

Nitrogen Metabolism in Bears: Urea Metabolism in Summer Starvation and in Winter Sleep and Role of Urinary Bladder in Water and Nitrogen Conservation

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Three bears were studied under conditions of (1) no food but access to water for 2 weeks and (2) no food or water for 3 weeks. During starvation in summer, the bears could not inhibit the net production of urea but used lean body mass; when denied access to water as well, the bears became dehydrated and azotemic. Urea was continuously formed and degraded in the winter. Arginase activity in liver increased in winter sleep; hepatic steatosis and inflammatory reactions were also noted. The urinary bladder reabsorbed labeled urea and D_2O in winter; the rate of absorption of urea was equal to the rate of excretion of it into the bladder. The ability to preserve lean body mass during winter sleep apparently is a special mechanism associated with the induction of winter sleep. Bears cannot duplicate this feat during summertime starvation. In winter sleep, urea is formed and degraded but the nitrogen produced is conserved in some manner that maintains the total nitrogen pool constant. The urinary bladder plays a central role in maintaining the state of winter sleep by absorbing water and solute at a rate equal to their entry into the urinary bladder.

This study in bears had three aims: (1) to determine if starvation inhibits urea production when the bear is not in winter sleep; (2) to determine if urea is produced and metabolized in winter sleep, although there is no net production;^{1,2} and (3) to determine the role of the urinary bladder in maintaining the state of winter sleep through reabsorption of water and urea.

It has been shown² that bears in winter sleep can go without food and water at near-normal body temperatures (35°C) for 3 months without urination or defecation, because of the complete inhibition of net production of urea. If urea is injected intravenously during the winter sleep, diuresis occurs, and this disrupts normal winter sleep by producing dehydration.² Because starvation also decreases but does not completely abolish net urea production in humans,³ the possibility exists that the inhibition of net urea production in the bear may not involve special mechanisms peculiar to winter sleep but is a consequence of the starvation associated with it.

METHODS

Studies were done on three bears (*Ursus americanus*) (no. 1, 3, and 7). Starvation experiments were done several times in bears 1 and 3. To obtain blood, catheterized urine, and biopsy specimens, they were immobilized as previously described² with phencyclidine HCl and promazine HCl. Experiments were done in summer or fall, well before the onset of winter sleep. Three types of studies were done, separated by at least several months to 1 year to allow the animals to recover completely from the effects of starvation.

In the first study, done in the fall, the bears were placed in an outdoor metabolic cage, sheltered from sun and rain, with access to food and water; then, food was removed for 2 weeks. Twenty-four-hour collections of urine were obtained before and during starvation and were analyzed for total nitrogen⁴ and urea.⁵ In the second study, performed in the summer,

both bears were placed in an enclosed den in a root cellar without food or water for 17 days, at 10°C. In the third study, also done in the summer, bear 1 was housed in a den in a cold room, maintained at 5°C, for 21 days. All dens were dark, similar to winter conditions. The bears were observed each morning, and the experiment was terminated when sufficient time had elapsed for an adequate test of the hypothesis without producing an irreversible situation from which the animals could not recover. Body weight and blood hematocrit, creatinine,* and urea⁵ were determined in all starvation experiments. Total body water⁶ was also determined before and after the cold-room study. The same two bears were studied in winter sleep and similar determinations were made.

To study formation and degradation of urea before and during winter sleep, tracer quantities of ¹⁴C-labeled urea (220 μ Ci) were injected intravenously as a slug dose into each of two bears (1 and 3) in the fall, at a time when they were eating and drinking normally, and in winter sleep. The bears were immobilized and samples of blood, urine, and expired air were obtained at timed intervals over a 24-hour period. The activity of plasma samples was determined in a liquid scintillation counter (Beckman LS-100). By using a specially designed face mask and tubing,² expired air was bubbled through a scintillation vial containing Hyamine solution with an indicator; when the solution turned from blue to colorless, 2.0 mmol of CO₂ had been trapped.⁷ Percutaneous biopsy specimens of liver were obtained in the fall and winter from the two bears, and the tissue was analyzed for arginase activity⁸ and examined microscopically.

¹⁴C-labeled urea and D₂O were instilled into the bladder of bears 1 and 7 in winter sleep and the appearance of these substances in the blood was followed for 7 to 24 hours. In bear 1, the radioactivity from these substances was determined in blood and urine 1 week after the initial experiment. All urine and blood samples were counted in a liquid scintillation counter (Beckman LS-230). Several blood and urine samples were examined further by separating the urea from other blood constituents by ion exchange column chromatography⁹ and measuring its radioactivity by using a flow-through cell in a liquid scintillation counter (Beckman LS-100). Urea isolated from blood and urine samples was compared with known creatinine and urea standards.⁹

RESULTS

Large quantities of urinary nitrogen and urea were excreted daily while the bears were eating and

Table 1.—Urinary Excretion Before and During Summer Starvation* in Bears

Period	Urine volume (ml/24 h)	Total nitrogen (g/24 h)	Urea nitrogen (g/24 h)
Bear 1			
Before	16,400	69.2	45.3
During:			
Day 2	3,540	8.6	4.9
Day 7	1,450	11.4	9.5
Bear 3			
Before	8,140	104	94.8
During:			
Day 7	1,475	11.5	10.0
Day 14	590	7.0	5.4

*Water available ad libitum.

drinking normally (Table 1). When they were deprived of food but given water, urinary nitrogen loss continued, most of it as urea. When both food and water were withheld, the bears failed to demon-

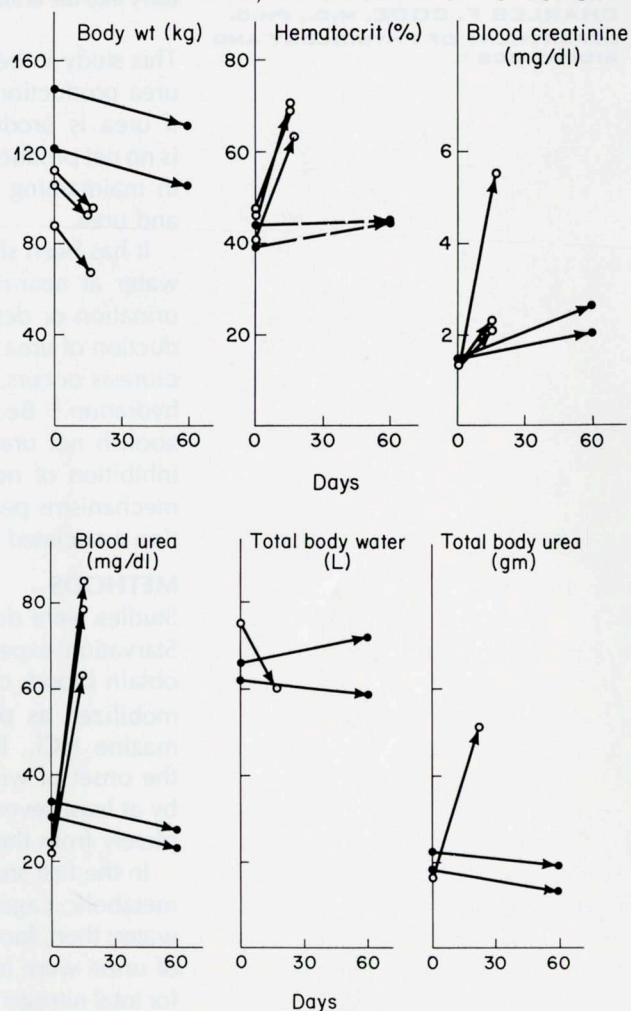


Fig. 1. Measurements in bears denied access to food and water in summer (○) and in winter (●).

*Technicon AutoAnalyzer File N-11b, Technicon Instruments Corp., Chancery, NY.

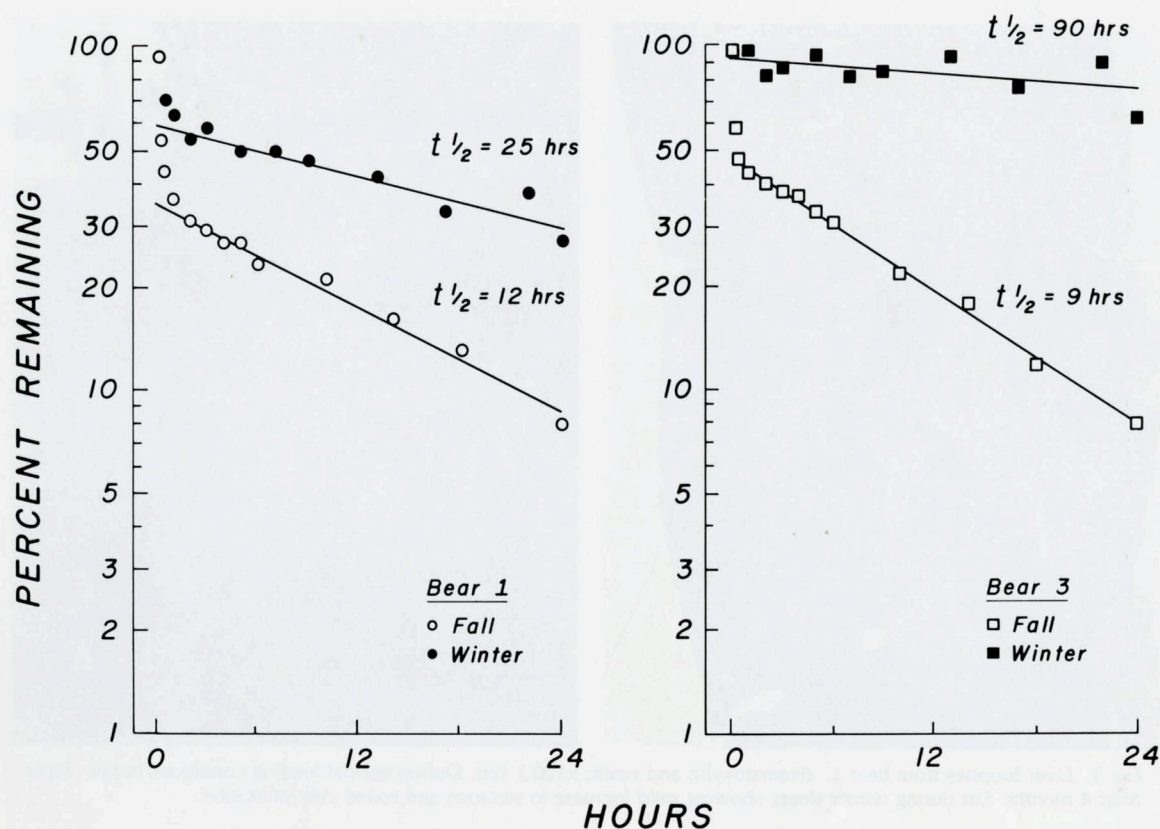


Fig. 2 Disappearance of ^{14}C -labeled urea from plasma in fall and winter in two bears.

strate winter sleep adaptation. Body weight decreased and blood hematocrit, urea, and creatinine increased more rapidly and to greater extents than observed in winter sleep (Fig. 1). However, summer experiments lasted only one-third as long as winter sleep studies. In summer, total body water decreased but the increase in blood urea was enough to produce a large increase in calculated total body urea (only the bear studied in the cold room received D_2O). On the other hand, in winter, because total body water remained relatively constant while blood urea decreased slightly, there was a slight decrease in calculated total body urea. Because of the failure of total body water, which reflects the quantity of lean body mass,⁶ to change significantly, the data support previous impressions² that lean body mass remains constant in winter sleep.

Although total body urea decreased slightly in winter, urea apparently was formed and degraded during this time because the labeled urea in plasma steadily decreased over the 24-hour test period (Fig. 2). This interpretation of the data was confirmed by the finding that $^{14}\text{CO}_2$ was always detected in expired air after the administration of labeled urea (Table 2). Turnover rates for urea were higher in

fall than in winter, as demonstrated by the great differences in the half-life of the urea molecule (Fig. 2).

Activity of arginase was increased in winter compared to fall (activities, in mg urea produced/g fresh liver per h: bear 1, 2,300 in fall and 8,800 in winter; bear 3, 3,700 in fall and 9,500 in winter).

On percutaneous biopsy, liver from the bears appeared flabby and stringy compared to human liver tissue on gross inspection. Microscopically, no collagen could be detected after staining with van Gieson's stain but otherwise the samples were nearly identical to human liver. In both fasting bears in winter sleep, biopsies showed minimal but definite changes when compared with samples obtained in the fall (Fig. 3). The winter sleep biopsies showed a mild increase in fat, presumably triglyceride, and clusters of round cells that were thought to represent an inflammatory response. There was an impression of a generalized increase in cellularity related to a decrease in the internuclear spaces, suggesting some loss of cytoplasmic elements.

