that, in case 19, hormone measurements from both sides of the neck are elevated. These results indicate that, in case 4, there is a left-sided adenoma and that, in case 19, there is multiglandular involvement. These facts were confirmed during surgery.
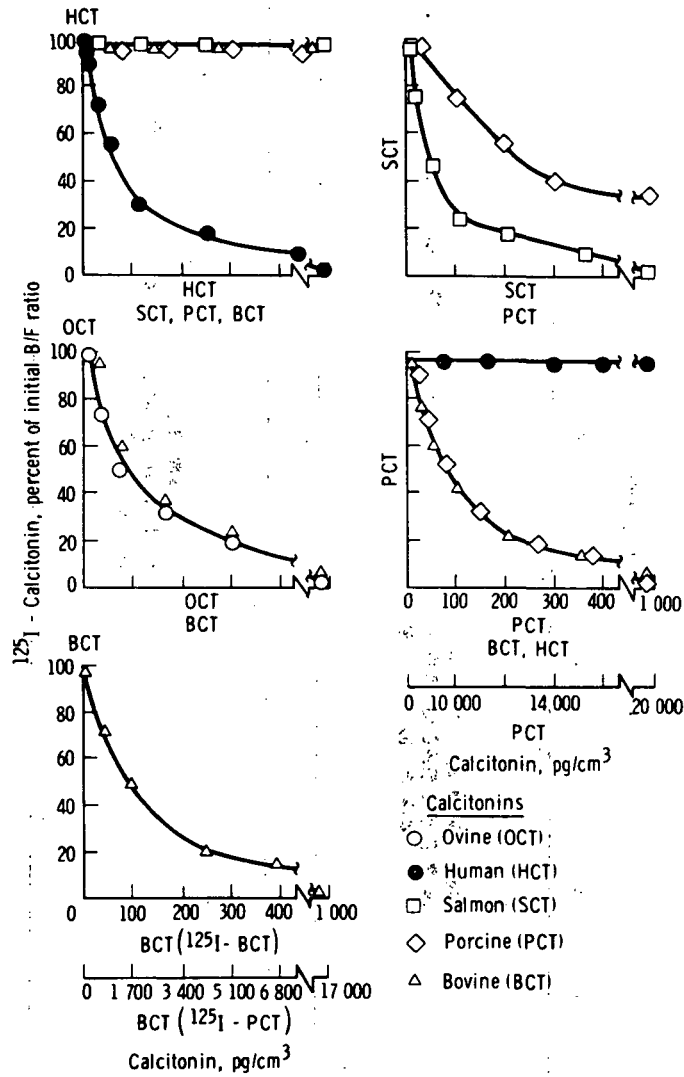
Figure 8-10.- Each of the calcitonin immunoassays is sufficiently sensitive to detect peripheral concentrations of calcitonin in the appropriate species. Note that significant cross-reactivity is shown among the structurally similar porcine, bovine, and ovine calcitonin; there is little cross-reactivity among salmon, human, and the other calcitonins.
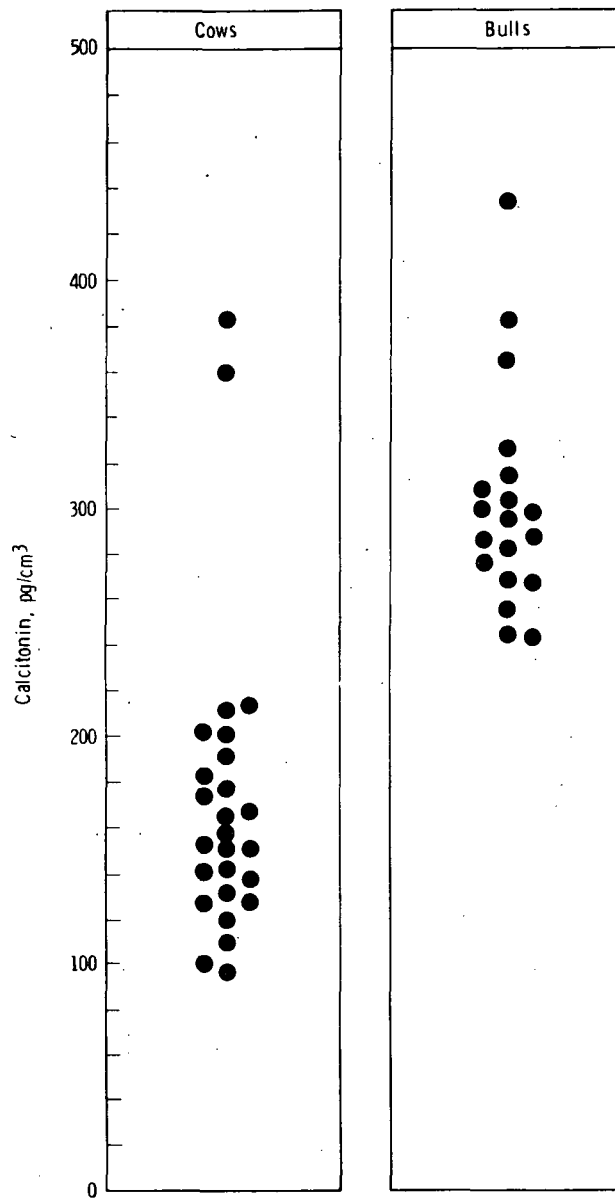
Figure 8-11.- Calcitonin concentrations in plasma samples taken randomly
from 29 bulls and 16 cows. The mean (±S.D.) concentration in bulls,
303 ± 13 pg/ml, is significantly (p < 0.01 (by student's t test)) higher
than the mean concentration, 165 ± 12 pg/ml in cows. There is no sig-
nificant difference in plasma-calcium concentration between cows and bulls.
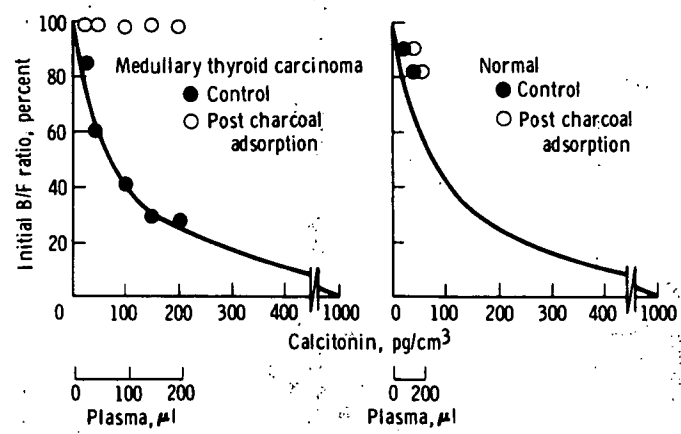
Figure 8-12.- The use of charcoal adsorption to preclude artifactual dis-
placement of calcitonin from specific antibody by plasma samples. Char-
coal, demonstrated to adsorb > 10 000 pg/ml of calcitonin from plasma,
removed all detectable calcitonin from a plasma sample of a patient with
medullary thyroid carcinoma. As a result, the sample, which progres-
sively displaced tracer from antibody before charcoal adsorption, fails
to do so after adsorption. By contrast, there is no difference in the
displacement of tracer produced by a normal plasma sample before and
after charcoal adsorption. Therefore, the apparent calcitonin concen-
tration in this sample was artifactual and probably was caused by non-
specific displacement of tracer from antibody.
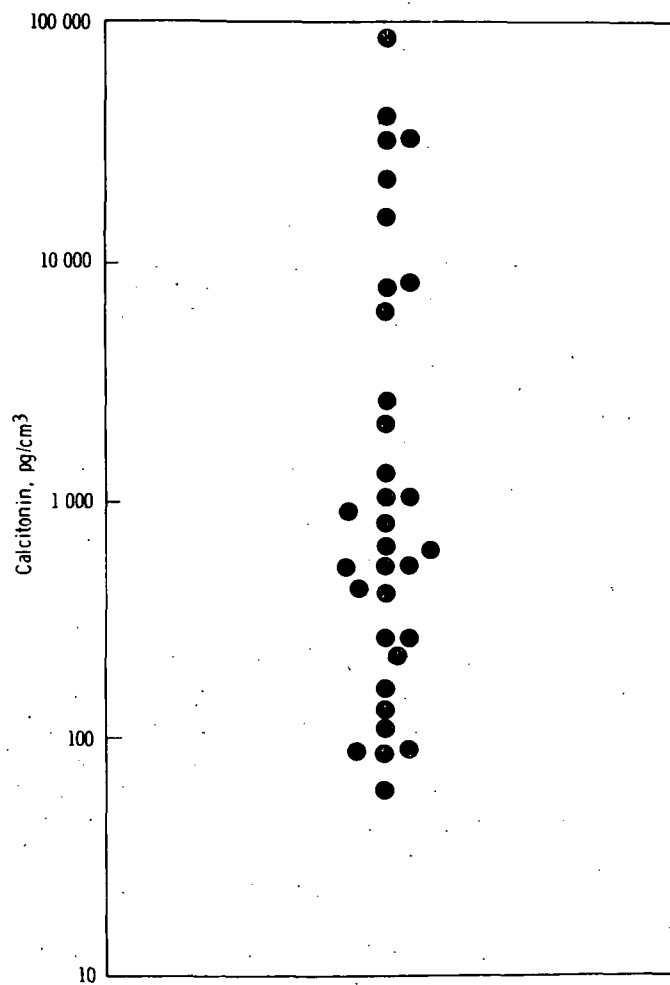
Figure 8-13.- Plasma calcitonin in patients with medullary thyroid carcinoma
(surgically proven) and elevated hormone concentrations. Plasma calci-
tonin in normal subjects is less than 100 pg/ml, the detection limits of
the immunoassay system.
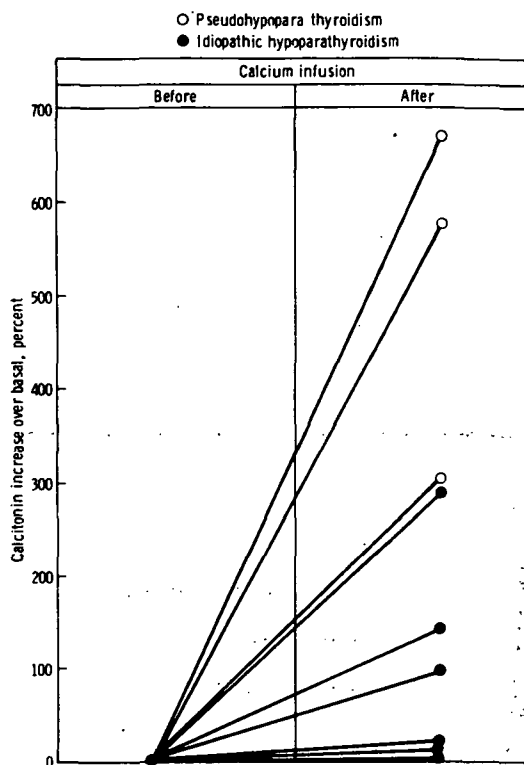
Figure 8-14.- The increase in plasma calcitonin after calcium infusion in patients with pseudohypoparathyroidism and idiopathic hypoparathyroidism. In contrast to normal and hypercalcemic subjects, the patients have post-infusion levels of hormone that can be detected readily.
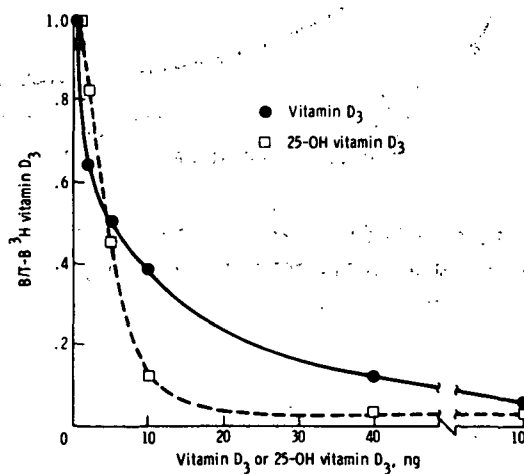
Figure 8-15.- Competitive binding assay standard curves with vitamin D_3 and 25-OH vitamin D_3.
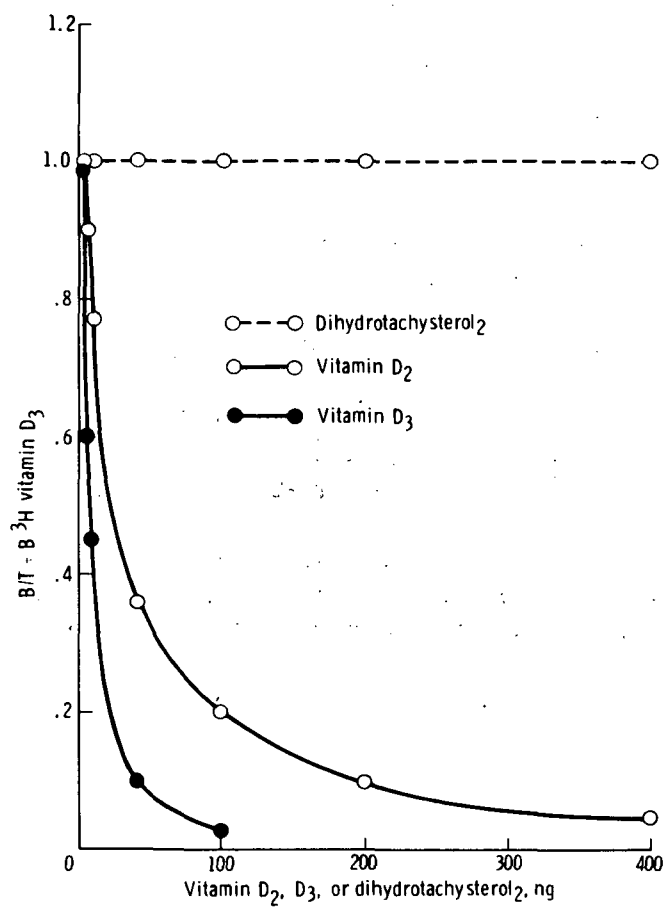
Figure 8-16.- Competitive binding assay standard curves with vitamin D_2, vitamin D_3 and dihydrotachysterol$_2$.

9. DISSOCIATION OF EFFECTS OF PROLONGED CONFINEMENT

AND BEDREST IN NORMAL HUMAN SUBJECTS:

HEART RATE AND BODY TEMPERATURE

By Charles M. Winget, Ph. D., Joan Vernikos-Danellis, Ph. D.,
Carolyn S. Leach, Ph. D., and Paul C. Rambaut, Sc. D.

The daily rhythm of human physiological functions is a complex re-
action, produced at least in part by adaptation of the organisms to daily
changes in the earth environment. The complex, dynamic interrelation-
ships produced by social and physical environmental factors underlie the
formation of diurnal rhythms in human physiological functions. To eval-
uate the effects of simulated weightlessness on physiological systems,
the effect of restriction of muscular activity (bedrest) on certain
physiological rhythms was investigated in eight healthy male subjects
who were maintained in a defined environment. The results of this study
have been reported previously (ref. 1). That study included a 6-day
ambulatory, prebedrest period; 56 days of bedrest; and a 10-day postbed-
rest recovery period. Four of the subjects exercised during bedrest.
Body temperature (BT) and heart rate (HR) and circulating cortisol,
triiodothyronine (T_3), and thyroxine (T_4) concentrations were measured
at four hourly intervals throughout the study.

The results were indicative of the following changes. During bed-
rest, mean HR increased, whereas BT and steroid outputs decreased. Nei-
ther exercise nor the 10-day postbedrest ambulatory period prevented or
corrected this effect. The HR remained more stable throughout bedrest
than did the other rhythms studied. By contrast, the amplitude of the
T_4 rhythm appeared to increase as bedrest progressed, and the total
serum T_3 concentrations increased during the latter part of the bedrest.
The data are suggestive that the daily change in phase and amplitude or
desynchronization of BT, T_3, and T_4 rhythms were caused by the position
of the body and that the observed low-grade hypothermia and minor tachy-
cardia were characteristic of the hypokinetic syndrome in man. In ad-
dition, most parameters did not show complete recovery by the end of the
experiment; that is, 10 days after the last day of bedrest. Furthermore,
on approximately day 20 of bedrest, all parameters showed some changes.

For example, the level of cortisol increased, as did the mean level of T_3, and the phase angle of BT and HR shifted suddenly. A possible social influence of the rhythms of one individual on those of another also was suggested by the results.

In a study of this type in which continuous data are obtained from eight subjects during a period of 72 days, the problems of data analysis and optimal representation becomes enormous. Therefore, to readily inform the observer concerning day-to-day dynamic phase and amplitude changes, it was necessary to develop a method that expresses, in chart form, rhythmic data that may be nonstationary in time. The summation-dial method (ref. 2) was developed to meet the requirements. This is a method in which the curve best fitting the data is derived mathematically, assuming a specified period (for example, $\tau = 24$ hr). Each point on the curve represents the end of a vector, which has a certain magnitude and direction describing the phase of the rhythm for that day (fig. 1). The summation of these vectors or train of vectors produces the summation dial (fig. 2). The direction of the vector train is determined by the hour of the day at which estimated peak activity of that parameter occurred. The length of the vector indicates the amplitude of the rhythm.

Because this was the first study of this duration and because certain parameters changed radically over time and did not return to prebedrest values, it was necessary to conduct a second experiment to better explain certain of these changes. The second study was designed to include a longer prebedrest control and postbedrest recovery period. Because the second bedrest study involved confinement of young, healthy males for prolonged periods, an equal group of equal number subjects that were not put to bed were included. These subjects remained ambulatory and confined to the experimental area and were maintained on an identical schedule.

Because exercise appeared to have little or no effect in the previous study, no exercise was included. In the previous study, the question was raised whether some of the observed changes, particularly those around day 20, may have been caused by the bleeding schedule. Therefore, two subjects, one in the ambulatory and one in the bedrest group, were not bled in this study. Only BT, HR, and urine data were collected from these subjects. Several additional parameters were added to the original study to better assess the changes and mechanisms in physiological function produced by prolonged bedrest.

Twelve healthy males, age 20 to 26 years and weighing approximately 55.33 to 98.41 kilograms (122 to 217 pounds), were divided into two groups. The six subjects in group I followed an experimental procedure that included a 20-day ambulatory prebedrest control period, 56 days of absolute

bedrest, and a 20-day postbedrest recovery period. The six subjects in group II remained ambulatory for the entire 96 days but were confined to the metabolic ward. The selection of the 12 subjects from the 50 to 60 applicants was based on interviews and psychological tests. All subjects were on a 14-hour-light/10-hour-dark regimen (lights on at 0900 hours) and were fed a balanced diet of 10 460 joules (2500 calories) per day. Data were collected on HR and ear-probe temperature (BT) 12 times daily throughout the study.

The main characteristic of the data obtained from the ambulatory subjects was the remarkable consistency. The HR was particularly consistent, showing a peak at approximately 1700 hours in all six subjects, and did not deviate throughout the study. The BT of this group was somewhat less stable; however, the peaks occurred in the same quadrant, between 2045 and 0245 hours (figs. 3 and 4).

In the bedrest group (fig. 5), the HR data can be divided into four segments. In the prebedrest period, the subjects showed stable rhythms that were similar to the ambulatory group. In the first 3 weeks of bedrest, a small but obvious phase shift of approximately 2 hours occurred. Next, a second phase shift of approximately 4 hours occurred and lasted for the remaining 5 weeks of bedrest. Finally, the rhythms of postbedrest subjects almost immediately resynchronized with the original prebedrest rhythms. The mean HR dropped initially and then increased and remained elevated during the 20 days postbedrest.

During bedrest, the BT data were highly variable (fig. 6). The temperatures of three of the subjects peaked in the same quadrant as those of the ambulatory subjects, and peaks for the other three occurred in the morning (180 degrees out of phase). The BT data for all subjects showed considerable rephasing throughout the bedrest; the BT data for three subjects showed random-walk distribution. Only two subjects had resynchronized relative to baseline values at the end of the 3-week postbedrest period. The mean BT decreased throughout the study.

This study confirms previous findings indicating that internal biologic time, more than light/dark cues, is highly important in the physiology of hypokinesis (for example, BT rhythms). The experimental desynchronosis observed in the bedrest group and not in the ambulatory group threatens entrainment to periodic stimuli; therefore, a better understanding of environmental fluctuations, including social "Zeitgebers," is essential in forestalling a catastrophe of the circadian clock.

The relatively constant phase relationship between HR rhythm and the light/dark cycle in this study suggests that the HR rhythm is not an endogenous rhythm but is exogenous. In ambulatory subjects, HR may be determined largely by the BT rhythm and, thus, may appear to be an endogenous rhythm. Mills (ref. 3), in his comprehensive review of

human circadian rhythms, concluded that the rapid adaptation of the HR rhythm to abnormal time schedules or shifts in sleep and activity suggests that it is not an endogenous rhythm.