The bladder experiments indicated that urea and water were transported from bladder urine into blood during winter sleep (Table 3). Both labels were

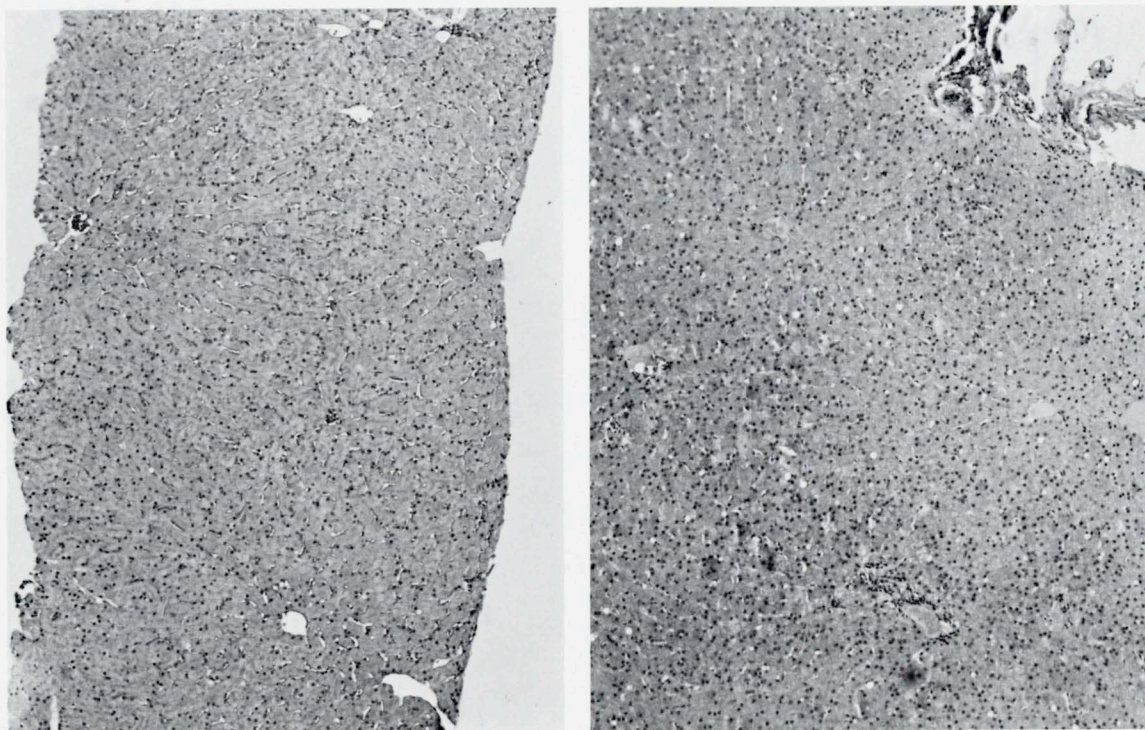


Fig. 3. Liver biopsies from bear 1. (Hematoxylin and eosin; $\times 100$.) *Left*, During normal feeding conditions in fall. *Right*, After 4 months' fast during winter sleep, showing mild increase in steatosis and round cell infiltration.

Table 2.— $^{14}\text{CO}_2$ in Expired Air and ^{14}C in Plasma After Intravenous Injection of $[^{14}\text{C}]\text{Urea}$

Fall			Winter		
Time (h)	Exp. air (dpm/vial*)	Plasma (dpm/ml)	Time (h)	Exp. air (dpm/vial*)	Plasma (dpm/ml)
Bear 1					
0	0	14,100	0	0	13,490
0.1	585	12,670			
1	643	4,900	1	124	7,968
3	660	3,670	3	212	7,279
6	712	2,720	5	268	6,291
18	53	1,220	7	214	5,918
24	61	810	9	211	5,556
			13	207	4,661
			17	44	4,015
			22	64	3,899
			24	79	3,112
Bear 3					
0	0	12,994	0	0	10,140
0.5	110	5,900	1	26	10,118
1.5	642	4,780	3	275	8,860
3	263	3,880	5	185	9,549
6	103	2,810	7	232	8,062
18	19	770	9	163	8,573
24	14	470	13	97	8,585
			17	55	6,706
			22	84	7,011
			24	94	5,776

* Each vial trapped 2.0 mmol of CO_2 .

detected in the first sample of blood, taken 20 minutes after injection of label into the bladder. Bear 7 died 2 hours after the 7-hour sample was taken. The death was attributed to anesthesia; the sample taken 2 hours prior to death was obtained while the bear appeared to be reacting normally to anesthesia.

From the specific activity of the urea isolated from samples of blood and urine, more than 99% of the radioactivity in blood and urine was in urea. $^{14}\text{CO}_2$ most likely represented the remaining activity. These findings indicate that urea molecules passed across the bladder wall unchanged and entered the blood. Data collected in the bladder experiments were used to estimate the quantity of urea transported across the bladder epithelium in winter sleep. The equations for these calculations were derived in the same manner as those used to calculate transport of water and solute between gut lumen and blood.¹⁰ The following assumptions were made:

1. The volume of urine in the bladder was constant during the study. Earlier data² support this assumption; residual volumes of bladder urine checked weeks apart in winter revealed a fairly constant volume per bear, usually in the range of 50 to 150 ml. In the present study, the bladder of bear 1 contained approximately 77 ml at the start of the

Table 3.—Disappearance of [¹⁴C]Urea and D₂O From Bladder Urine and Appearance in Plasma

Bear 1					Bear 7				
Time (h)	Specific activity (dpm/mg urea)		D ₂ O (mol % excess)		Time (h)	Specific activity (dpm/mg urea)		D ₂ O (mol % excess)	
	Blood	Urine	Blood	Urine		Blood	Urine	Blood	
0	0	467,708	0.9		0	0	377,615	0	
0.33	271		1.7		0.5	2,720		4.8	
0.67	376		1.3		1	2,413		5.2	
1	376		2.8		1.17	2,344		8.1	
1.33	333		4.2		1.5	1,481		9.5	
1.67	300		3.3		2	2,767		11.0	
2	253		3.3		2.5	2,663		11.1	
2.33	333		3.7		3	3,100	368,800	13.1	
2.67	368		6.6		4	3,293		13.1	
3	...	338,572	5.4		5	5,113		18.5	
4	479		11.2		6	4,338	340,047	18.4	
5	447		12.4		7	...	271,273		
6	495	259,767	12.1						
24	532	76,234	17.1	635					
7 days	356	259	19.3	15.4					

experiment and 84 ml 1 week later. When all urine possible was drained through the bladder catheter on two occasions 3 hours apart, in bear 7, volumes of 91 and 84 ml were obtained. These differences were assumed to be due to the difficulty of completely extracting urine from the bladder via a catheter.

2. Although urea excreted by the kidneys entered the bladder during the 3-hour study period, it was assumed that no significant quantity of labeled urea did because of the extremely low specific activity in blood. (This assumption was made in previously published equations as well.¹⁰)

3. The change in specific activity of bladder urine was linear over the 3-hour test period. This assumption appeared reasonable when specific activity changes with time were analyzed over 6-hour intervals.

The following equations were derived:

$$*U \times V_{(t=3)} = *U \times V_{(t=0)} - \Theta R \quad \text{eq. 1}$$

in which $*U$ = [¹⁴C]urea in dpm/ml, V = volume of urine in ml, Θ = mean specific activity of urea in dpm/mg urea, R = 3-hour rate of urea reabsorption by bladder epithelium, and t = time in hours. Equa-

tion 1 states that the number of counts present after 3 hours is equal to the quantity instilled at time 0 minus the quantity transported from bladder lumen to blood during the 3-hour test.

After R is calculated, the following equation is used to determine E , the quantity of urea both excreted by the kidneys into the bladder and diffusing from blood into the bladder lumen:

$$U \times V_{(t=3)} = U \times V_{(t=0)} - R + E \quad \text{eq. 2}$$

Equation 2 states that the quantity of urea left in the bladder after 3 hours is equal to the quantity present initially minus what was reabsorbed plus the quantity excreted into it. U is urea concentration expressed as mg/ml urine.

Table 4 shows that the amount of urea reabsorbed by the bladder is about equal to the amount excreted into it by the kidney over a 24-hour period. The calculated rate of urea excretion by the kidney of bear 1, 3.4 g/24 h, is of interest; a previous measurement,² obtained by 24-hour drainage of the bladder during winter sleep, was 4 g/24 h.

DISCUSSION

The ability of the bear to inhibit the net production of urea and to maintain lean body mass constant during winter sleep while metabolizing only fat as a source of energy is a special mechanism associated with the induction of winter sleep. The bear could not duplicate this feat during the summer when starved outside under ambient temperature or housed in winter sleep-like conditions (in the dark and in the cold). Similar to other starving, warm-blooded animals including humans, when not in winter sleep

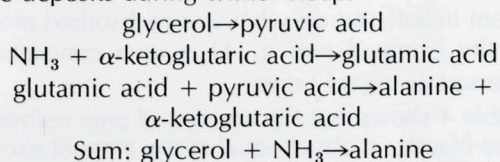
Table 4.—Urea Transport Into and Out of Bladder in Bears in Winter Sleep

	Bear 1	Bear 7
Into:		
Measured (g/3 h)	0.402;0.394	0.125;0.203
Estimated (g/24 h)	3.2	1.3
Out of:		
Measured (g/3 h)	0.500;0.358	0.035;0.113
Estimated (g/24 h)	3.4	0.6

the bear utilized muscle as a source of energy continuously and became dehydrated and azotemic when denied access to water.

The mechanism by which lean body mass is preserved during starvation in winter sleep (confirmed in this study) is not clear. It is not due to inhibition of protein catabolism because urea was shown to be continually formed and degraded during winter. The appearance of ^{14}C in expired air after the injection of ^{14}C urea was most likely a result of ^{14}C urea diffusing across the gut epithelium into the gut lumen, where urease is present in bacteria. The ammonia and carbon dioxide produced must have diffused back into blood.¹¹ The fate of the ammonia is not known, although it must have been recycled into the body nitrogen pool. Ammonia was not fixed in the gut lumen; previous studies have shown that fecal nitrogen does not accumulate in winter sleep.² Further studies are necessary to trace nitrogen metabolism in winter sleep.

One biochemical reaction that may be operative in fixing nitrogen in the bear in winter sleep is glutamic acid synthesis.¹² This reaction exceeds all other forms of ammonia fixation in mammals.¹³ If incorporated into glutamic acid, the ammonia released in the degradation of urea would have many diverse pathways of metabolism available to it, yielding compounds such as amino acids for protein synthesis. In the following model, pyruvate is generated from the glycerol released from adipose tissue deposits during winter sleep:



These reactions would involve several different organs (adipose tissue, liver, muscle, kidney). Although further studies are needed to test the hypothesis, previous studies have shown that, in the bear in winter sleep, plasma concentrations of alanine and glutamic acid increase,² rather than decrease as has been shown for alanine in prolonged starvation of humans.¹⁴

The steatosis and inflammatory reaction noted in the liver of bears in winter sleep resembled similar but more marked changes noted in human liver tissue after periods of fasting or after jejunoileal shunts. In the latter instance, mobilization of peripheral fat stores results, in some poorly understood way, in histologic abnormalities that sometimes

are severe and occasionally result in liver-related deaths.^{15,16}

The urinary bladder plays a central role in maintaining the state of winter sleep. Previous studies have shown that about 100 ml of urine and 4 g of urea enter the bladder each 24 hours during winter sleep.² These rates are about equal to the rates at which the bladder wall can reabsorb the urine and water and thus prevent the distention that would result in urination. This movement of urea and water across the bladder wall of the bear also occurs in a non-hibernating mammal; Levinsky and Berliner¹⁷ showed that, under conditions of low urine flow (1 ml/min), urea and water are reabsorbed by the bladder of the dog. These investigators found that sodium, potassium, chloride, creatinine, and hydrogen ion also move across the canine bladder wall. The extremely low urine flow in the bear (0.07 ml/min) in winter, therefore, is most conducive to reabsorption of the water and solute excreted by the kidney.

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JAMES I. McNITT*, G.E. FOLK, M.C. BREWER, and J.B. EHRENHAFT.

Arctic Research Laboratory and University of Iowa.

The day-night activity of polar bears exposed to continuous light.

Polar bears have been sighted throughout the Arctic as far north as 87° . They thus are exposed to as long as 3 to 6 months of continuous light, and the same period of near-darkness. This necessitates an activity rhythm independent of the sun. The bears however, must relate their rhythms to those of seals- the bears' main food-which are in large part dependent on tides. Two yearling, male polar bears which had been reared together in the same cage at the Arctic Research Laboratory, Barrow, Alaska, were observed for six 24-hour periods in continuous daylight. The two siblings (423 and 383 lbs.) were found to have nearly identical daily rhythms with a single daily period of activity. They were highly active from 10 a.m. to 6 p.m. and were usually inactive from 9 p.m. to 9 a.m. The remainder of the time was spent in transition from one setting (baseline) to the other. The greater length of time required to go from the high to the low setting (3 hours) compared to that required to go from the low to the high (1 hour) is due to the greater susceptibility of the bears to outside noise while in a light sleep compared to deep sleep. The daily rhythm did not appear to depend on a daily change in environmental temperature, light intensity or barometric pressure, nor did this rhythm follow that of camp activity. If this rhythm had been observed in the temperate zone, it would be concluded that these animals were day active, although they were in especially deep sleep from 6 a.m. to 9 a.m.

The heart rates of the bears were determined using Iowa radio-capsules. The changes in heart rate paralleled those for activity with a high of 132 b/m in the afternoon and a low of 56 b/m early in the morning. These are high rates for animals of this size, but are similar to those found previously at the same laboratory in grizzly bears of comparable weight and age. The patterns were the same for both species although the timing of the rhythms varied. (Supported by AINA under ONR) *CIC Fellow of USPHS.

REFERENCE-- AMER. ZOOL.
VOL. 7 : 807. 1967

What time of day were bears fed?



DEPARTMENT OF MICROBIOLOGY

COLLEGE OF LETTERS & SCIENCE MONTANA STATE UNIVERSITY, BOZEMAN 59715

October 17, 1975

Dr. Frank C. Craighead, Jr.
Environmental Research Institute
Moose, Wyoming 83012

Dear Frank:

The work you request on elk rumen bacteria will not be published for about a year. unfortunately I became involved in University administration before I could get it all written and am just getting back to it now.

There are some interesting changes that occur in the types of dominant bacteria as the nitrogen intake falls below a certain level, thus reducing the ability of the elk to digest the cellulose from the roughage which makes up a large part of his diet during periods of starvation. The rumen, therefore, becomes compacted with undigestible material which cannot be moved and the animal dies with a full rumen, unable to eat any appreciable amount of hay or other supplementary feed. An experiment that needs doing is to see whether protein cubes such as are fed to cattle under stress would help to relieve this situation.

I shall be glad to send a reprint of this work when it is published.

Sincerely,

A handwritten signature in cursive script, appearing to read 'Mac'.