The frequency of sampling the plasma pool (for example, 6 samples/subject/24 hours) did not appreciably influence the circadian frequency of BT and HR rhythms. This seemingly simple demonstration indicates that both short-term transverse design and the much longer longitudinal study can be carried out simultaneously and meaningful results can be derived from both experimental designs.

The results further confirm (ref. 3) that the primary influence of bedrest on BT and HR periodicity is to reduce the amplitude and change their phase relationships. The normally entrained rhythms are altered after approximately 20 days in the hypokinetic environment and are expressed in changes of amplitude and phases. Bedrest induced low-grade hypothermia and minor tachycardia. This, according to Selye (ref. 4), is characteristic of acute stress, regardless of the cause of stress.

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$R = \sqrt{a^2 + b^2} \cdot L$

$\phi = Tan^{-1}\left(\frac{b}{a}\right)$ Direction of the vector

R = vector length (amplitude)
ϕ = phase angle
(x, y) = vector end point
 = period length
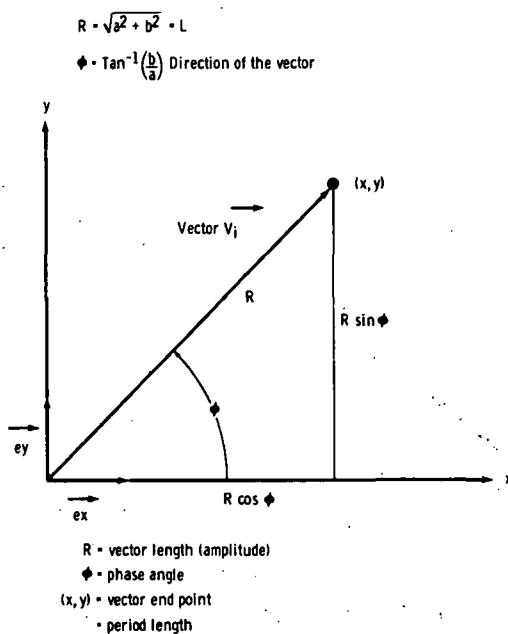
(a)   Graphic representation of the components of a vector.

$f(t) = R \cos\left(\frac{2\pi}{\tau} t - \phi\right) + M$

f(t) = data points
t = time
$\frac{2\pi}{\tau}$ = circadian frequency (1 cycle/24 hours)
M = mean of y
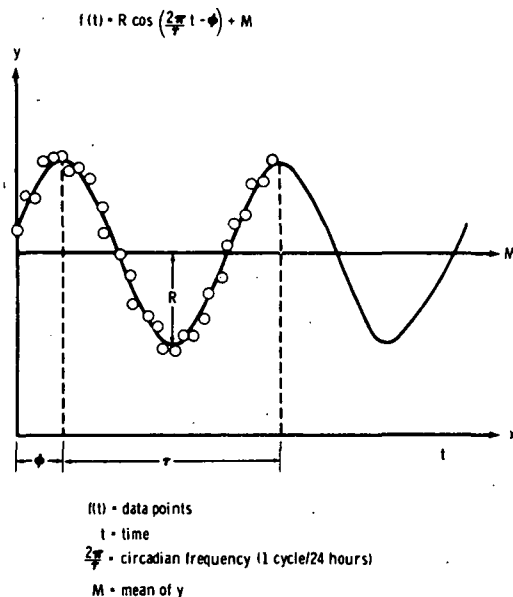
(b)   The least-squares method is applied to fit equally spaced discrete
values.

Figure 9-1.- The summation-dial method, a vector representation of two
components x and y.
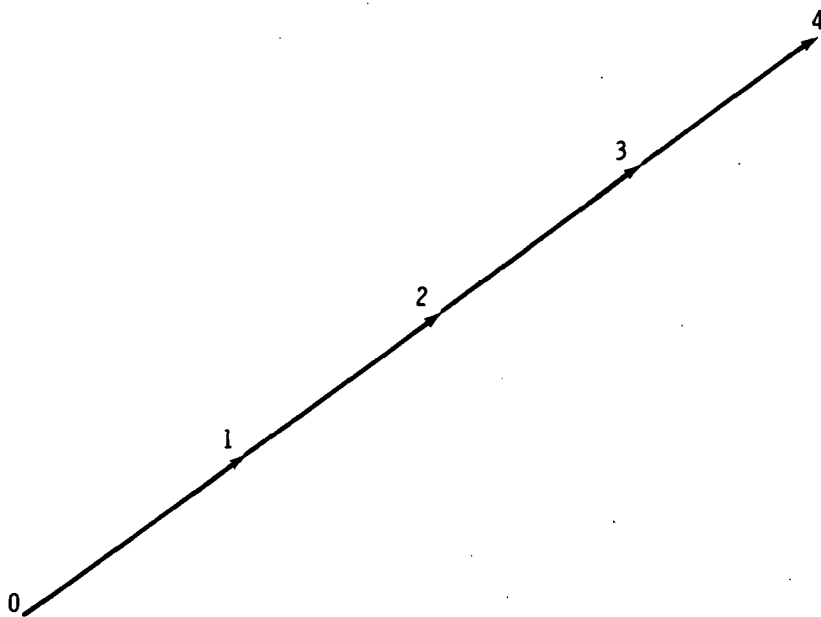
(a) Data stationary in time.
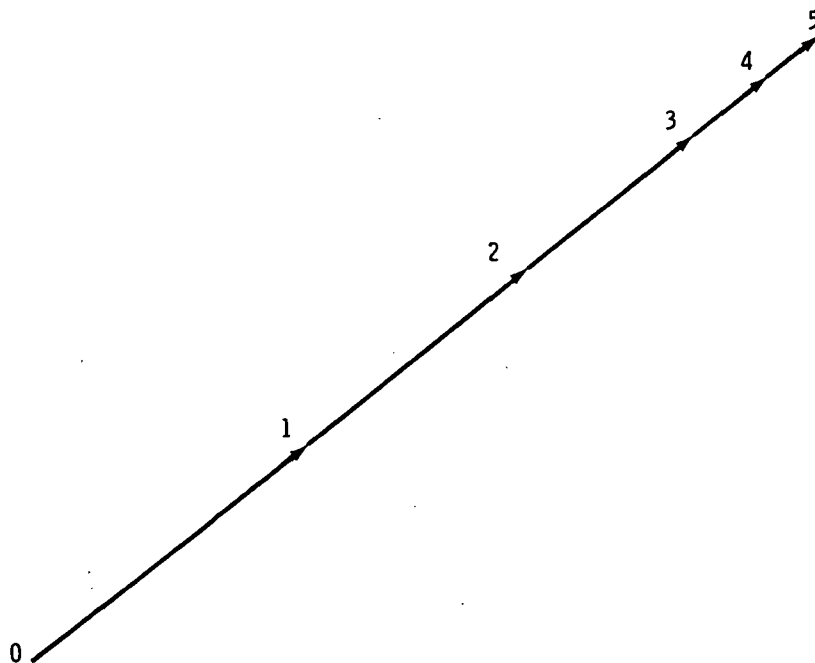
(b) Amplitude damping with constant phase angle.

Figure 9-2.- Schematic illustrations of certain features encountered in the
construction of summation dials.
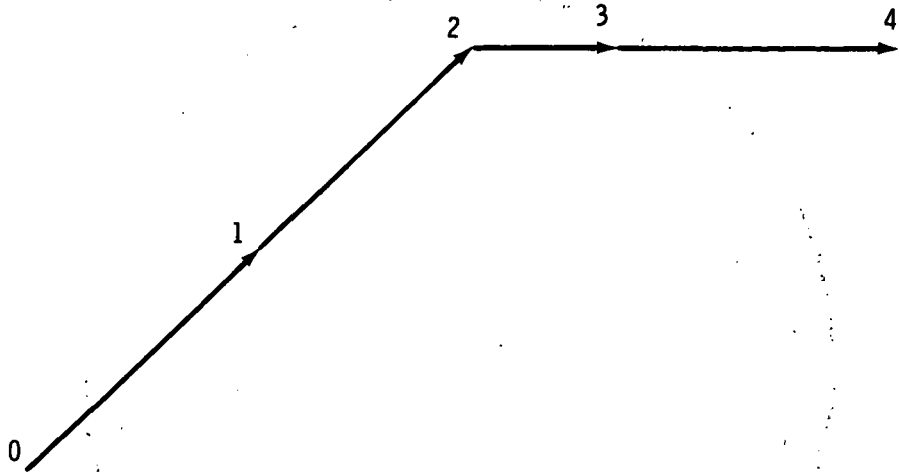
(c) Change in phase angle.
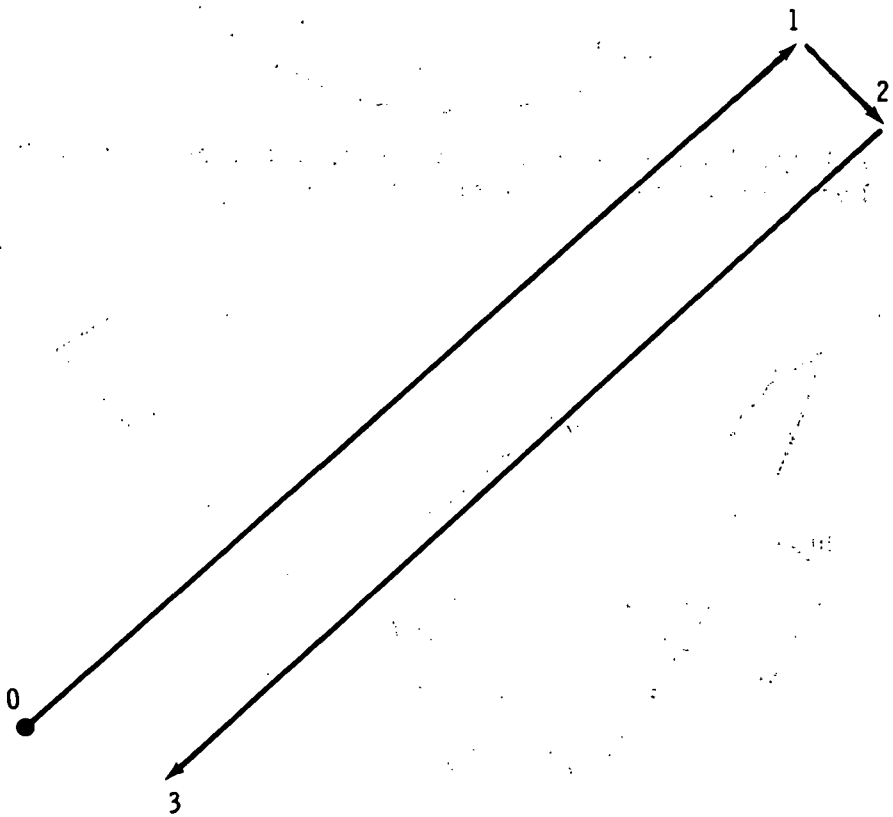
(d) Complete (180-degree) phase reversal.