Richard H. McBee
Professor of Microbiology

RMc/jb



DEPARTMENT OF MICROBIOLOGY

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Richard H. McBee
Professor of Microbiology

RMc/jb

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SOME COMPARATIVE ASPECTS OF MILK
FROM FOUR SPECIES OF BEARS

ROBERT JENNESS, ALBERT W. ERICKSON, AND JOHN J. CRAIGHEAD

Reprinted from JOURNAL OF MAMMALOGY
Vol. 53, No. 1, 24 March 1972
pp. 34-47
Made in United States of America

SOME COMPARATIVE ASPECTS OF MILK FROM FOUR SPECIES OF BEARS

ROBERT JENNESS, ALBERT W. ERICKSON, AND JOHN J. CRAIGHEAD

ABSTRACT.—Milk specimens obtained from 26 individual bears representing *Ursus americanus*, *U. maritimus*, three subspecies of *U. arctos*, and *Helarctos malayanus* were analyzed for gross composition and were characterized for carbohydrates, fats, and proteins. Bear milks contain high concentrations of fat, proteins, and minerals, but are low in carbohydrates. The lactose content of milk from bears maintained in zoos was much higher than that from wild bears. *Myo*-inositol was identified as a prominent constituent. The principal fatty acids are palmitic ($C_{16:0}$) and oleic ($C_{18:1}$). Polar bear milk fat, however, has significant concentrations of palmitoleic ($C_{16:1}$) and long chain fatty acids, probably derived from food fat. The caseins of bear milk consist of two principal groups of proteins readily separable by gel electrophoresis. The whey proteins contain five groups of proteins not found in blood. These exhibit evidence of great species specificity and, in some cases, of genetic polymorphism within species.

The milk of many species of mammals has been investigated but relatively little is known about milk of Ursidae. In view of the immaturity of the young at birth and the long nursing period, it appeared of interest to secure information on the composition of the milk of bears. We are aware of previous publications on ursid milk by three groups of workers. Hock and Larson (1966) reported analyses for one specimen from a captive black bear and Baker *et al.* (1963a, 1963b, 1967) and Cook *et al.* (1970) for three specimens from wild polar bears. The compilation by Ben Shaul (1962) includes analyses for the milk of brown bears (*Ursus arctos* subspecies) and grizzly bears (*Ursus arctos horribilis*) made by her. It also contained an analysis of polar bear milk (*U. maritimus*), stated to have been taken from the compilation of Spector (1956). We were unable to locate this analysis in Spector's handbook and judge that it may be another of Ben Shaul's analyses.

MATERIALS AND METHODS

Single specimens of milk were obtained from each of 26 individual lactating bears (Table 1). Some of the grizzly bears were weighed and aged (Craighead *et al.*, 1970). Animals 72, 112, 119, 34, 39, 42, 170A, and 172 weighed 385, 350, 325, 325, 415, 290, 235, and 262 pounds, respectively. Animals 112, 34, 39, 42, and 172 were estimated to be respectively 16.5, 14.5, 8.5, 7.5, and 8.5 years old. All of the bears were immobilized by intramuscular injection of either M-99, succinylcholine chloride followed by pentobarbital sodium or a combination of phencyclidine hydrochloride (sernylan) and promazine hydrochloride (sparine)—see Seal and Erickson, 1969. An injection of 2 milliliters of oxytocin (20 units per milliliter) was then given intramuscularly and the animal milked by manual expression. All milk specimens were frozen and kept frozen until analyzed at the University of Minnesota. The following analytical methods were employed:

Total solids.—Desiccation at 70°C in a vacuum oven to constant weight.

Ash.—Incineration in a platinum dish in a muffle furnace at 650°C for 16 hours.

Fat.—Roese-Gottlieb extraction (Horwitz, 1965:224).

TABLE 1.—Sources, volume, and pH of specimens of bear milk.

Specimen no.	Species	Animal designation	Date collected	Locality	Cubs		Milk	
					No.	Age (mo.)	Vol. (ml)	pH
1	<i>U. americanus</i>	1	1 Aug 61	Y. N. P.*	—	—	12	6.6
2	"	2	4 Aug 61	"	2	6	19	6.6
3	"	3	13 Aug 61	"	—	—	32	6.4
4	"	50146	18 Aug 67	Chippewa Co., Michigan	Unknown	—	25	6.6
5	"	50257	18 Aug 67	Alger Co., Michigan	2	7-8	25	6.6
6	<i>U. arctos horribilis</i>	72	3 Sep 60	Y. N. P.	1	7	44	6.2
7	"	112	15 Aug 61	"	3	6.5	32	5.9
8	"	119	29 Aug 61	"	2	7	19	6.4
9	"	34	23 Jul 64	"	2	18	—	—
10	"	39	19 Aug 64	"	3	18.5	—	—
11	"	42	1 Jul 64	"	2	5	—	—
12	"	170A	2 Jul 64	"	2	5	—	—
13	"	172	8 Jul 64	"	3	17.5	—	—
14	"	9	9 May 66	Pittsburgh Zoo	3	3	—	6.7
15	"	Tessie	25 Mar 68	St. Paul Zoo	2	2	16	6.1
16	<i>U. arctos yesoensis</i>	14	9 May 66	Pittsburgh Zoo	1	3	—	6.3
17	"	15	9 May 66	"	1	3	—	6.2
18	<i>U. arctos middendorffi</i>	7	9 May 66	Pittsburgh Zoo	1	3	—	6.3
19	<i>U. maritimus</i>	1	6 Aug 66	Kong Karl's Land	1	7-8	25	6.2
20	"	318	26 Jul 67	77°30' N, 28°15' E	1	7-8	25	6.1
21	"	308	19 Jul 67	77°28' N, 27°00' E	2	7-8	60	6.4
22	"	305	14 Jul 67	77° N, 28°30' E	2	7-8	35	6.3
23	"	—	7 Oct 67	Hudson's Bay	Unknown	—	50	6.2
24	"	552	23 Oct 68	"	2	18-19	14	6.5
25	"	557	29 Oct 68	"	2	10	12	6.5
26	<i>Helarctos malayanus</i>	—	21 Jan 71	St. Paul Zoo	1	3	30	6.4

* Yellowstone National Park

Apparent lactose.—Phenol-sulfuric acid method (Marier and Boulet, 1959).

Nitrogen.—Wet digestion with H_2SO_4 and H_2O_2 followed by direct Nesslerization of the digest. Total nitrogen was determined on an aliquot of whole milk, noncasein nitrogen on the supernatant after precipitating casein at pH 4.6 and nonprotein nitrogen on the supernatant after precipitating protein with 12 per cent trichloroacetic acid. From these values, casein and whey protein contents were calculated using the factor 6.38 to convert N to protein.

Calcium.—On a few specimens by the EDTA titration of Jenness (1953) but on most specimens by the colorimetric method of Nickerson *et al.* (1964) applied to solutions of milk ash after passage through a column of Dowex-2-acetate ion exchanger.

Phosphorus.—On solutions of milk ash by the method of Sumner (1944).

Citrate.—Colorimetric method of White and Davies (1963).

Myo-inositol.—Microbiological method of Campling and Nixon (1954).

A portion of each specimen was fractionated into fat, casein, whey protein, and dialyzable fractions as follows. Milk was centrifuged to form a cream layer, which was removed and extracted either with the Roese-Gottlieb method or with mixture of equal parts methanol and chloroform. Fat was recovered by evaporating the solvent with a stream of N_2 . Casein was precipitated from the centrifuged milk by acidification with HCl to pH 4.6. It was washed with water and freeze-dried. The supernatant at pH 4.6 (whey) was dialyzed

against water to remove the dialyzable fraction, which was concentrated and saved for analyses of the carbohydrates. Dialyzed whey proteins were freeze-dried.

Electrophoretic analyses of the milk proteins were made on polyacrylamide gels in the apparatus of the E-C Apparatus Corp. Caseins were dispersed in tris (hydroxy methyl) amino methane buffer of pH 9.2 containing 7 M urea and 2 drops 2-mercaptoethanol per milliliter. They were run on gels containing 7 per cent acrylamide and 4.5 M urea in a tris buffer at pH 9.2, ionic strength = 0.08. For whey proteins, gels containing 10 per cent acrylamide in veronal buffer pH 8.6, ionic strength 0.008 were used. Sugar solutions for qualitative chromatography were prepared by deionization of dialysate with a monobed resin (Amberlite MB-3) and concentration to a small volume. Chromatograms were developed with a solvent consisting of ethyl acetate, pyridine, and water in the ratio of 10:4:3 on Whatman no. 1 filter paper for 16 to 20 hours. The components were detected by a dipping technique consisting of successive treatments with saturated AgNO_3 in acetone, 0.75 N NaOH in methanol and Kodak fixer F-24.

Gas-liquid chromatography of carbohydrates in the form of trimethylsilyl derivatives was performed on 3 per cent silicone SE-52 on Gas chrom Q in columns 3 millimeters I.D. $\times$ 6 feet long (Sweeley *et al.*, 1963).

Fatty acid composition was determined by transesterification of samples of fat as described by Glass *et al.* (1965) followed by gas-liquid chromatography (GLC) on 5 feet $\times$ $\frac{1}{4}$ inch O.D. columns of 10 per cent diethylene glycol succinate on Diaport W or Anakrom A.

RESULTS AND DISCUSSION

Gross Composition

Analyses for the major constituents, presented in Table 2, show that bear milk tends to be highly concentrated in solids. In comparison with these data, the analyses for solids reported by Ben Shaul (1962) (11, 11.1, and 24 per cent) seem remarkably low. It seems possible that some error in sampling, analyzing or reporting might be involved in her data, particularly those for *Ursus arctos* subspecies and *U. a. horribilis*. The data of Hock and Larson (1966) are similar to ours for black bear with the exception of somewhat lower fat content and the data of Baker *et al.* (1963a) and Cook *et al.* (1970) for polar bears resemble those in Table 2. In general, half or more of the solids in bear milk are fat; polar bear specimens, averaging 33 per cent fat, have higher fat contents than milk from the other species. Bear milk also can be classified as high protein milk because it contains 6 to 17 per cent protein. The concentration of protein in the aqueous phase is particularly high, ranging up to about 30 per cent. The ratio of whey proteins to casein is relatively high, 1:1 to 1:2. Considerable variation in protein content is evident among the 26 specimens. Perhaps these reflect inherent differences among individuals although specimens taken from individuals at intervals during lactation would be needed to prove the point.

The caloric contents of bear milk are high, ranging up to nearly 500 kilocalories per 100 grams in the case of specimen 23—calculated as $(9 \times \text{fat}) + (4 \times \text{lactose}) + (4 \times \text{protein})$. Caloric contents as high as this are found in the milk of cetaceans, pinnipeds, and certain rodents, but not in those of fissipeds other than bears (see Jenness and Sloan, 1970).

Bear milk is low in apparent lactose content but there is a remarkable contrast between wild bears and zoo specimens, the milk of wild bears ranging

TABLE 2.—Composition of bear milk.

Species	No. of specimens	Total solids	Fat	Lactose g./100g.*	Casein	Whey protein	Ash	Ca	P
<i>U. americanus</i>	5	44.5 (40.9–50.5)	24.5 (23.3–26.5)	0.4 (0.2–0.5)	8.8 (5.2–11.9)	5.7 (5.2–5.9)	1.8 (1.4–2.1)	0.41 (.20–.51)	0.28 (.14–.36)
<i>U. arctos horribilis</i> wild	8	40.6 (35.8–45.2)	22.3 (18.3–26.5)	0.6 (0.4–0.7)	6.7 (4.3–8.2)	4.4 (2.2–5.7)	1.5 (1.0–1.8)	0.35 (.31–.44)	0.23 (.16–.32)
zoo	2	29.1 (28.5–29.7)	15.9 (12.9–18.8)	2.4 (1.9–2.8)	5.3 (4.3–6.2)	2.1 (1.3–2.9)	1.4 (1.2–1.6)	0.26 (.15–.37)	0.18 (.17–.18)
<i>U. arctos yesoensis</i>	2	40.3 (39.7–40.8)	23.2 (19.6–26.8)	2.2 (2.0–2.4)	3.5 (2.8–4.2)	4.4 (4.4–4.4)	1.6 (1.3–1.8)	0.31 (.28–.34)	0.24 (.22–.25)
<i>U. arctos middendorffi</i>	1	29.4	14.4	2.4	7.7	4.1	1.6	0.31	0.27
<i>U. maritimus</i>	7	47.6 (35.8–59.6)	33.1 (23.8–48.4)	0.3 (0.2–0.5)	7.1 (5.2–9.6)	3.8 (2.2–4.6)	1.4 (0.8–1.9)	0.29 (.21–.34)	0.23 (.16–.28)
<i>H. malayanus</i>	1	27.2	10.8	3.2	4.6	3.8	1.5	0.37	0.24

* Means and ranges are reported in cases where more than one specimen was analyzed.

from 0.2 to 0.7 per cent as opposed to 1.9 to 3.2 in zoo bears. These analyses, performed by the phenol-sulfuric method (Marier and Boulet, 1959), include all of the sugars commonly found in biological material, even those that are bound to protein. This method does not include *myo*-inositol. However, chromatographic evidence indicates that lactose itself is responsible for the large difference. Low lactose content seems to be characteristic of milk from the four species studied and independent of the age of the young. The data are consistent with the values of 0.5 to 1.1 per cent lactose for wild polar bears (Baker *et al.*, 1963a, and Cook *et al.*, 1970, picric acid reduction method) and 1.5 per cent for a captive black bear (Hock and Larson, 1966, probably a reduction method). The carbohydrate contents of 3, 4, and 4 per cent reported by Ben Shaul (1962) are not comparable to our data as they appear to have been calculated by difference—that is, total solids – (fat + protein + ash).

The reason for the differences in the lactose content of milk of wild and zoo bears is not at all evident. We are not aware of reports of any such difference in any other species. There are, of course, large interspecific differences in the lactose content of milk. Of the Carnivora studied, only the Pinnipedia have low lactose (Jenness and Sloan, 1970)—in some cases, even an undetectable concentration (Pilson and Kelly, 1962).