Figure 9-2.- Continued.
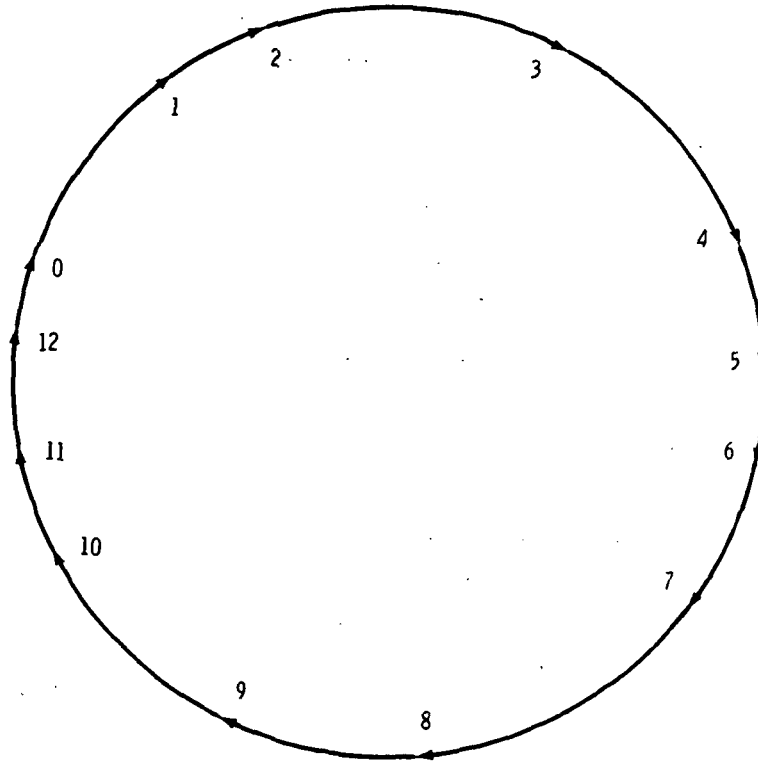
(e) A loop, indicating either a linear phase shift or a 26-hour, rather than a 24-hour, period.
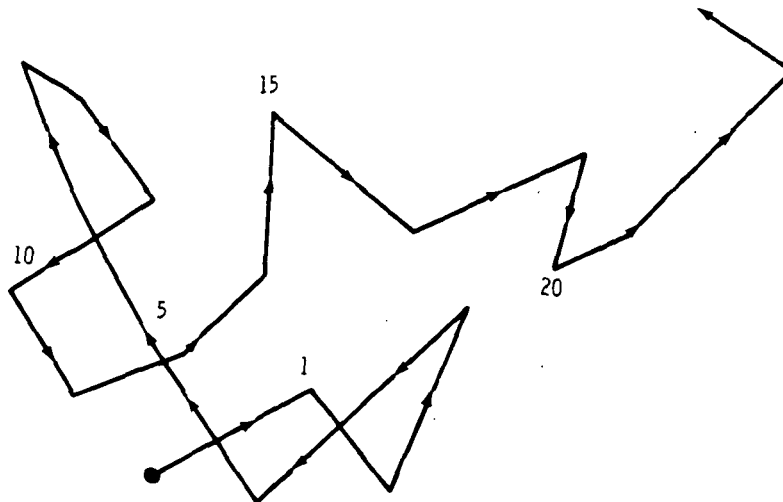
(f) Small signal-to-noise ratio (random walk).

Figure 9-2.- Continued.

2100   2200   2300   2400   0100   0200   0300

2000   0400

1900   0500

(0200)   (1200)

1800   0600

1700   0700

(1800)

(2100)

1600   0800

1500   0900

1400   1300   1200   1100   1000

Numbers represent time of day
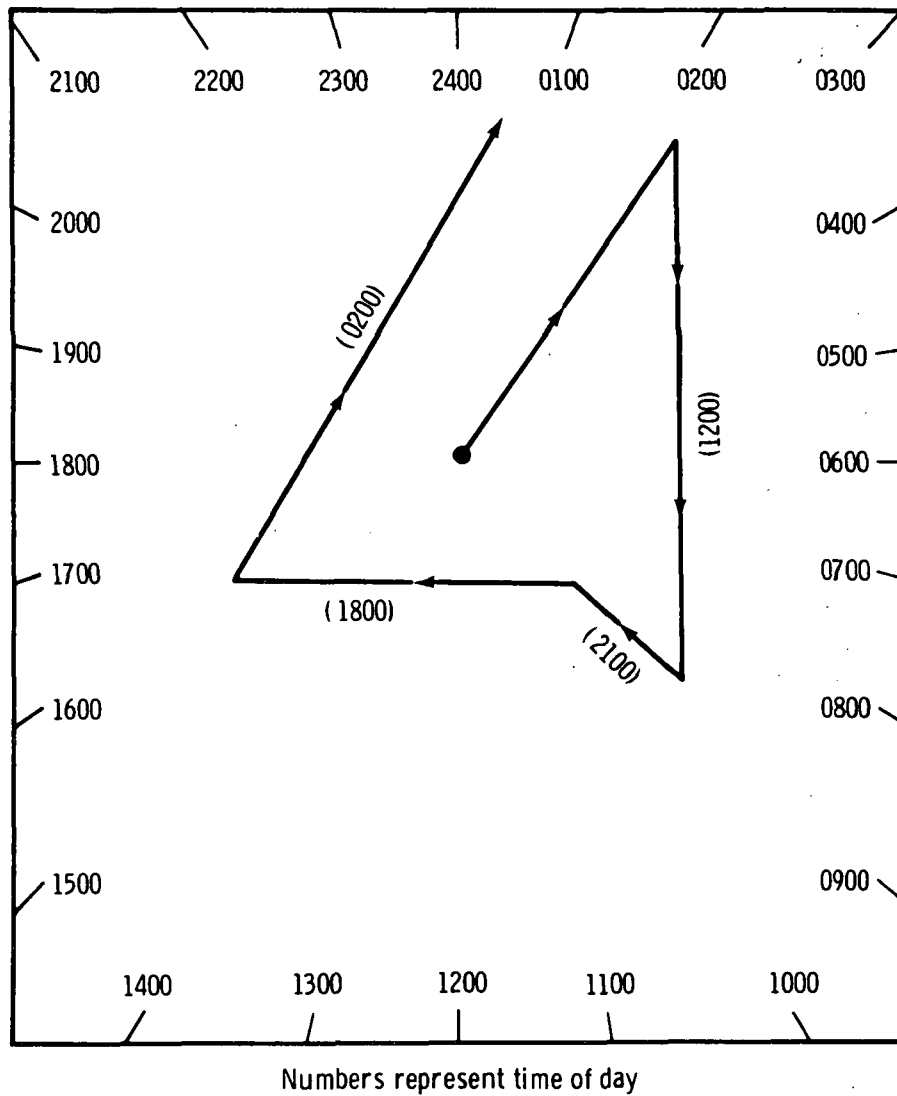
(g) Completed dial. Numbers represent time of day.

Figure 9-3.-- Concluded.
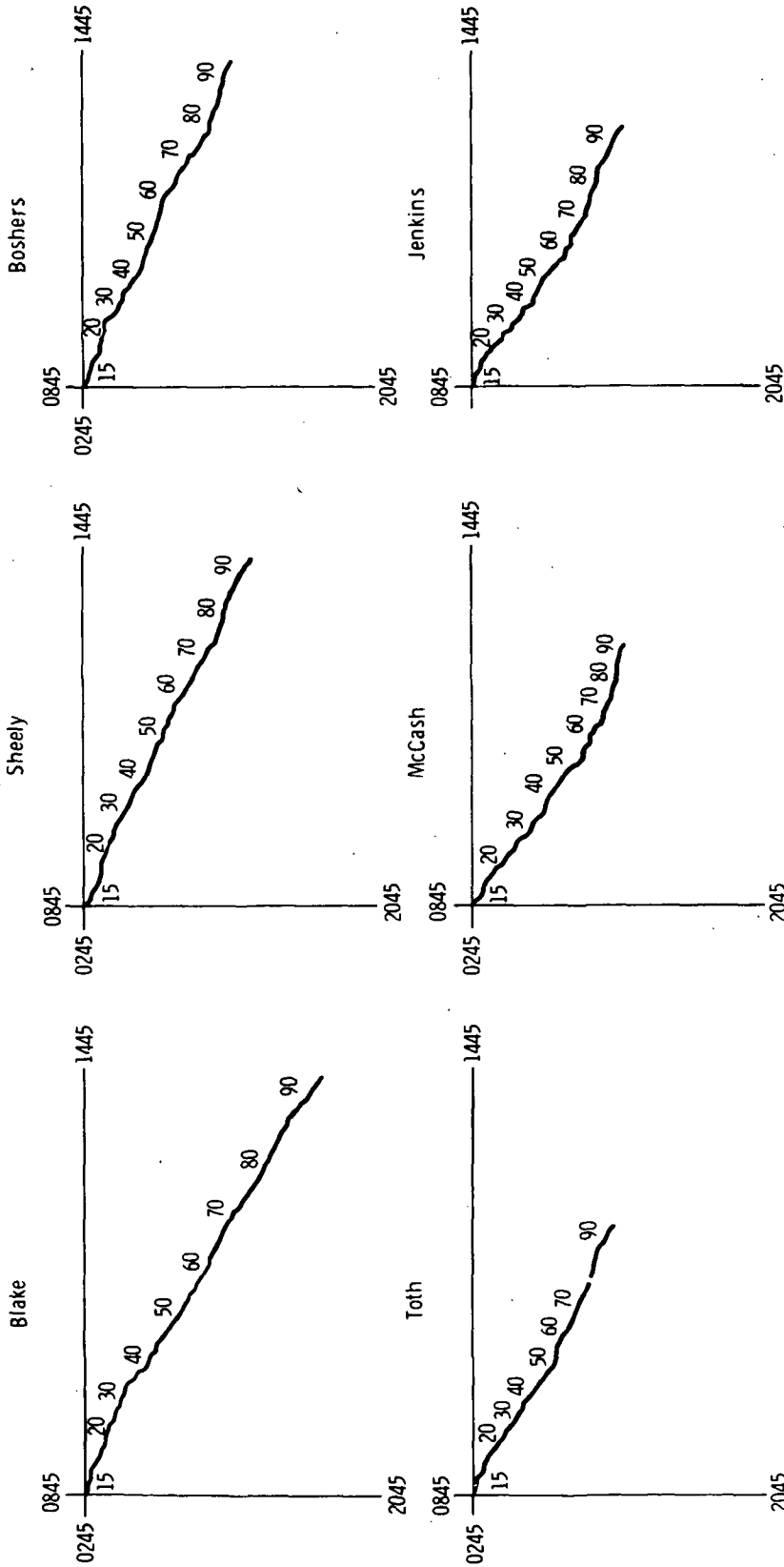
Figure 9-3.- The summation dial for each of the ambulatory subjects showing the successive addition of heart-rate daily vectors in order of experiment day.
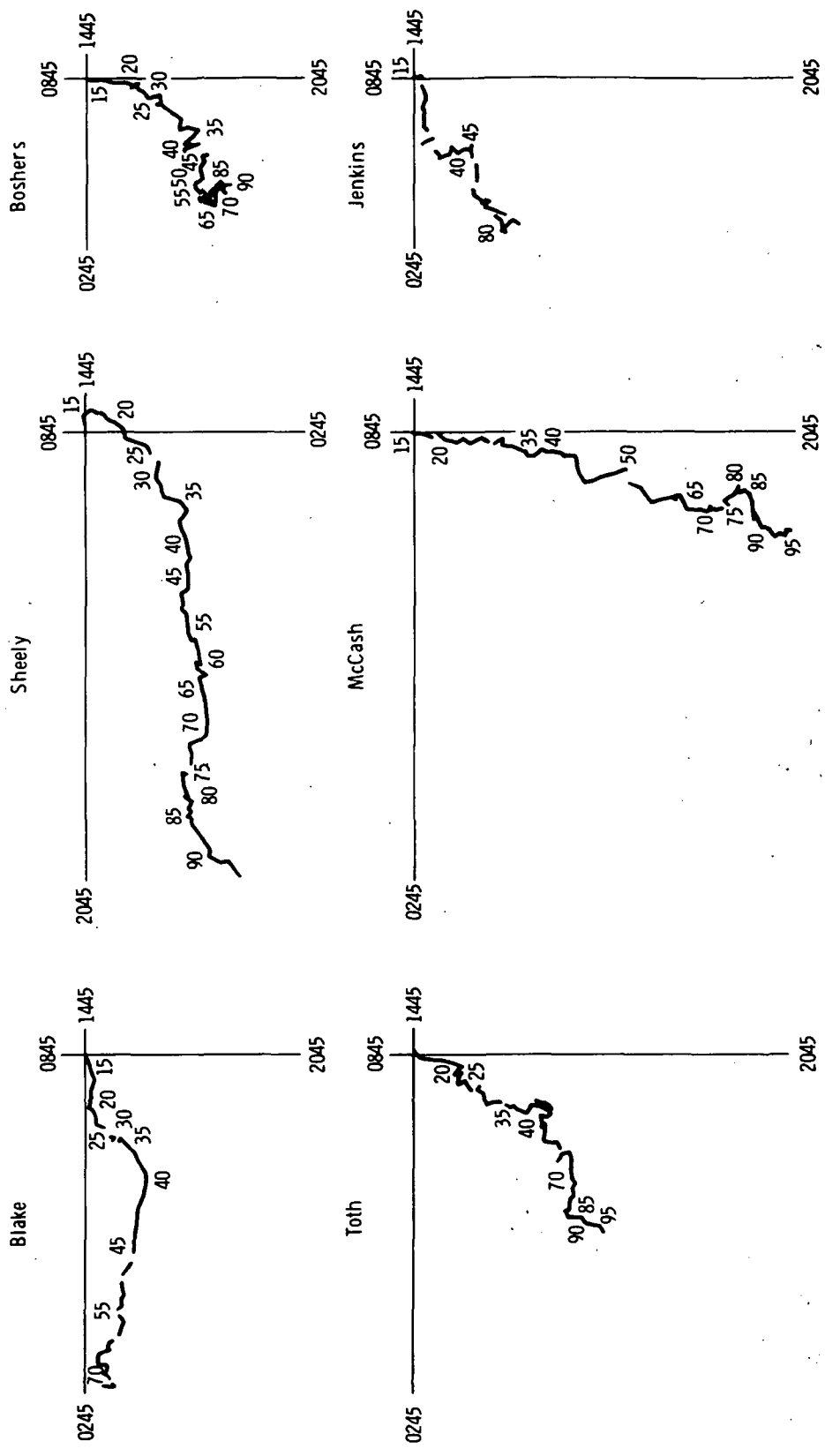
Figure 9-4.- The summation dial indicating the successive addition of body-temperature daily vectors in order of experiment day for the ambulatory subjects.
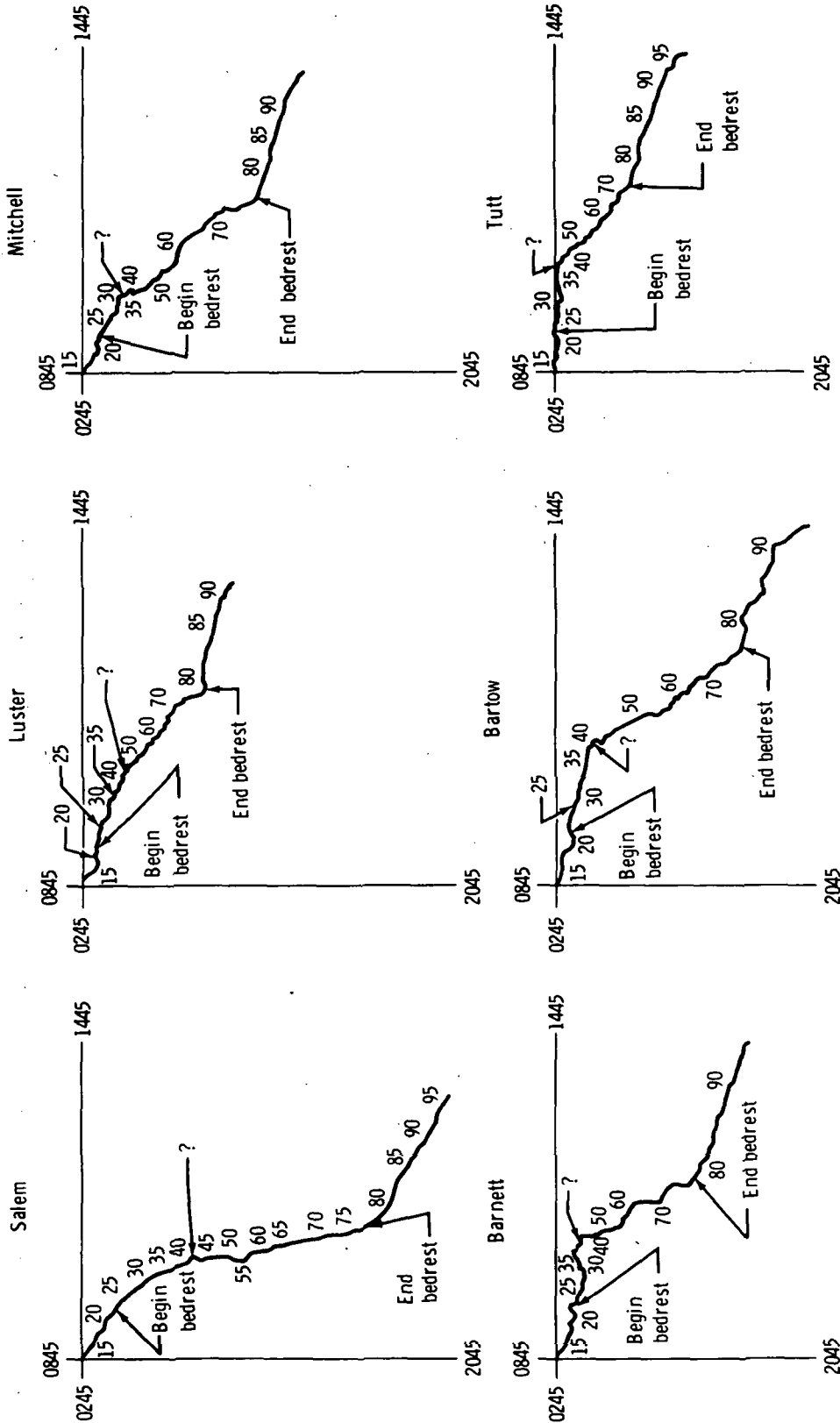
Figure 9-5.- The summation dial indicating the successive addition of heart-
rate daily vectors in order of experiment day for the bedrested subjects.
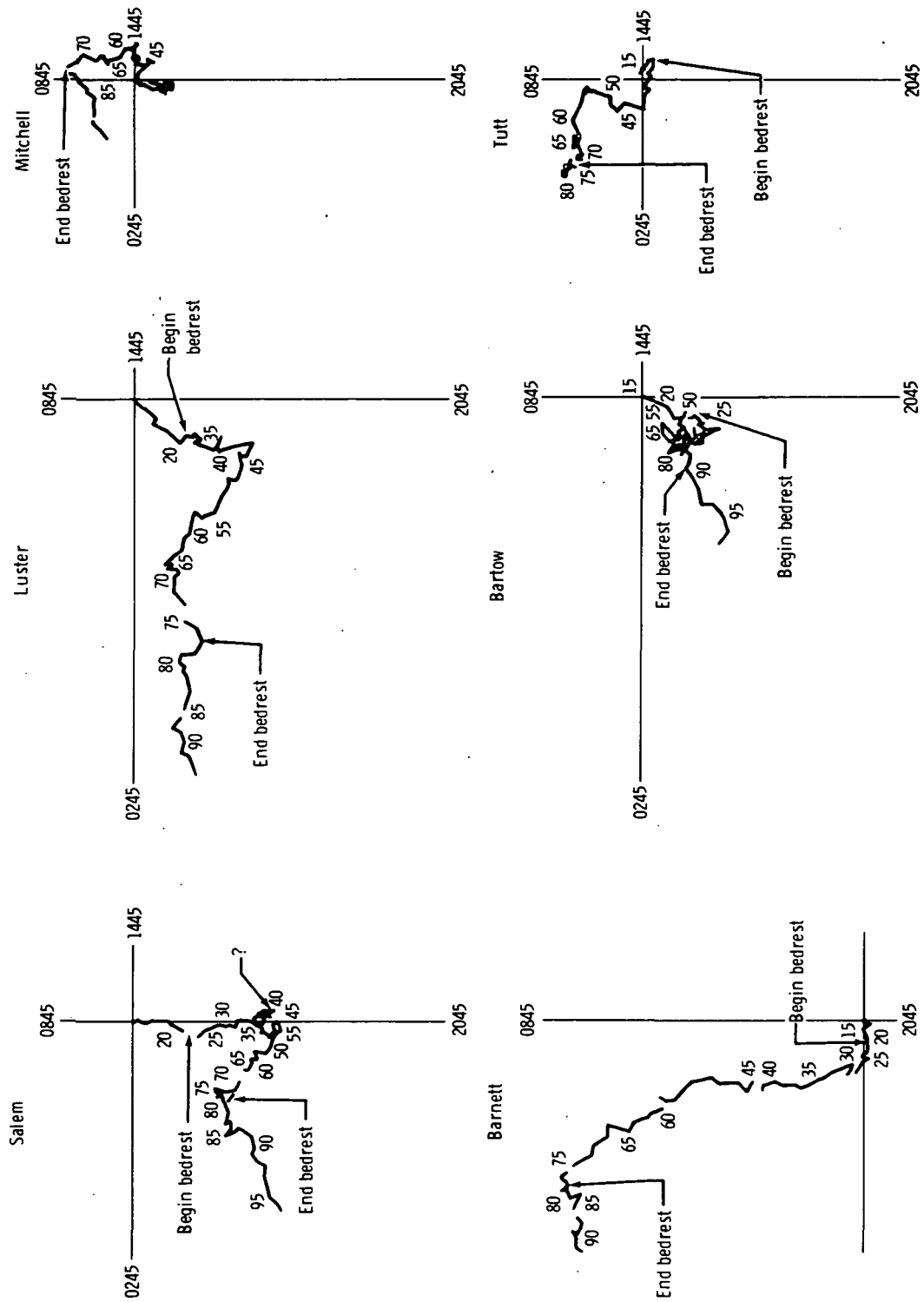
Figure 9-6.— The summation dial for each of the bedrest subjects showing the successive addition of body-temperature daily vectors in order of experiment day.