Parmiter (1969) speculated on several possible mechanisms by which the lactose content might be regulated and Brew (1969) postulated that the rate of synthesis of α -lactalbumin, a crucial component of lactose synthetase, is the determinant of lactose concentration. However, present knowledge is inadequate to invoke the ideas of Parmiter or Brew as explanation for the higher lactose contents of the milks of zoo bears.

The diet of wild bears undoubtedly differs markedly from zoo rations, although the wild grizzly and black bears reported here (specimens 1 to 13) were getting a considerable portion of their nutriment from garbage dumps. In domestic cows and goats, the level of nutrition and composition of the diet have little influence on the lactose concentration of milk (Rook and Wheelock, 1967). Starvation or production of hypoglycemia by injection of insulin lowers the yield of milk and the yield of lactose, but does not drastically influence the lactose concentration. Indeed, current thinking is that lactose production operates through osmotic mechanisms to regulate the amount of water secreted in milk (Rook and Wheelock, 1967). The situation in bear milk appears to be quite different than in the domestic ruminants in that some environmental factor, as yet unidentified, can greatly influence lactose concentration.

The mineral content of bear milk as reflected in the percentage of ash is relatively high. This might be expected on the basis of the generally reciprocal relation in milk between lactose and dissolved salts, and the high content of casein, which contains phosphorus and binds calcium and phosphate. Calcium and phosphorus are present in a weight ratio of about 1.3:1 (Table 2), which corresponds to an atomic ratio of approximately 1:1. They are principal contributors to the ash; the sum of calcium and the $\text{PO}_4^{=}$ equivalent of the phosphorus represents 44 to 88 per cent of the ash.

TABLE 3.—Vitamin content of bear milk (mg per liter).

Vitamin	Black bear ¹	Grizzly bear ²	Cow ³
Biotin	0.0094	0.0054	0.031
Nicotinic acid	5.4	5.0	0.94
Ca pantothenate	ca. 0.27	ca. 0.22	3.5
Riboflavin	1.8	1.3	1.75
Thiamin	0.58	0.63	0.44
Vitamin B ₆	0.11	0.09	0.64
Vitamin B ₁₂	0.009	0.010	0.004
Vitamin A	1.0	0.73	0.4
Vitamin E	3.4	3.9	1.0

¹ Composite of equal parts of specimens 1, 2 and 3.² Composite of equal parts of specimens 7 and 8.³ Hartman and Dryden (1965).

Samples of milk from four species of bears are similar in gross composition; no prominent differences among species are evident. Furthermore, milk composition seems not to be greatly influenced by stage of lactation at least within the periods of 2 to 18 months postpartum represented by the specimens analyzed.

Vitamins

Composite specimens of milk from black and grizzly bears were freeze-dried and submitted to Dr. Margaret E. Gregory, National Institute for Research in Dairying, Reading, England, for analysis for certain vitamins. Dr. Gregory's analyses (Table 3) indicate no important differences in vitamin contents between the two species. Both specimens were taken from wild bears from Yellowstone National Park in August 1961. Thus, dietary influences on vitamin contents would not be likely.

Insofar as the B vitamins are concerned, bear milk is strikingly higher in nicotinic acid and lower in calcium pantothenate than cow milk. On a fat-free basis, the nicotinic acid content of the bear specimens is about seven times that of cow milk. The contents of fat-soluble vitamins A and E, although higher than that of cow milk on the basis of milligrams per liter, are actually considerably lower per unit of fat.

Fatty Acid Composition

Fatty acid compositions calculated from gas-liquid chromatograms of the methyl esters of fat from black, grizzly, and polar bear milk have been published previously (Glass *et al.*, 1967). Like other members of the Carnivora, these bears produce milk fat characterized principally by C_{16:0} and C_{18:1} acids. The milk fat of polar bears also contains large amounts of palmitoleic acid (see also Cook *et al.*, 1970) and some unidentified acids with chains longer than C₁₈. In this respect, polar bear milk fat resembles that of cetaceans and pinnipeds. It is likely the C_{16:1} and longer chain acids are derived directly

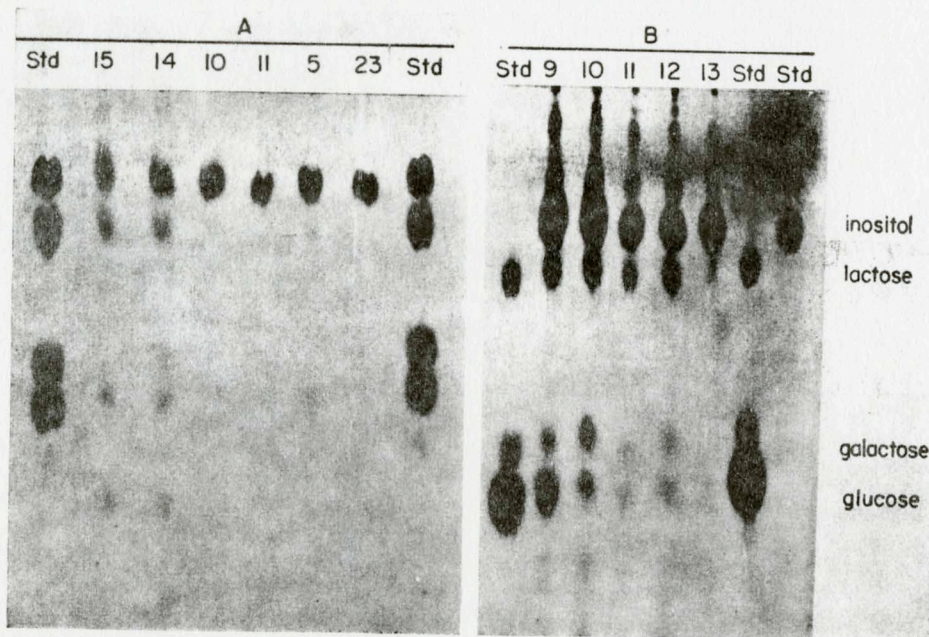


FIG. 1.—Paper chromatograms of dialyzable carbohydrates of bear milk. Application heavier and development time longer in B than in A. Numbers designate specimens as in Table 1.

from dietary fat. We detected no butyrate in a specimen of polar bear milk fat, although Baker *et al.* (1963a) reported 13.9 per cent for one specimen. This discrepancy does not appear to be due to deficiencies in our method inasmuch as it readily detected butyrate in ruminant milk fats and recovered quantitatively butyrate, added as tributyrin, to polar bear milk.

Carbohydrates

Paper chromatograms of concentrated deionized dialysate preparations from certain of the specimens of bear milk (Fig. 1) reveal the presence of a component moving with the mobility of lactose ($R_G = 0.50$), small amounts of glucose and galactose, a prominent component at $R_G = 0.30$ to 0.34 , and components at $R_G = 0.24$ to 0.29 and $R_G = 0.11$. In a paper (Jenness *et al.*, 1964) devoted to dialyzable carbohydrates, the component with $R_G = 0.30$ to 0.34 was noted in preparations from a number of species and was tentatively designated as a trisaccharide. Because it appears prominently in bear milk, we attempted to identify it more definitely. A sample of deionized dialysate from specimen no. 9 was streaked on Whatman 3 MM paper and chromatographed. The zones corresponding to components $R_G = 0.31$ and $R_G = 0.24$ were cut out and eluted. A portion of each was applied to Whatman no. 1 paper and another portion was refluxed with 1 N H_2SO_4 for 6 hours, neutralized with $BaCO_3$, filtered, concentrated and spotted on Whatman no. 1 paper. As shown in Fig. 2, the com-



FIG. 2.—Paper chromatogram of dialyzable carbohydrates from grizzly bear specimen no. 9 and the fraction of $R_G = 0.31$ therefrom. A, fraction $R_G = 0.31$ after hydrolysis; B, fraction $R_G = 0.31$; C, specimen 9; D, standard lactose, glucose, and galactose.

ponent of $R_G = 0.31$ was not affected by the hydrolysis, and thus is not a trisaccharide. It moved with the mobility of *myo*-inositol (Fig. 2) so would be unaffected by the hydrolysis used. Additional evidence that it is *myo*-inositol is that the eluted component did not react in the phenol-sulfuric acid test and that it supported the growth of the yeast *Kloeckera brevis* when inoculated according to the procedure of Campling and Nixon (1954).

A number of specimens of skimmed milk from bears were assayed for *myo*-inositol content by bioassay with *K. brevis* (Table 4). The method responds only to free *myo*-inositol; it appears that there is a high but somewhat variable content of this component in the milk of bears. Gas-liquid chromatograms of the trimethylsilyl derivatives of the dialysates from specimens no. 8 and no. 14 revealed that the former (wild) contained much more *myo*-inositol in relation to lactose than the latter (zoo).

TABLE 4.—Free *myo*-inositol and citrate contents of bear milk (mg per 100 ml skimmed).

Specimen no.	<i>Myo</i> -inositol	Citrate
5	—	145
9	28	196
10	12	—
11	55	—
12	32	—
13	40	—
14	15	122
16	34	—
17	20	—
18	13	—
19	43	136
26	—	234
Cow (<i>Bos taurus</i>)	4	150–200
Human (<i>Homo sapiens</i>)	30	20–80
Rat (<i>Rattus norvegicus</i>)	60	3–10

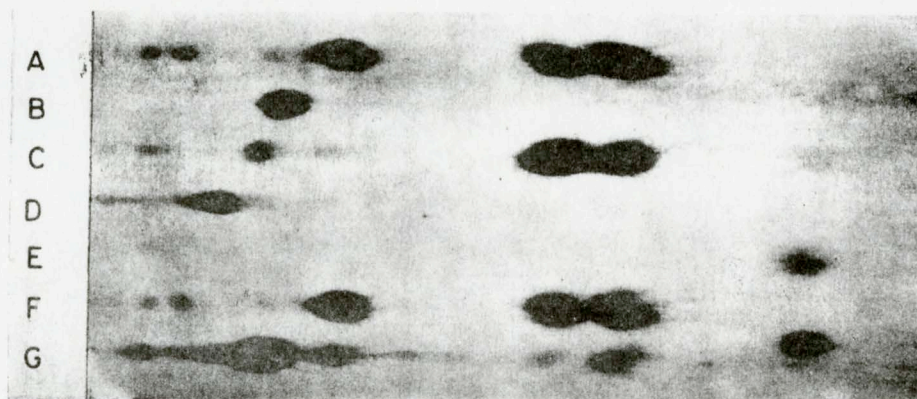


FIG. 3.—Paper chromatogram of dialyzable carbohydrates from grizzly bear specimen no. 9 and the fraction $R_G = 0.24$ therefrom. A and F, standard lactose, glucose and galactose; B, *myo*-inositol; C, fraction $R_G = 0.24$ after hydrolysis; D, fraction $R_G = 0.24$; E, fucose; G, specimen no. 9.

The eluted component with $R_G = 0.24$ yielded, upon hydrolysis (Fig. 3) largely glucose and galactose. Upon analysis of the hydrolysate for each of these sugars with specific glucose and galactose oxidases (Worthington Laboratory Manual), a ratio of galactose : glucose = 2:1 was found. The material is thus tentatively identified as a trisaccharide consisting of one glucose and two galactose residues. Such a compound has not been described from any natural source but 6- β -galactosyl-lactose and 4- β -galactosyl-6- β -glucosylgalactose have been synthesized (Bailey, 1965).

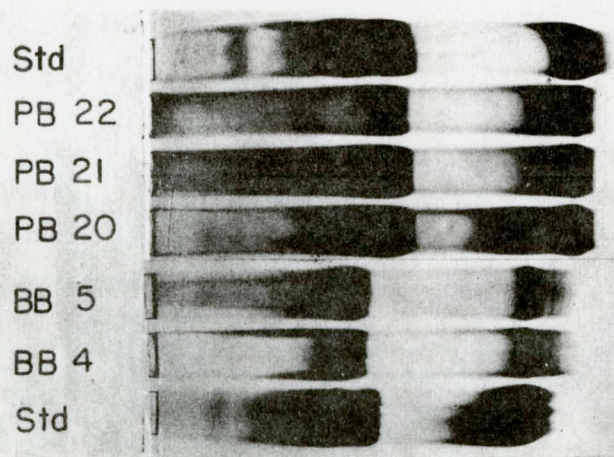


FIG. 4.—Polyacrylamide gel electrophoretic patterns of bear caseins: PB, polar bear; BB, black bear. Numbers designate specimens as in Table 1. "Std" is casein from cows (*Bos taurus*) milk.

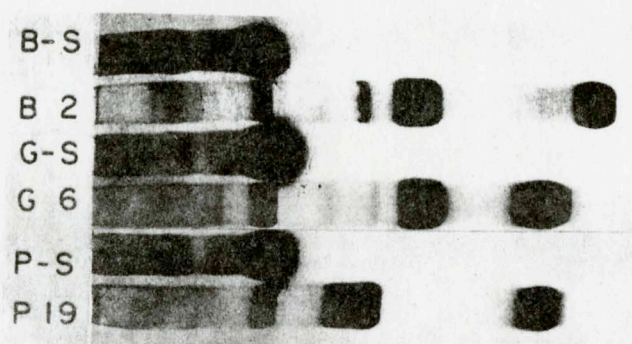


FIG. 5.—Polyacrylamide gel electrophoretic patterns of bear blood serum and whey proteins. P-S, G-S, B-S are polar, grizzly, and black bear serums, respectively. Numbers designate specimens as in Table 1.

Citrate

A few specimens were analyzed for citrate (Table 4). The levels of this constituent seem to be comparable to those in the milk of many other species, particularly other carnivores.

Proteins

Electrophoresis of the caseins on polyacrylamide gel separated them into two principal classes (Fig. 4). This is consistent with the results of Sloan *et al.* (1961) for the casein of a grizzly bear subjected to electrophoresis on paper. The resolution of bear caseins is not as good as for the bovine casein standard. Thus, it has not been possible to determine if there are significant differences between species or polymorphism within species. Baker *et al.* (1963b) resolved polar bear casein into three principal components on acrylamide gel containing urea but not mercaptoethanol. On starch gel with both urea and mercaptoethanol they found eight components but the resolution was not clear cut.