10. DISSOCIATION OF EFFECTS OF PROLONGED CONFINEMENT AND

BEDREST IN NORMAL HUMAN SUBJECTS; CORTISOL,

INSULIN, THYROXINE, AND TRIIODOTHYRONINE

By Joan Vernikos-Danellis, Ph. D., M. Winget, Ph. D.,
Carolyn S. Leach, Ph. D, and Paul C. Rambaut, Sc. D.

INTRODUCTION

In the same subjects discussed in the previous presentation, endo-crine and metabolic information on the relative effects of confinement and prolonged bedrest was obtained by assaying blood and urine samples for a variety of parameters. This report is preliminary because only the blood changes in cortisol, insulin, thyroxine (T_4), and triiodothy-ronine (T_3) will be discussed. Furthermore, only the determinations on the bedrest subjects have been completed, so comparison with the ambula-tory group will not be possible at this time. Eventually, information on plasma growth hormone, and urinary hormones, and electrolytes will be available. Also, the data will be compared to the subjects' own prebedrest values as well as to those of the ambulatory control group.

DISCUSSION

Urine samples from all subjects were pooled at 4-hour intervals, except during night hours when an 8-hour pool was collected. On days of bleeding, 4-hour pools (150 cubic centimeters maximum) were collected throughout the 24-hour period and stored frozen or cold. No preservatives were added. At each nine periods throughout the study, five subjects in each group were bled by repetitive venous punctures every 4 hours for a 48-hour period. The nine periods occurred during two 48-hour periods before bedrest; at 10, 20, 30, 42, and 54 days after confinement to bed; and at 13 and 20 days after the subjects had become ambulatory again. Group II subjects were bled on the same schedule as those in group I. Fifteen cubic centimeters of blood were removed at each bleeding time to obtain approximately 5 cubic centimeters of plasma and 2.5 cubic centi-meters of serum. The samples were kept in crushed ice during collection and separation, frozen promptly, and stored frozen.

Once during each 48-hour bleeding period (at 0800 hours on the second day), hemoglobin, hematocrit, red-blood-cell, and white-blood-cell parameters were determined in all 12 subjects. No appreciable changes were observed throughout the study.

Plasma free hydrocortisone levels were determined by Murphy's competitive protein-binding radioassay and expressed as $\mu g/100 \; cm^3$ plasma (ref. 1). Serum total thyroxine was expressed as $\mu g/100 \; cm^3$ serum (ref. 2), and serum triiodothyronine was estimated by determining the binding capacity of serum to the hormone and was expressed as relative percent update (ref. 3). Immunoreactive levels of insulin were estimated by the radioimmunoassay technique of Herbert et al (ref. 4). The data were analyzed by usual statistical techniques and by the method of Winget et al. (ref. 5).

Several observations made in a previous study (ref. 6) were confirmed. Diurnal rhythms existed in all four hormone levels during the prebedrest control period, with hydrocortisone showing a peak at approximately 0800 hours, and thyroxine and triiodothyronine reaching maximal levels at 0400 hours. Insulin levels started rising at noon and attained maximal levels between 2000 hours and midnight and dropped to the lowest levels at 0400 hours. Between noon and midnight insulin levels were maintained generally high (fig. 1). Lambert and Hoet (ref. 7) have reported cyclical variations in plasma insulin levels in nonfasting subjects and found high values occurring at night. On the other hand, Freinkel et al (ref. 8) used subjects who fasted for 3 or 4 days. Insulin was measured either at 0700 to 0800 hours in the morning or at 1500 to 1600 hours in the afternoon, and significantly higher levels were found in the morning samples. In the present study, meal time and caloric content were constant throughout. Although the levels of insulin increased dramatically during bedrest (fig. 2) and the time of peak shifted (fig. 1) from midnight to noon and back, the lowest values always were recorded at 0400 and 0800 hours throughout the study.

The hydrocortisone rhythm was affected little by bedrest. As mentioned in this symposium, the peak usually occurred at 0800 hours, anticipating lights on. During bedrest and in the postbedrest period, a secondary peak at approximately 1600 to 2000 hours was observed in these subjects (ref. 9) (fig. 3). During the first half of the study, a 50- to 75-percent increase occurred in the mean cortisol level (fig. 2). The increase was greatest on or about day 20 of bedrest (ref. 10) and was mostly caused by an increase in the usually low evening and night levels. Subsequently, these low points returned to normal and decreased to below control levels by the end of bedrest. A second increase in mean plasma hydrocortisone occurred after approximately 40 days of bedrest. However, the second increase was caused by a 100-percent increase in the usually high morning levels. A marked decrease in the amplitude of the steroid rhythm developed by the end of the study.

In figure 4, a correlation is shown between the mean 24-hour prebed-rest hydrocortisone levels and the mean 24-hour level during bedrest for each of the five subjects. Those subjects with lowest initial levels showed the greatest increase during bedrest and vice versa, as would be expected according to the negative feedback mechanism regulating the pituitary/adrenal system.

As had been noted, thyroid rhythms were the least stable (figs. 4 and 5). The rhythm in either T_3 or T_4 either disappeared or showed considerable phase shifting as bedrest progressed and returned during the postbedrest period.

The increases in mean 24-hour T_3 levels during bedrest (fig. 2) were not as evident as in the previous study (ref. 10), but decreases occurred in both T_3 and T_4 levels during the early part of the study (10 days bedrest), confirming earlier observations by C. Leach (ref. 11). The previously observed sharp rise in T_4 when the subjects got out of bed was not evident in this study.

Recently, Nicoloff et al (ref. 12) have postulated a negative feed-back action of circulating hydrocortisone in regulating the diurnal rhythm in thyroid function as measured by thyrodial iodine release and serum thyroid-stimulating hormone (TSH) values. Their hypothesis was based on the evidence that pharmacological doses of glucocorticoids suppress TSH secretion, and a rebound in TSH release follows withdrawal of the steroid (ref. 13). Although the inverse relationship between adreno-cortical and overall thyroid function has been demonstrated by several investigators, the data on which this report was based show a dissocia-tion between T_3, T_4, and cortisol rhythmicity caused by bedrest, which does not support the thesis that the diurnal rhythm in thyroid function is under corticosteroid regulation.

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     coids on Thyrotropin Secretion. J. of Clin. Invest., vol. 48,
     1969, pp. 2096-2103.

Figure 10-1.- Plasma insulin rhythm before, during, and after 56 days of bedrest. Stippled areas represent lights off periods. Each point is the mean of five subjects ± standard error.
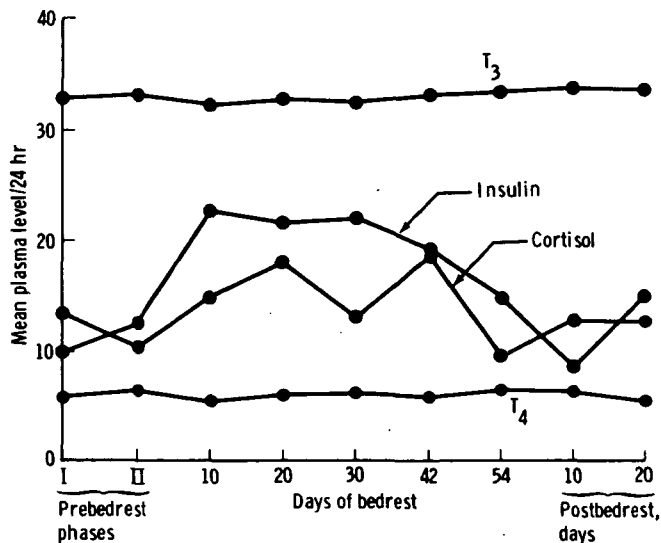
Figure 10-2.- Mean plasma circulating levels of triiodothyronine, thyroxine, insulin, and cortisol per 24-hour period in five subjects before, during, and after 56 days of bedrest.

Figure 10-3.- Plasma cortisol rhythm before, during, and after 56 days of bedrest. Stippled areas represent lights off periods. Each point is the mean of five subjects ± standard error.

Figure 10-4.- Correlation of mean 24-hour prebedrest cortisol levels and percent increase of mean 24-hour level of entire bedrest period for each of the five subjects.