Comparison of electrophoretic patterns of blood sera and whey proteins (Fig. 5) indicates that whey contains a component corresponding to blood serum albumin, but little of the slower moving blood serum proteins. It does contain, however, a number of faster moving proteins that appear specific for milk. Baker *et al.* (1963b) also observed several components in electrophoretic patterns of polar bear whey on acrylamide gel without urea. For purposes of description and discussion, the major components are designated numerically 1, 2, 3, 4, and 5 as indicated in Figs. 6 and 7. Comparison of the patterns for various species demonstrates some outstanding differences that may be summarized as follows: protein 1 was prominent only in the yezo brown bear (*U. a. yezoensis*) and in one black bear specimen (no. 4); protein(s) 2 is a pronounced doublet in yezo brown and polar bear specimens and a more or less prominent single band in black and grizzly bears (it seems to be lacking from the kodiak and sun bear specimens and from polar bear colostrum); protein 3

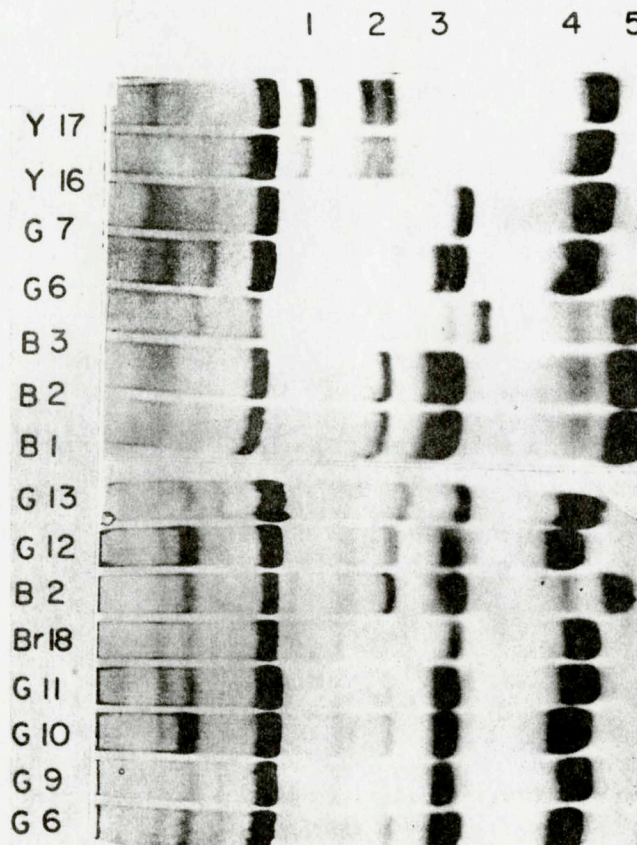


FIG. 6.—Polyacrylamide gel electrophoretic patterns of bear whey proteins. Letters refer to taxa: Y, yezo; G, grizzly; B, black; Br, kodiak. Numbers designate specimens as in Table 1.

is absent from yezo brown and polar bears, but is pronounced in black, grizzly, and sun bears; protein 4 is present in all species; protein 5 is present only in black bears. The patterns suggest polymorphism in protein 3 in the case of grizzly and black bears.

The electrophoretic patterns indicate a great deal of divergence among the specific whey proteins in bear milk. This is in marked contrast to the hemoglobins and blood serum proteins of the Ursidae. Serum albumins from each of the six genera of bears (*Thalarctos* being considered a genus) could not be differentiated by the microcomplement fixation reaction against rabbit anti *U. americanus* serum (Seal *et al.*, 1969). A single hemoglobin of identical electrophoretic mobility, composition, and properties is present in six families of Carnivora, including all six ursid genera (Seal, 1969; Seal *et al.*, 1967).

The fact that the whey proteins of the three genera studied exhibit considerable differences in electrophoretic patterns suggests that they might be

Dr. Margaret Gregory, National Institute for Research in Dairying, Reading, England, is responsible for the vitamin analyses and Dr. Robert Glass for the GLC analyses of fatty acids. Their willingness to make these analyses is greatly appreciated. This contribution is paper no. 7354, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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Hibernation

Returning to Den of No. 164

Returning to Maman's Den

Danger of working with grizzlies

Decision to work with black bears - compare black & grizzlies

Black Bear article

Descrip. of hibernation - preparing for hibernation 6 months

Physiology of hibernation

Unique adaptiveness of bear

Bear physiology as compared with man

Blood chemistry studies

milk

arteriosclerosis etc.

Grizzly incident - charging W. Yellowstone - while working on Black bear

Last Chart
Notes

Homing instinct - referred to chart II p. 18 Ignatz

The grizzlies with their efficient absorption system in summer and fall store more energy than they ^{use} ~~use~~. If the weather gets colder and snow covers food sources, they gradually reach a point where they use more energy in seeking food than they take in through eating. This initiates lethargy and hibernation. In spring the warmth and light from the increased solar energy makes available energy to bears in the form of grass, ~~xxxx~~, tubers, seeds, fruits, and so on. The bear has masterfully learned (instinctive) to regulate the use of available energy. ~~xx~~ As such, he has been successful. Man has learned to exploit the use of energy and in doing so has attained mastery over his co-dominant, the bear. He has done this not through instinct, but through his intellect. The task delegated to any intelligent form of life is to guide the use of the energy of the universe in a way that intellegent life and life complexes and patterns on which it is dependent also survive. Failing in this the bear will perish and other life complexes as well. Man will follow and if intellegent life is to attain its almost limitless capabilities, it will do so only in some other solar system where greater tolerance has been built into a social animal resulting in a life form where status and greed (possessiveness) is not self-destructive.

Man is a part of an aggressive submissive-hierarchical system. Like grizzlies, the more dominant individuals once fought for leadership. As we developed from hunting bands to tribes to city-states and nations, leaders did not vie with one another in physical combat. Instead they used others to fight for them. It was easier and less dangerous. Thus wars and the art of warfare developed. If we to some extent equalized the acquisition of worldly possessions we still will not eliminate the threat of war and ~~xxxxxxxxxxxxxxxxxxxx~~ ~~xxxxxxxxxxxxxxxxxxxx~~ eventually self-destruction unless we can greatly temper and modify the desire for status and dominance or in-other-words, power; power to control others. Can man do this? Only if he understands the problem. It is one that he is admirably equipped to ignore.

In the chapter on Dens ~~MM~~ -- further emphasize the unique adaptations of the bear for hibernation. Discuss the ~~year~~ (?) capabilities, the conversion of fat during winter sleep to various energy forms. Further point out the unusual survival value of winter sleep to this large carnivore. Somewhere in this book, or perhaps in one of the letters I'm preparing - ~~ix~~ point out that the Park Service civil servants, particularly the superintendents, through the power they have ~~attained~~ attained & often arrogantly use have become civilian dictators, not civilian servants.

Add to the grizzly bear range tables, the data for 1968.

Winter Sleep

Returning to dens of 164, 40, 101,
Grizzlies leaving den in winter (old 7.)

Black bear den (article) Old 7.

Rousing - p. 11 Learning W of B. (Sc. Yr.)

Waller, M.D.

9. Gradient Layer Calorimetry and Human Calorimetry

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Calorimeters for measuring directly the heat produced and lost in animals or man have been applied as major tools of medical or biological research for almost two centuries. The names of Antoine Lavoisier¹ and Max Rubner² stand out in this field of scientific technology. Its classical applications in this country are associated with the names of Graham Lusk,³ Francis G. Benedict,⁴ and Eugene F. DuBois.^{5, 6} Together with J. D. Hardy, E. F. DuBois has carried out the first extensive calorimetric study of human temperature regulation using the classical methods.

Today many laboratories (Prouty, Barret, and Hardy,⁷ Lawton, Prouty, and Hardy,⁸ Pullar,⁹ Stewart,¹⁰ Tasker, Humphreys, Parmelee, and Schutrum,¹¹ Huebscher, Schutrum, and Parmelee¹²) apply instead the modern method of gradient layer calorimetry designed by the authors of this article in 1946, submitted for approval of construction of a human calorimeter in 1947, and published in 1949 with the quantitative results of extensive testing.¹³

When the method of gradient layer calorimetry (then designated "gradient calorimetry") became rather widely accepted, considerable interest was shown in comparing this method with previous approaches to calorimetry in which a difference in temperature (gradient) was essential to the measurement. Prouty, Barret, and Hardy in their introduction to reference 7 call attention to the following earlier work:

"In 1942 Day and Hardy¹⁴ described a simplified type of heatflow or gradient calorimeter for premature infants based on the principle that the rate of heat transfer from the air inside the calorimeter to the air in a constant temperature room is proportional to the measured air temperature difference."

Accordingly, Hardy¹⁵ contends that "the idea of measuring heat loss by means of a gradient calorimeter is not new." However, Bothe in 1946¹⁶ compares gradient layer calorimetry with an even earlier instrument, namely Rubner's air calorimeter of 1899, as follows: "The Schicht*-Calorimeter (after Benzinger, Bothe, and Maier-Leibniz) and the air calorimeter² have one feature in common, i.e. a stationary thermal gradient is set up between the calorimeter chamber and the surrounding thermostat. They differ in that the air calorimeter measures a certain mean value of the temperature in the heat conducting medium (air) whereas the Schicht-Calorimeter records the value of the difference of temperature between the inner and the outer surface of the (solid) conducting layer. The resulting advantages of the Schicht-Calorimeter are: the compensating chamber may be dispensed with and the stationary thermal state is reached fairly rapidly." Hardy,⁷ in reviewing our method, points out that our use of thin layers of solid insulating material produced the speed, and the use of several hundred thermocouples in series across a gradient layer maintained the sensitivity of the gradient layer measurement. It may be permitted to add what is perhaps the most important characteristic: using solid gradient layers instead of air spaces, and measuring at many narrowly and uniformly spaced sites, are prerequisites of the high precision and reproducibility of calorimetric measurements with the gradient layer principle. In solid matter, thermal conductivity of the layer is not subject to changes induced by thermal convection. A large number of gradient measuring sites ascertains a

* Schicht is the German word for the English "layer."

reproducible response to total heat transfer under varied conditions. It excludes deviations that otherwise occur when the heat producing object—or certain parts of it with different surface temperature—are exchanging heat with more sensitive or with less sensitive areas of the layer.

In *human* calorimetry, Atwater and Benedict in 1905¹⁷ applied thermocouples at various sites to detect differences in temperature between the inner and outer surfaces of a wide air space surrounding their calorimeter. Heating or cooling was then applied to these places until the difference became zero. In this way, "adiabatic" calorimetry was accomplished. Burton, in 1934,¹⁸ chose to measure rather than eliminate differences of temperature at the inner and outer surfaces of an insulating layer which surrounded his calorimeter on the outside. The quantities so measured were of the order of 5% of the total heat loss as measured inside the calorimeter with a water circuit. This method was also described in a paper by Murlin and Burton.¹⁹ Prouty, Barret, and Hardy⁷ quote, furthermore, the following work as having extended the application of the gradient calorimeter: Forster *et al.*²⁰ on the relationship between total heat exchange and blood flow in the hand; Love and Bazett²¹ on heat loss and blood flow in the feet; and unpublished data by Yaglou on thermal insulation of clothing.

Although this list of references to "gradient" measurements is certainly not complete, it may be sufficient for viewing against it as a background the principles of gradient layer calorimetry outlined in our previous publications^{18, 22} and re-stated here as follows.

METHOD

Principles of Gradient Layer Calorimetry

When a source of heat is accommodated in a cavity (four pi enclosure) and the surface of that cavity is completely lined with a layer of insulating material, uniform in thickness and in specific thermal conductivity (Fig. 1), the integrated average difference, or gradient, of temperatures between the inner and outer surfaces of this layer is proportional to the rate of loss or gain of heat from the source inside, once a steady state of heat flow has been established. In response to a sudden transition from one constant rate of heat flow to another, the tempera-

ture gradient will show an exponential rise or fall to a new level which represents the new steady rate of heat flow. The time constant of this exponential response decreases with the first power of the specific thermal diffusivity of the layer. It increases with the square of its thickness. Thin layers respond more swiftly, thick layers produce higher gradients.

The response to changing total heat flow from the source, by changes of the average gradient, is independent of the size, shape, location, emissivity, and distribution of temperature over the source of heat, and also of the shape of the layer surface, since the geometry delineated by the gradient layers has the essential properties of a blackbody for any incident thermal energy (Fig. 1). Provided there is no change of total enthalpy within the space enclosed, any closed surface, irregular or regular, large or small, surrounding the source of heat and intersecting its total thermal gradient field will intercept on its passage the total energetic output from the source. The response is independent of the way in which the heat is transferred to the layer, whether by radiation, conduction-convection, or by condensation of water vapor previously generated at the surface of the source of heat.