Figure 10-5.- Serum triiodothyronine rhythm before, during, and after
56 days of bedrest. Stippled areas represent lights off periods.
Each point is the mean of five subjects ± standard error.
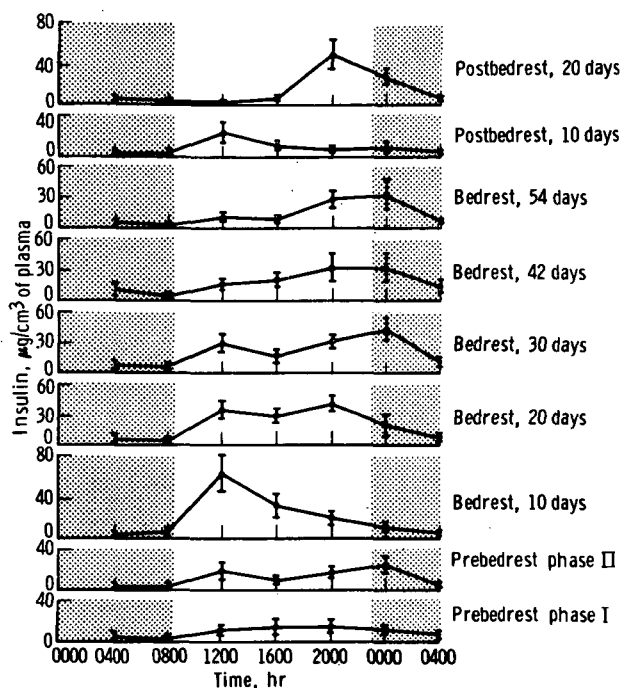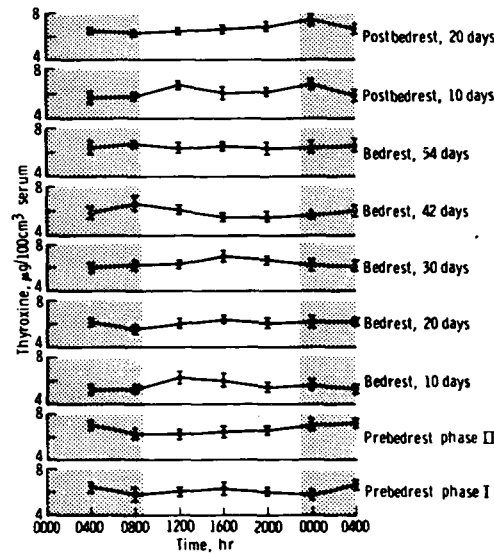

Figure 10-6.- Serum thyroxine rhythm before, during, and after 56 days of
bedrest. Stippled areas represent lights off periods. Each point is
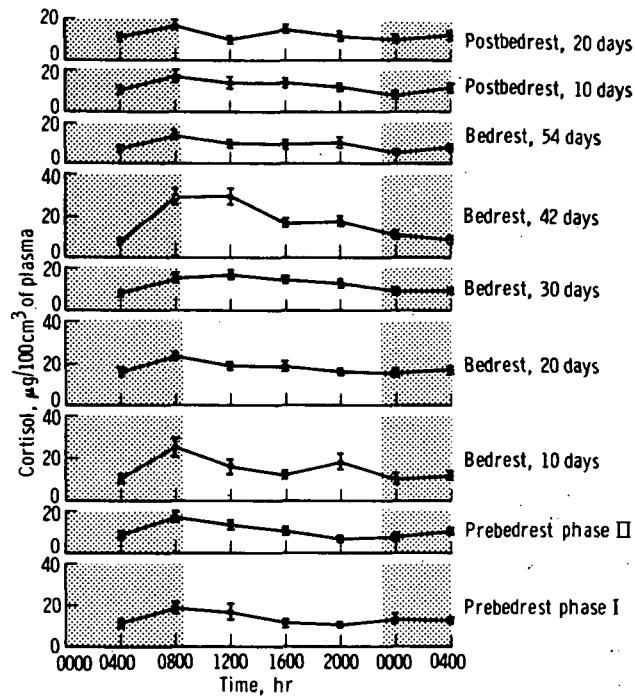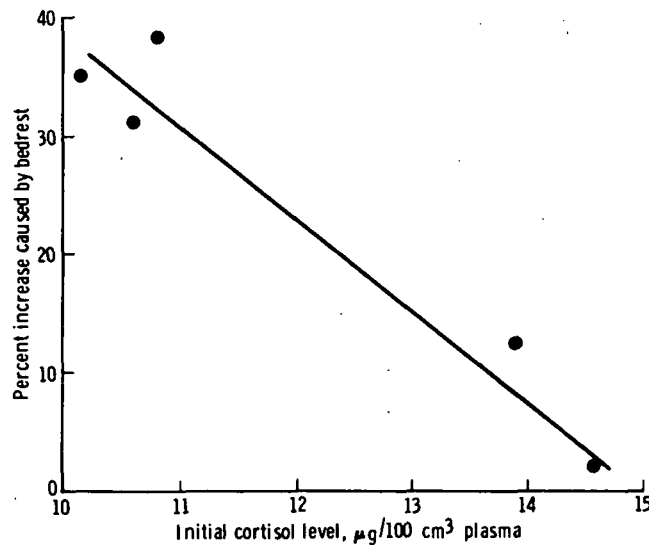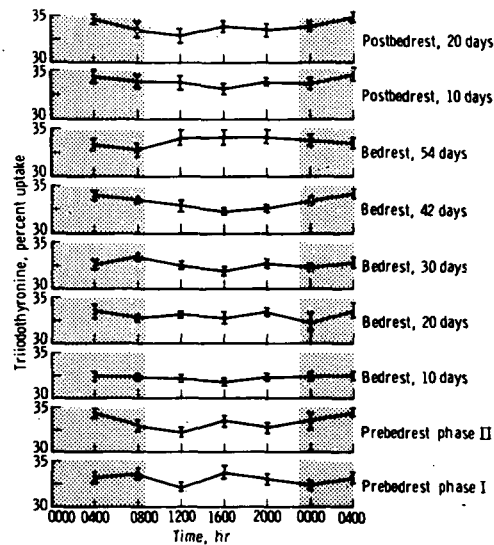the mean of five subjects ± standard error.

11. NUTRITION AND MUSCULOSKELETAL FUNCTION:

SKYLAB EXPERIMENT SERIES NUMBER M070

By Paul C. Rambaut, Sc. D.

INTRODUCTION

Early in 1973, the Skylab Program is scheduled to begin with the insertion into low earth orbit of the Skylab orbital workshop containing approximately 10 000 cubic feet of habitable space. One day after the workshop is established in orbit, it will be joined by an Apollo command module carrying a crew of three men, including a medical doctor, who will live and work in space for 28 days. Three months later, this crew will be followed by another three astronauts, who will occupy the spacecraft for 56 days. The third crew of three men also will occupy the workshop for 56 days.

The Skylab crewmen will perform a variety of experiments in physics, astronomy, engineering, and earth resources. Foremost among the experiments that the crewmen will perform are those designed to support the medical mission of Skylab. The primary purpose of Skylab is to obtain precise information on the long-term effects of weightlessness on physiological and biochemical functions. Unlike any other United States space mission, Skylab is designed primarily to gather medical information. For the first time, space-medicine specialists will have the opportunity to explore, in depth, the subtle changes that have occurred on the shorter Gemini and Apollo flights.

One of the medical investigations to be pursued intensively during the Skylab Program is designed to assess the effects of space flight on the integrity of bone and muscle. A metabolic-balance study is to be performed to quantitate the effect of space flight on the rate of gain or loss of key body-chemical constituents. In conjunction with this study, an exhaustive endocrinological investigation also is being conducted to examine the changes in control functions that accompany or precipitate changes in body composition and fluid and electrolyte metabolism. A series of investigations designed for the study of these factors has been formally designated as an experiment series entitled "Nutrition and Musculoskeletal Function" and has been assigned the number M070 in the Skylab Program.

PURPOSE

The M070 experiments are designed for the acquisition of kinetic data concerning changes in body chemistry as a function of time of exposure to weightless flight. The experiments differ qualitatively from similar Apollo experiments, which were dependent, for the most part, on preflight and postflight measurements only.

Alterations in calcium metabolism constitute one of the major threats to the health of astronauts during long-term exposure to weightlessness (refs. 1 and 2). Studies of immobilized subjects indicate that the clinical disorders most likely to be encountered during prolonged space flight are primarily a consequence of an imbalance between bone formation and resorption. Under these conditions, a loss of skeletal mass occurs, leading to osteoporosis, hypercalcemia, hypercalciuria, and possibly nephrolithiasis (refs. 3 and 4). Available data from inflight studies tend to support the use of immobilization as a terrestrial method for simulation of the alterations in calcium metabolism that occur during space flight (refs. 5, 6, and 7).

During periods of bedrest lasting from 30 to 36 weeks, calcium losses from the skeleton average 0.5 percent of total body calcium per month (ref. 8). Tenfold greater rates of loss from the central portion of the calcaneus were observed by X-ray transmission scanning (ref. 9). Mineral loss during bedrest is probably caused by a reduction in the forces that are applied to the skeleton during normal activity. These forces would also be absent in the hypogravic environment of space flight. Therefore, theoretically, loss of bone mineral during space flight is expected (ref. 10).

PROCEDURES AND HARDWARE

The M070 experiments were designed to obtain definitive information on the rate of occurrence of these losses in flight. For the experiments, a complete input and output measurement on all Skylab astronauts will begin 21 days preflight and continue throughout the inflight phase and for 18 days postflight. All nutrient and water intake will be measured precisely. All fecal material and urine samples will be returned to earth for analysis, and samples of blood will be taken preflight, inflight, and postflight.

The M070 experiments will be the most rigorous metabolic studies ever conducted in manned space flight and are among the prime objectives of the mission. However, a secondary program objective of Skylab is to test the environmental conditions thought to be necessary to optimize

psychological performance of the crewmen. As a design goal, the Skylab
spacecraft is to be made so pleasant a living and working environment
that the crewmen will be reluctant to leave it. Therefore, two conflict-
ing goals were presented to spacecraft designers. One was to design
equipment to support an intensive metabolic investigation, and the other
was to design equipment that was to make the spacecraft as habitable as
the technology permitted.

SKYLAB FOOD SYSTEM

Foremost among the conditions of life known to influence behavior
is the type and variety of the food system. The food system to be em-
ployed in Skylab is composed basically of 72 different food items. These
items are packed in flip-top cans and include a variety of frozen, ther-
mostabilized, dehydrated, and compressed foods. A facility exists on
Skylab to heat some foods before consumption and to refrigerate others.

The energy requirements of each astronaut are estimated on the basis
of his age and body weight and are adjusted for some of the known effects
of weightless flight. Individualized menus are formulated and offered
to the crewmen for a series of 5- or 6-day metabolic test periods. After
several iterations of this testing program, menus are finalized for
flight. A number of nutrients are controlled within rigid day-to-day
tolerances, as depicted in table I.