At any given rate of heat flow the average gradient increases or decreases in proportion to the surface area of the layer. If the surface area is divided into equal units and if every such unit is instrumented to produce a defined electromotive force in response to the local gradient, and if these responses are made additive to represent the average gradient over the total (four pi) surface area of the enclosure, then the total voltage response to heat flow—though not the gradient itself—becomes independent of the size of the calorimeter. The voltage is then determined uniquely by the established pattern of the measuring layer. (The gradient in response to a given heat flowrate decreases with increasing total surface area, while the number of voltage-producing units increases proportionately.) The number of measuring units, as long as it is very large, is important only to the response sensitivity of the layer. However, if the number of units is reduced too far, the surface area represented by each one of them is no longer appropriately defined, and the instrument would respond correctly only to heat flow of uniform distribution, a quite exceptional condition. Division into numerous small units is, therefore,

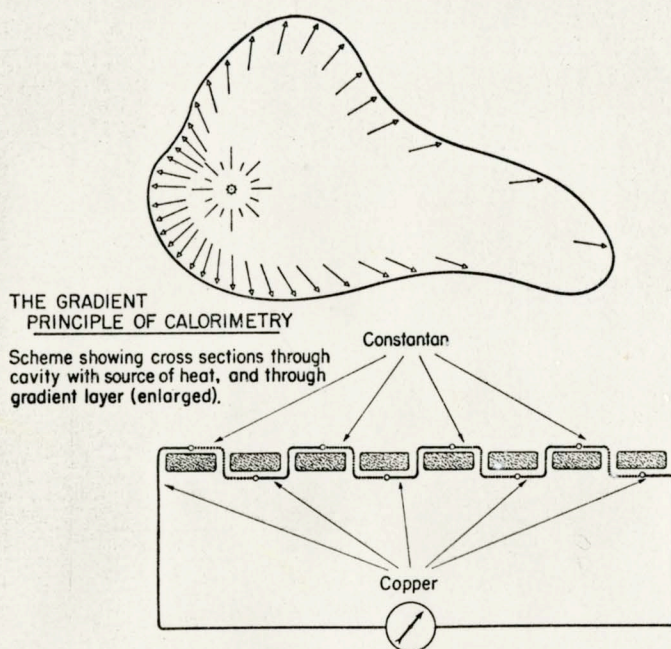


Fig. 1. Gradient principle of calorimetry. A "gradient layer" shown below, lining completely and uniformly the entire inner surface of a cavity (above) records correctly the total heat from a source within, regardless of its location and the size or shape of the cavity.

essential to the gradient layer method. It also helps in eliminating statistically any minor variations in individual voltage responses to heat flow by different units of the layer. These principles of gradient layer calorimetry are best translated into workable design and construction of a calorimeter with a continuous chain of alternating links between two thermoelectrically different metals, interlaced in a regular pattern through the entire layer and lining completely and in a uniform manner the cavity or four pi enclosure. From the two terminals of such a chain a single potential is derived which represents with high precision the total energetic loss from any source of heat within the cavity.

Gradient Layer Design

In the design of gradient layers two main objectives must be attained: (a) uniformity in response of the product, and (b) easy serial production without soldering of individual units. For uniformity of response there must be a strong and well-defined thermal contact between the thermoelectric junctions and the insulating layer material. Therefore, all layer designs were based on foils, not wires, of copper and constantan. (Wires, with their point-shaped, tangential touch would not provide a sufficiently

reproducible thermal contact for this purpose.) The foils were firmly bonded to the layer material, to the basal calorimeter shell, and to foils of anodized aluminum covering the layers in the calorimeter and protecting them against the penetration of moisture. Water in the layer with traces of electrolytes would form galvanic elements between the two thermocouple metals, and render the layer useless.

Individual soldering of thousands of junctions in a gradient layer is neither feasible nor necessary. Instead, repetitive patterns of alternating constantan and copper links are readily obtained with running ribbon material in which the two metals are joined in a soldered¹³ or welded¹² seam, resembling a medal ribbon of two colors. By making alternating cross incisions at regular distances, which cut through the seam, while leaving a narrow bridge at the edge of the ribbon (Fig. 2), the double ribbon is transformed into a chain of alternating thermoelectric junctions between the two metals. Sheet material for the insulating stratum is then cut into ribbons of a width equal to the distance between two incisions in the metal ribbon, and laid out at right angles to parallel strips of incised metal ribbon. By placing the strips of insulating stratum alternately on top or below

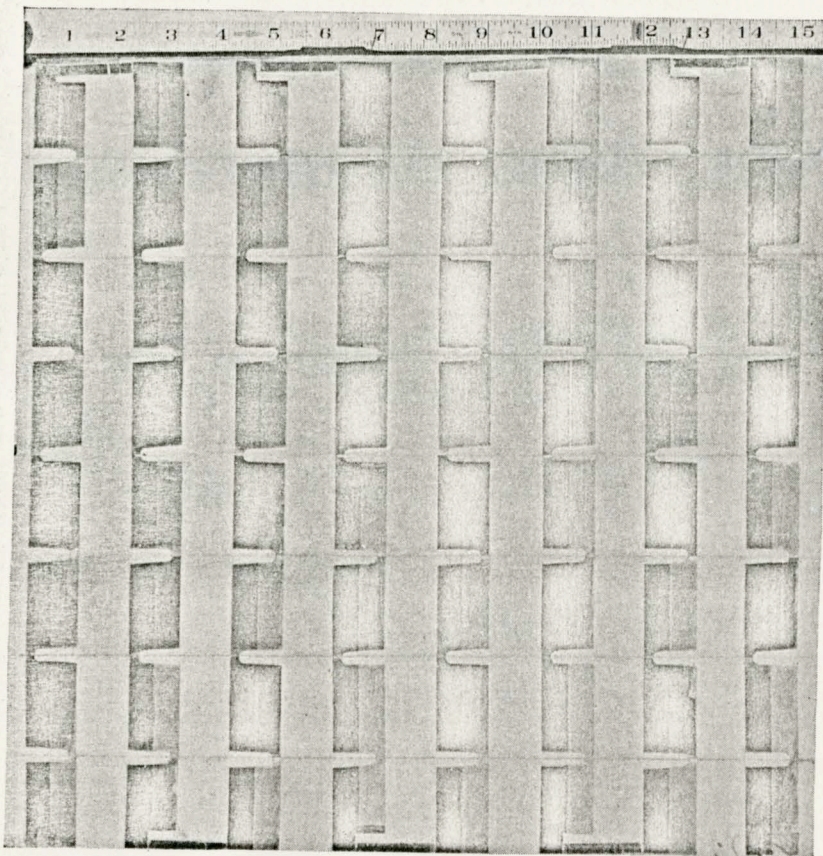


FIG. 2. Gradient layer. Ribbons of constantan and copper are so interlaced through a layer of squares of a transparent plastic that all copper-to-constantan junctions are located above, and all constantan-to-copper junctions below, the plastic sheet. Plastic and metal are firmly bonded by adhesive foil.

the metal ribbon, an interlaced pattern is achieved in which all metal *A* to metal *B* junctions are located on one side, and all *B* to *A* junctions on the other side of the insulating stratum (Fig. 1). In the human calorimeter, each junction so constructed covers an area of 2 inches $\times$ 2 inches with a section of ribbon 2 inches long and 1 inch wide, consisting of $\frac{1}{2}$ inch of constantan and $\frac{1}{2}$ inch of copper. The thickness of the insulating stratum, dilecto plastic, is $\frac{32}{1000}$ inch, of the copper constantan foil, $\frac{5}{1000}$ inch. Scotch Weld foil was used to firmly bond the component foils among themselves, with the aluminum base plates, and with an anodized aluminum foil which covers the outside of the layer and prevents the penetration of moisture. The materials and techniques employed have resulted in satisfactory characteristics, reproducible over a period of several years. This type of layer and its performance

characteristics were first described in reference 13 and later in more detail in reference 22. It has also been applied in the calorimeters of Lawton, Prouty, and Hardy⁸ and of Tasker, Humphreys, Parmelee, and Schutrum.¹¹

An alternative way to obtain a repetitive pattern of thermoelectric junctions for gradient layers was designed for our infant calorimeter.^{23, 24} This is based on winding a flattened spiral of constantan ribbon on a strip of insulating stratum, and subsequent partial electroplating with copper or silver. The technique employed with constantan ribbon resembles a procedure used by Gier and Boelter²⁵ for a silver-plated thermopile of constantan wire. This design of gradient layer has the advantage not to require special ribbons consisting of two metals or repetitive cutting of the metal. The animal calorimeter of Pullar⁹ has been equipped with layers of this type, and Stewart¹⁰ employed

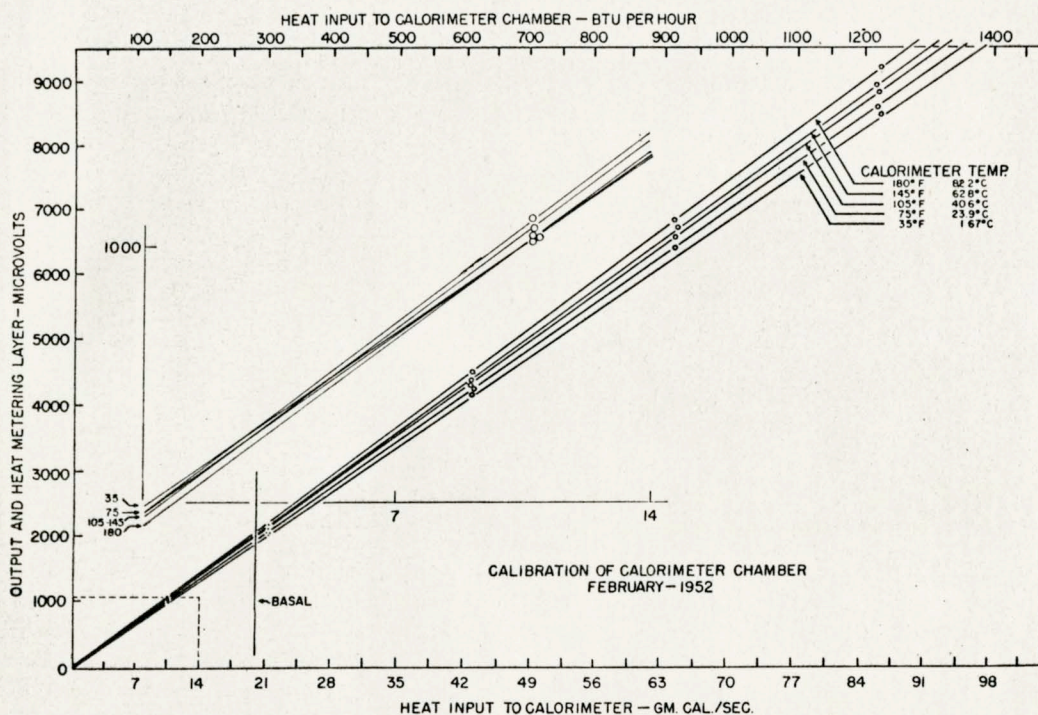


Fig. 3. Linearity of response of calorimeter. Response in voltage to input of heat in cal/sec is linear for all practical purposes at any given temperature of operation.

the spiral arrangement with "edgelay foil" ribbons.

For calibration, we need not know the number of junctions, the specific thermal electromotive force of the metals, nor the thermal conductance of the layer (although approximate data were, of course, required for planning a layer of suitable sensitivity and speed). It is only necessary to measure once, under equilibrium conditions, with the gradient layer applied to a cavity, the response in volts to a known electrical input in watts. The relationship between input of heat and response of the layer is linear for all practical purposes at any given temperature of operation, as demonstrated in Fig. 3 by measurements with the human calorimeter. The sensitivity is higher than required for recording with commercially available potentiometers, 100 microvolts per calorie per second, with the materials and dimensions of the present example. The speed of response of the layer, 3 seconds for 50%, is almost one order of magnitude above the speed at which a source of heat in an air space of the present calorimeter dimensions attains a steady state of heat flow with reasonable rates of ventilation. Since more than adequate sensitivity

and speed of response are readily obtained, the quality of the layer rests to a large extent on its uniformity of response. Voltage observed with a given caloric input must not appreciably vary when the source of heat is shifted from one position to another within the calorimeter. The variation of gradient responses from the calorimeter in such tests was less than $\frac{1}{2}\%$ as shown in Fig. 4.

The voltage response of a layer calorimeter is, of course, slightly different at substantially different temperatures of operation. The specific thermoelectric potential of the constantan-copper combination increases with the temperature of these metals. A minor influence is also exerted by changes in thermal conductance or dimensions with the absolute temperature of the layers. The response sensitivity is, therefore, best determined experimentally in a series of calibrations with known energetic input rather than by calculation from the temperature coefficients of the thermoelectric electromotive force. On the basis of such a series of calibrations as shown in Fig. 3 shunts are prepared which set the sensitivity of the calorimeter and plates with a switch at one

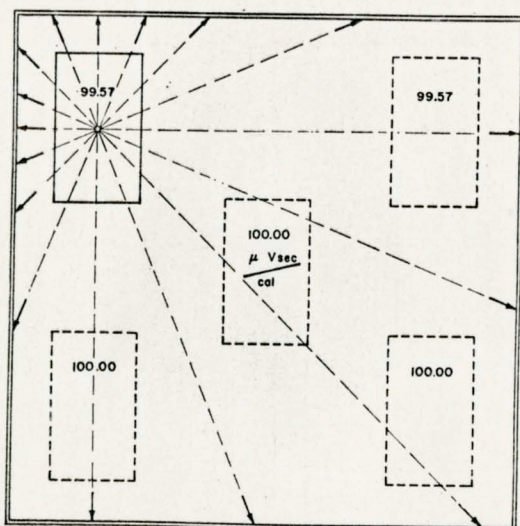


FIG. 4. Uniformity of response of calorimeter. To a given source of heat, the calorimeter responds with voltage to the same degree, regardless of the location of the source of heat in the cavity. This proves that the layers are practically uniform.

defined value, 70 microvolts per cal/sec, with every temperature of operation.

Calorimeter Shell and Subject Support

The shell of the human calorimeter consists of rectangular aluminum plates $\frac{1}{4}$ inch thick held together in a frame of angle steel, forming a cavity of $32 \times 32 \times 84$ inches, which may be opened at both ends by swinging out the 32×32 inches walls. At their inner surfaces the six walls are completely and uniformly covered with gradient layers. To the outer surfaces of the plates, a piping system of aluminum tubes is brazed (with good thermal contact) for circulation of cooling liquid at constant temperature. In order to avoid an undesirably large temperature increase at the discharge end of this circuit, caused by the thermal output of the subject, the piping is divided into a large number of parallel loops. For the same reason, the water circuits of the six walls are arranged in parallel rather than in series. Thus, the total flow rate of water through the walls of the calorimeter is 8000 liters/hour.

Paired air inlets, 1×4 inches, are provided in the lateral walls, one for a ventilatory and the other for a respiratory air circuit. Three windows 1×4 inches, made from several layers of glass separated by air spaces to provide additional thermal insulation, and two panels for electrical connections are installed in the walls for communication with the subject.

In the angles of the lateral walls with bottom and ceiling, 12 eyes are fastened for holding nylon cords to suspend—with low thermal conduction and without rigid and heavy structural elements—the light-weight frame of steel tubing that supports the subject (Fig. 5). For low added capacity, good thermal conduction, and minimum occlusion of evaporation, the body rests on metal screens supporting only the head, shoulders, buttocks and heels, while a sling of gauze through the knee bends holds the legs. In this way, it was attempted to avoid as far as possible thermal inertia, capacity, and shielding of body radiation.

Temperature Control of the Calorimeter Shell

Although the heat capacity of the shell is substantial, a uniform and constant temperature cannot be maintained in it for any extended period without additional control. Changes induced by a changing environment or by the varied heat load from the subject must be prevented by circulation of controlled liquid within the walls of the shell, for two major reasons: (a) to provide reproducible conditions for physiological experimentation, and (b) to provide a physical reference basis for the gradient measurement. The latter requirement is more stringent and is, therefore, discussed first.

Changes of shell temperature would inevitably result in heat flows out of or into the gradient layer, producing undesirable responses in proportion with the thermal capacitance of the layer.* Total thermal capacitance of the layer, from the top cover foil to the midline, is $3660 \text{ cal deg}^{-1}$ calculated from weight and specific heat of the components. (Independently, the thermal capacitance may be derived from the total conductance and the measured time constant of 7.46 seconds for 63.2% response. Total conductance through the 88.8 square feet of layer, calculated from conductivities of components, is $493 \text{ cal sec}^{-1} \text{ deg}^{-1}$. The 50% thermal capacitance of the layer was in this way found to be $3680 \text{ cal deg}^{-1}$.) At a rate of change of 0.2°C/hr , $0.234 \text{ cal sec}^{-1}$ would be added to, or withdrawn from the heat capacity of the layer. This rate of change, producing a response equivalent to 1% of human

* Additional exchanges of heat with the thermal capacitance of the air adjacent to the layer may be disregarded since they are of a lower order of magnitude.

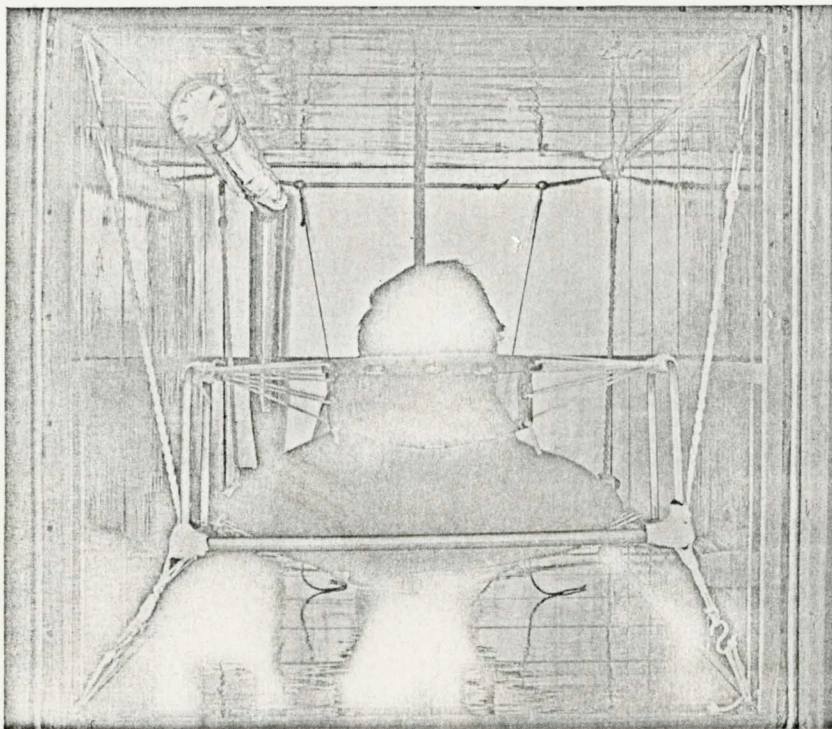


FIG. 5. Human subject in the calorimeter. Subject is suspended on a frame with wire nets held by nylon cords. The layers at the walls are covered with anodized aluminum foil.

basal metabolic rate, was not to be exceeded in the shell as a design criterion.

While the characteristics of the layer make it desirable to minimize the rate of change of temperature, as shown, physiological reasons call for minimum absolute deviation of the shell temperature from determined levels. As an example, for an average cutaneous temperature of 32°C in a 22°C environment, radiated and conducted heat losses would change approximately 1% for purely physical reasons if the shell temperature were increased or decreased by 0.1°C . It was, therefore, required that the control equipment maintain the shell temperature within $\pm 0.1^{\circ}\text{C}$ and the rate of change within 0.2°C/hr , or $0.000055^{\circ}\text{C/sec}$ at any desired temperature of operation, against considerable external and internal heat loads. For physiological experiments on stress, an operating range from $+2$ to $+80^{\circ}\text{C}$ was contemplated. The method used for fulfilling these designed criteria was as follows.

The temperature of the bath supplying water to the calorimeter shell is controlled by overcooling (a manual adjustment) and then reheating under automatic control. Water is

circulated with a $1\frac{1}{2}$ -hp pump through the piping in the walls of the calorimeter, at a rate of 130 l/min. This water is supplied from, and returned into, a bath of 500-liter capacity. A second pump and circuit remove water from the bottom and return it into the top of the bath after passing the flow through a shell and tube heat exchanger in which heat is withdrawn from the circulating water of temperature $T^{\circ}\text{C}$ by thermal contact with circulating liquid of a lower temperature $T-4^{\circ}\text{C}$. Heat in accurately controlled quantities is intermittently supplied by two 5-kw heaters (located in this circuit and cycling on and off) at an approximately constant frequency of 3/min. The quantity of heat added to the bath is regulated by means of a "duration adjusting type" control, which changes the ratio of the durations of "on" and "off" periods of the heaters without appreciably changing their frequency. The sensing element of the control mechanism, a platinum resistance thermometer, is located in the line supplying water to the calorimeter.

The $T-4^{\circ}\text{C}$ liquid, an ethylene glycol mixture of freezing point -16.5°C , is drawn from a storage tank of 180-liter capacity. Temperature

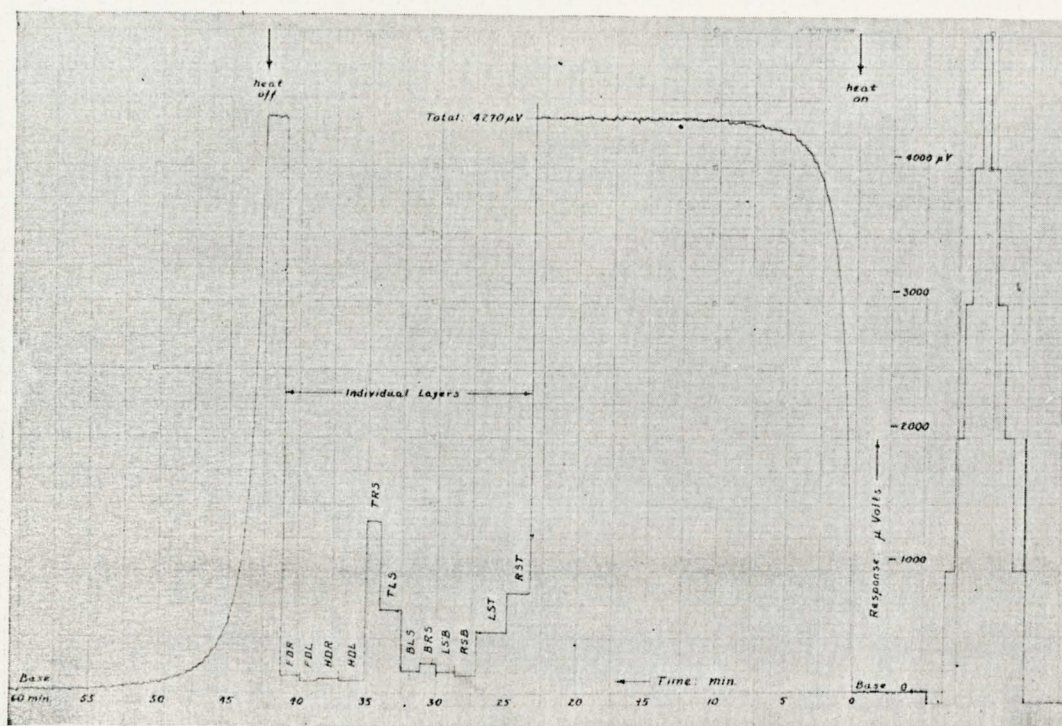


FIG. 6. Stability of calorimeter response. This pen recording shows the degree of stability afforded by the temperature control of the calorimeter shell, and the degree of reproducibility of the zero base line. In the middle section, the widely different rates of heat transfer through the individual walls of the calorimeter are separately recorded.

in this tank is maintained at $T-4^{\circ}\text{C}$ by a "proportioning adjustment type" control. In this instance, however, the control acts not upon a heater, but on an adjustable port control valve which admits to the tank a varying but continuous flow of liquid at $-10 \pm 1^{\circ}\text{C}$. This latter liquid, of freezing point -16.5°C is supplied at -10°C by means of equipment comprising a 15-hp 7-ton freon condensing unit and a storage tank of 900-liter capacity. The maximum size of the port control valve can be adjusted over a wide range, depending on the desired temperatures T and $T-4^{\circ}$ for operation of the calorimeter between $+2$ and $+80^{\circ}\text{C}$. The rate of flow of the $T-4^{\circ}\text{C}$ liquid through the exchanger—where it cools the water returning from the calorimeter—may be adjusted within wide limits, depending on the heat load expected during experimentation. The usual flow rate is 80 l/min.

The purpose of the cooling and reheating procedure is, of course, to counteract thermal disturbances in both directions arising from load or from external sources. The use of controlled $T-4^{\circ}$ liquid makes it possible to obtain optimum

control at any desired level of calorimeter temperature T from $+2$ to $+80^{\circ}\text{C}$. In summary, the entire system first adjusts roughly a low level of $-10 \pm 1^{\circ}\text{C}$, then with increased precision a level of $T-4^{\circ}$, which may be varied over the wide range from -2 to $+76^{\circ}$, and then within $\pm 0.01^{\circ}$ the final level, at calorimeter temperatures T between $+2$ and $+80^{\circ}\text{C}$. Remaining fluctuations are damped in the large heat capacity of the storage tank and finally in the heavy aluminum shell to which the gradient layers are attached. External and internal loads approaching the capacity of the two heaters, 2500 cal/sec, can thus be handled. A 15-hour record of temperature in the water supplied to the calorimeter showed less than $\pm 0.01^{\circ}\text{C}$ maximum variation and 0.0053°C maximum change in any 1-hour period. The efficiency of the control system in providing a stable reference temperature for the gradient measurement (rates of change of temperature less than $0.00005^{\circ}\text{C/sec}$) is best demonstrated with a pen recording of the layer output from the entire calorimeter (Fig. 6).

Attention is called in this recording to the un-

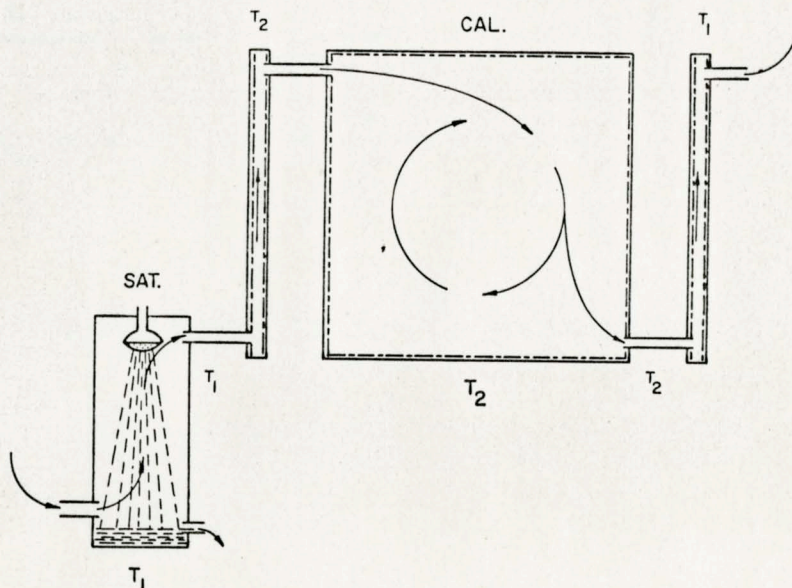


FIG. 7. Gradient calorimetry of circulating air. Ventilatory air, forced through the calorimeter in an open circuit, exchanges heat at equal rates of opposite sign in two plate-shaped gradient calorimeters P_1 and P_2 if no heat is added in the calorimeter. Any added heat from the subject induces a net excess potential in the "plate meter" P_2 which is recorded with P_1 and P_2 in series electrically. Saturation at temperature T_1 of the air entering and leaving is ascertained by a saturator (SAT) and by condensation of excess vapor in P_2 . This circuit serves to remove vapor and to exchange the respiratory gases.

even distribution of heat flow from a symmetrically placed source of heat. The top layers receive by thermal convection a multiple of what the layers at the wall and bottom are required to handle. This emphasizes the importance of uniformity in layer design and response.