Menus are designed according to 6-day cycles. The menus contain a
core set of foods that provide the required levels of nitrogen, calcium,
phosphorus, magnesium, potassium, and sodium. This core diet is approxi-
mately 12.552 joules (300 kilocalories) less than the caloric requirement
established for one-g. All additional calories are provided by food items
that are low enough in controllable elements so they will not perturb
the prescribed intake ranges. The items that furnish the additional
calories are termed empty-calorie items.

The crewmen will be encouraged to consume completely the items on
their nominal menus. A system of negative reporting will be employed so
that the crewmen will report, at the end of each day, any deviation from
the nominal menu. The only permissible deviations are the imcomplete
consumption or omission of an item on the nominal menu, the use of an
off-nominal rehydration quantity, or the consumption of empty-calorie
items.

To maintain controlled intakes of calcium, phosphorus, magnesium,
sodium, and potassium in conjunction with these possible deviations, the
crewmembers also are supplied with a series of mineral supplements. By

the use of the supplements, the intake of each of these elements can be adjusted independently of the others. The mineral supplements are listed in table II. A computer program has been designed to calculate mineral and caloric deficits from information transmitted to earth by the crew. The quantity of mineral supplements equivalent to its deficit is evaluated in near real time and transmitted back to the crew.

The food system utilized for the Skylab Program is not only designed to meet the requirements and the constraints imposed by metabolic and psychological demands of the crewmembers and the demands of medical experimenters, but it is also designed to withstand the physical and environmental characteristics of the vehicle during prelaunch, test, launch, orbit, and entry periods.

The microbiological composition of the foods complies with the required specifications (ref. 11). Each Skylab food item is analyzed for approximately 30 nutrients. These analyses are performed as the food is produced and after various periods of storage. In addition, information on the effect that heating, in the Skylab heater tray, has on the nutrients of interest is acquired.

The total supply of water for the entire mission will be launched in the orbital workshop. The water system provides the capability to supply and dispense water for food and beverage preparation and drinking with an accuracy of ±1 percent. The system also incorporates a capability to dispense and maintain an iodine biocide to ensure microbial control. A separate drink dispenser is provided for each crewman, and this dispenser contains a visual indication of the amount of water dispensed. The water that the crewman ingests is essentially free of calcium, magnesium, phosphorus, nitrogen, potassium, or sodium.

MASS-MEASUREMENT DEVICES

To measure mass in a weightless environment, a device is employed that measures and records the time associated with the period of a pendulum with a fixed displacement from a spring-supported restoring force. The masses to be measured are accelerated uniformally by this repeatable restoring force, and three periods of the pendulum are timed. Known masses are measured, and a nomograph is developed that can be used to determine the mass of other objects. This minimal response to gravitational force of the device is eliminated in orbital flight by recalibration with the same known masses that were used preflight. The difference is that the oscillating period which results between different masses is timed by interruption of a light train. A knife-edge device attached to the oscillating system intermittently cuts the light train, triggering a timing circuit in the mass-measurement device electronic subsystem. This

time is converted and presented in millisecond units. The display provides a six-digit readout. The linear spring oscillating arrangement is sufficiently responsive to small changes in mass to permit accurate discrimination over the expected range of mass. Temperature sensing of the springs is important to correct for thermal effects. A thermometer was incorporated to sense and digitally display the temperature of a thermal pathway, related to spring temperature. Correction values for temperature effects have been developed during one-g calibration.

Two types of mass measurement device are placed on board the spacecraft. One device will accommodate small masses up to 1 kilogram, and the other large enough to measure the mass of an astronaut.

WASTE MANAGEMENT SYSTEM

At a temperature below 288.15° K (59° F), the Skylab waste management system (WMS) provides for the collection, processing, storage, and disposal of feces, urine, and vomitus. It is designed to preclude mixing and cross-contamination of samples of urine, feces, vomit, and debris of the crewmembers and also prevents cross-contamination in excess of 1 percent between samples from the same crewmember obtained on different days. The WMS also provides the capability for transferring processed and identified samples of the collected urine, feces, and vomitus through the command module for subsequent return to earth for analysis.

Urine is collected in a device called the centrifugal urine separator assembly (CUSA). The CUSA permits urine from each micturition to be drawn into a centrifuge inlet line by means of entrained air from a downstream blower. The liquid is carried by centrifugal force to the outer diameter of a drum that is rotating at approximately 250 rpm. The rotating annulus of the urine impinges on a stationary pitot head, and a dynamic force pumps the liquid through the outlet line to an external urine-bladder collector assembly. The urine receiver completely encloses the urine stream during the collection process. The urine collector provides the capability to extract representative samples of 122 cubic centimeters from a homogeneous pool for freezing. The samples are frozen to below 254.00° K (-2.5° F) at the end of each 24-hour period. The urine-collection unit determines the volume of each 24-hour void to an accuracy of ±2 percent. A flushing capability is provided as a means of controlling cross-contamination between the 24-hour pooled urine collections for each user. Use of a flushing system limits the day-to-day cross-contamination between urine samples to less than 1 percent of the volume collected each day.

The 24-hour urine pool normally is maintained at a temperature below 288.15° K (59° F), and the temperature of this pool does not exceed 288.15° K (59° F) for more than an accumulated time of 3 hours during any 24-hour period. The urine collection system interfaces with the command module for transferring, collecting, measuring, and sampling of urine collected during the command module operations before the orbital workshop activation.

A lithium chloride tracer is incorporated as a backup method to determine the volume of urine collected in each 24-hour urine pooling period. Lithium is added in the amount of 30 ± 0.3 milligrams into each pooling bag before flight. The primary mode of volume determinations depends on displacement of a pressure plate with a calibrated readout. A contingency urine system also exists that utilizes the Apollo-type roll-on cuff and adaptors to accommodate urine collection directly into the urine-collection bags without the use of air entrainment. After each void, the urine collection bags would be stored, employing the chilling capability of the urine collector. Volume measurement will be accomplished by using the lithium chloride tracer. The contingency collection system also provides the capability for extracting urine samples.

The fecal waste processor provides for vacuum drying of fecal and vomitus collections before long-term storage.

ERROR ANALYSIS

An error analysis is being conducted on the M070 experiments to assess the effect that changes in design could have on the ultimate sensitivity of the experiments. As currently conceived, it is expected that the experiments will be able to detect, at the two-sigma level of confidence, changes as small as 5 percent in the body pool of calcium at the end of a 56-day period.

A mathematical model of the M070 experiments is being formulated and will incorporate each component of the experiment design and operation that can contribute to error. This model will be used to predict the effect of changes in any item of equipment or any mode of operation.

BLOOD COLLECTION AND EQUIPMENT

Blood samples will be collected inflight with the aid of a specially designed sampling device that will return approximately 11 cubic centimeters of plasma in a frozen state for ground-based analysis.

PREFLIGHT AND POSTFLIGHT METABOLIC CONTROL PERIODS

Metabolic control periods will be conducted for 21 days preflight and for 18 days postflight. During these periods, the crewmen will consume Skylab flight foods that will be supplemented, to a minor extent, with fresh food items to improve the acceptability of the flight diet. Complete collections will be made using a portable biological specimen kit that will enable the crewmember to collect urine and fecal samples and preserve them in a frozen state until they are returned to the laboratory for analysis.

BASELINE STUDIES

A variety of ground-based controlled studies have been conducted to determine the effect of various experimental variables other than weightlessness on the parameters of interest. To assess the effect of immobilization and hypogravia, the effect of long-term recumbency has been extensively studied. The effect of varying intakes of protein and of calcium and magnesium on the parameters of interest has been investigated, and the influence of different activity levels on mineral balance has been studied.

These baseline studies culminate in a 56-day manned chamber study that is designated the Skylab medical experiments altitude test (SMEAT). SMEAT is a highly-fidelity mockup of Skylab in which three crewmembers will be confined for 56 days and in which all Skylab medical experiments, including M070, will be performed in a manner as similar as possible to the way in which they will be performed in flight. Like Skylab, SMEAT has an atmosphere consisting of 70 percent oxygen and 30 percent nitrogen, $34\ 473.785\ \text{N/m}^2$ (5 psi). It contains carbon dioxide at a partial pressure of 5 millimeters of mercury. Primarily, the concern for this relatively high carbon dioxide partial pressure was the reason for conducting SMEAT before Skylab.

LABORATORY ANALYSIS

All fecal, urine, and blood samples from both SMEAT and Skylab will be returned to the NASA Manned Spacecraft Center for analysis. Extensive tests have been undertaken to establish the precise laboratory errors that are introduced in the analysis of these samples, as well as errors arising from any degradation that may have occurred in the constituents during prolonged storage in space and on the ground.

CONCLUSION

The M070 experiments are expected to give medical investigators precise information on a variety of biochemical changes occurring during exposure to space flight. Sufficient control data are being generated by baseline studies to differentiate those effects that are caused by weightless flight and those that are caused by other abnormal conditions that normally accompany spaceflight. However, some environmental conditions remain uncontrolled. For instance, fairly severe shifts in sleep cycles possibly will be necessitated by factors associated with the nature of the Skylab orbit. The unknown interaction of these factors with the physiological changes that the M070 experiments are designed to detect will introduce errors of unknown proportions. Nevertheless, the M070 experiments will provide essential data on which decisions will be based concerning the qualification of man, first on Skylab III and IV and then on missions of much longer duration.

The M070 and other Skylab experiments will eventually provide an answer for the question of whether man can live indefinitely in weightless flight. If physiological degradation appears to be severe under these conditions, then a new era of space medicine will evolve for the development of countermeasures to the deteriorative effects of weightless flight. Such countermeasures will be specific devices or procedures tailored to preclude the occurrence of one or more physiological difficulties.

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TABLE 11-I.- CONTROLLED-NUTRIENT DATA

Nutrient	Intake range	Tolerance
Calcium	750 to 850 mg	±16 mg
Protein	90 to 125 gr	±10 gr
Phosphorus	1500 to 1700 mg	±120 mg
Sodium	3000 to 6000 mg	±500 mg
Magnesium	300 to 400 mg	±100 mg
Potassium	At least 3945 mg	--

TABLE 11-II.- MINERAL-SUPPLEMENT DATA

Mineral supplement	Controllable element	Amount of element, mg
Calcium lactate	Calcium	32
Orthophosphate	Phosphorus	110
Magnesium lactate	Magnesium	25
Sodium chloride	Sodium	197
Potassium gluconate	Potassium	195.5