Gradient Calorimetry of Circulating Air

Human calorimetry would be restricted to very short periods and equilibrium could never be obtained, were it not possible to remove water evaporated from the lungs and the skin, and to exchange respiratory gases, O_2 and CO_2 by circulation of air through the cavity. Such circulation would, however, entail gains or losses of energy not recorded in the layer unless the air were caused to leave at the same temperature and with the same vapor content which it had when it entered the layer-lined system. Therefore, the air for ventilation flowing at some constant rate between 100 and 800 l/min is carried through the following sequence (see Fig. 7): (a) a saturator, where it assumes a low and constant temperature T_1 , and with it a low and constant absolute humidity (relative humidity 100%) in counterflow with a shower of pre-

cisely controlled chilled water (adjustable between $+2^\circ C$ and any desirable higher level); (b) a heat exchanger or plate meter P_1 , maintained at a controlled elevated level of temperature T_2 where heat is added to the circulating air (through a gradient layer lining the interface of exchange) until the leaving air has assumed temperature T_2 at the end of its pathway; (c) the calorimeter cavity, also maintained at temperature T_2 in which, so we assume for the present moment, no heat is added to or removed from the flowing air; (d) a second plate meter P_2 of design and construction identical with P_1 (however, maintained at the lower, initial level of temperature T_1) where heat is withdrawn from the circulating air until it has, at the end of its pathway, resumed a saturated condition at the initial temperature T_1 with no change in water vapor saturation. (Again, in plate P_2 , the total heat withdrawn from the air must pass through a gradient layer lining the entire interface of exchange which is wired in series with the layer in plate meter P_1 .)

Now, since the flowrate is the same through both plate meters P_1 and P_2 , the enthalpy changes in P_1 (where the air goes from tem-

DESIGN OF A PLATE METER

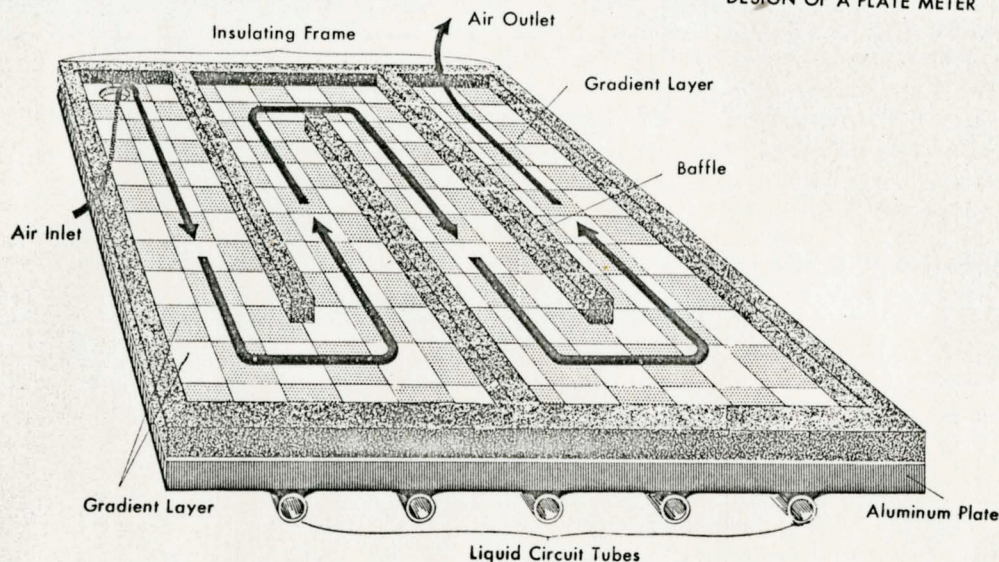


FIG. 8. Plate meter for air circuit calorimetry. The ventilatory air is forced through a narrow path between gradient layers where it exchanges heat and assumes the temperature of controlled water supplied in the tubes shown below. One-half of the assembly is removed to expose the gradient layer and baffles.

perature T_1 to T_2) and P_2 (where the air goes from temperature T_2 to T_1) are equal and of opposite sign, the output potentials of the gradient layers in P_1 and P_2 will cancel as long as no heat is added to or removed from the circulating air in the calorimeter cavity. However, if the human body adds heat to the circulating air by way of increasing either its temperature or its water vapor content, such excess heat over and above that transferred in the first plate meter will be correctly recorded as a net output potential from plate meters P_1 and P_2 wired in series. This net potential is then either separately recorded, or added to the main potential from the cavity for measurement of the total integrated heat loss of the human body.

In a ventilatory or respiratory air circuit as outlined, the "plate meter" (Fig. 8) is the principal element of construction. It is designed to bring about and to measure gains or losses of heat in circulating air by which the temperature of this air is changed from one constant level to another. Also, if the air contains water vapor in excess of the dew point level of the new temperature, such vapor is condensed and the rate of enthalpy change associated with this process is recorded. For this function, a pathway of approximately 15 m length and 20 cm² cross section lined with a gradient layer ap-

peared to be required, with flowrate maxima of 800 l/min. In the beginning of this pathway, the rate of heat transfer is maximal. It then decreases exponentially to a negligible fraction of the initial rate at the end of the pathway.

For economical construction of layers and in order to minimize the influence of extraneous temperatures, plates as shown were designed in 1947 and first applied in the 1949 Bethesda model and in the Naval Medical Research Institute baby-calorimeter. The air is guided through a maze of narrow pathways between two plates that are lined with gradient layers. The desired final level of temperature is maintained by circulation of controlled water in the aluminum shell of the plate. In Fig. 8, one-half of the assembly, an aluminum plate with water tubes on one side and a gradient layer on the other, have been removed to show the insulating frame, the baffles, and the pathway of the air.

In the ducts which connect the individual plates, gains or losses of heat must either be prevented or recorded. The ducts have, therefore, been lined with layers in this instrument.

Requirements for the control of temperature are, of course, as strict for the plate meters as they are for the calorimeter proper. Therefore, controlled water of the calorimeter supply circuit is circulated through the inlet plate meter

P_1 before it enters the calorimeter and thus maintains the temperature in this plate at calorimeter temperature T_2 . For the saturator, water of temperature T_1 is supplied from an independent control system built like the one described for the calorimeter shell, with a resistance thermometer sensing and controlling the desired level of absolute temperature in the line of supply.

The saturator (SAT in Fig. 7), which replaces in the human calorimeter a heat exchanger formerly used for the same purpose in the first models,¹³ consists of a sheet copper shell 95 centimeters high and 15 centimeters square in cross section. Air enters near the bottom and leaves at the top. Water flows from a spray nozzle on woven stainless steel mesh, packing the space of the saturator in several layers. Tests disclosed that 100% saturation is obtained with rates of water flow above 12 l/min, provided that the dew point of the incoming air is above the water temperature in the saturator. Preheating and humidification of the incoming air are, therefore,

sometimes required. The adjustment of the air temperature to the water temperature is only 98.5% complete after passage of the air through the saturator. This does not, however, introduce an error, because of the way in which the temperature of the second plate meter is regulated: instead of a sensing element for absolute water temperature as described above for the temperature control of the calorimeter shell, differential resistance thermometers are used for control of the water temperature in the second plate meter, with one element in the air stream between the saturator and the first plate meter and the other in the air stream at the outlet of the second plate meter. By this means the control makes certain that the air leaves the measuring circuit at precisely the same temperature at which it had entered and with a relative humidity of 100% at that particular temperature. A simplified layout of the controls and liquid circuits of the calorimeter, saturator, and plate meters is shown in Fig. 9.

The maximum rate of air flow in the ventila-

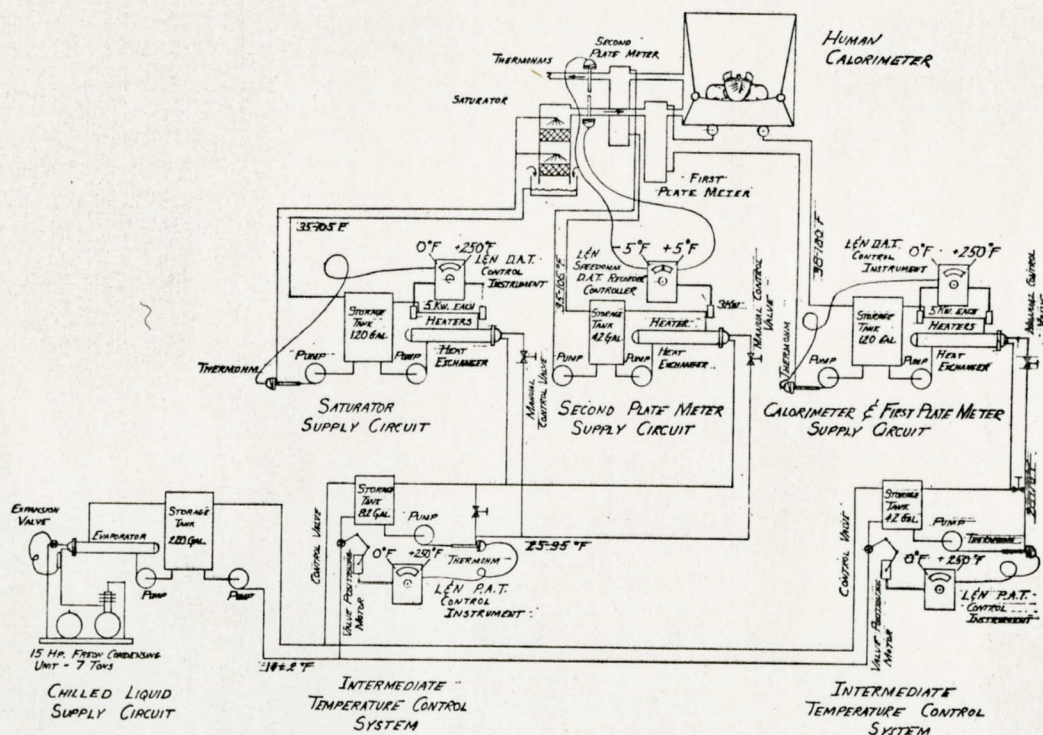


FIG. 9. Diagram of flow and control circuits for the calorimeter. Below the calorimeter, three circuits are shown which supply liquids of controlled temperature to the calorimeter, saturator, and plate meters. Below these are shown a unit supplying coolant of -10°C (left) and two circuits supplying " $T-4^{\circ}\text{C}$ liquid," 4°C cooler than the temperature of operation. Temperatures are controlled within $\pm 0.01^{\circ}\text{C}$.

tory circuit is 800 l/min. With the lowest dew-point temperature obtainable, $+2^{\circ}\text{C}$, the entering air has a water vapor tension of 5.3 mm Hg. At the maximum obtainable calorimeter temperature, $+80^{\circ}\text{C}$, the air could take up water from the subject to a limit of 355 mm Hg, and a maximum rate of 368 liters or 296 grams of water could thus be removed per minute until condensation would begin at the walls of the chamber. Known maxima of physiological water loss are approximately five times smaller. Even with an excessive sweating rate of 60 g/min, at a calorimeter temperature of $+80^{\circ}\text{C}$ and 800 l/min of air circulation, the ventilatory circuit could maintain a vapor tension of 71 mm Hg or 20% relative humidity in the calorimeter. With changing water output from the subject there would, of course, be alterations of humidity. It is desirable to prevent such changes in order to maintain a constant environment and also to prevent the storage or loss of heat which are associated with any enthalpy change of the air in the calorimeter. Therefore, an adjustable bypass has been installed between the in- and outlet for the ventilatory air. A control mechanism operating this valve from a humidity sensing element will be developed for the automatic control of constant humidity in the calorimeter.

The Measurement of Evaporative Heat Output With the Ventilatory Circuit

One purpose of the ventilatory circuit is to obtain the correct total of human heat loss in a ventilated layer calorimeter. The other is correct partition of the total into the evaporative and the "dry" (radiated-conducted-convected) components. This objective has special physiological significance. The evaporative fraction (when pulmonary heat loss is measured separately) yields the measurement of sweatgland action in calories (heat of evaporation) or weight of water secreted per unit time. The total heat loss (dry plus evaporative) minus the respiratory component is another important physiological quantity: total transfer of heat Q through the skin, in calories per unit time. The quotient $Q/T_i - T_s$ (where T_i is the internal temperature and T_s the average skin temperature) is "conductance" or the index of peripheral circulation (Burton¹⁸). In the measurement of conductance, the total output from the respiratory circuit may be taken for the pulmo-

nary component of the total heat loss. For the measurement of sweatgland activity the output from the ventilatory circuit cannot be taken without correction. It contains a fraction of "dry" heat transfer from the skin into the second plate of the ventilatory circuit whenever the subject either warms or cools the air as it passes through the calorimeter. Both of these situations are quite common, the first in moderate or cool, the second in hot environments.

The "dry" heat transfer into the ventilatory circuit, which must be subtracted from the ventilatory plate meter voltage to obtain the evaporative heat loss from the skin, is measured as follows.

At the inlet and outlet openings from the first ventilatory plate into the calorimeter and from the calorimeter into the second plate, a thermopile of 20 junctions is installed. The "cold" junctions are located at the inlet and the "hot" junctions at the outlet in the midst of the streaming air. This pile develops a potential of $20 \times 42 = 840$ microvolts per $^{\circ}\text{C}$ of air temperature difference. At an air flowrate of 800 liters per minute and a specific heat of 0.24 cal/g for the air, a difference of 1° will result in a "dry" heat transfer of 3.4 cal/sec. The sensitivity of this measurement in terms of voltage per unit heat flow is then $840:3.4$ or 247 microvolts per cal/sec, that is considerably higher than the shunted sensitivity of the calorimeter and its circuits, $70 \mu\text{v}$ per cal/sec. A shunt is, therefore, applied to the thermopile, which reduces the voltage response from 247 to 70 microvolts per cal/sec. The shunted pile is then wired in series with the ventilatory circuit output in proper polarity. A check of this calculated calibration is readily made with a known electrical source of heat installed in the air stream between the inlet and outlet at the calorimeter. After recalibration both the uncorrected and the corrected outputs from the ventilatory plates are continuously recorded in the experiments with human subjects. The various potentials are best superimposed upon one another in the following sequence:

- (1) ventilatory circuit
- (2) ventilatory circuit minus shunted thermopile (correction for "dry" output)
- (3) ventilatory circuit plus main calorimeter
- (4) ventilatory circuit plus main calorimeter plus respiratory circuit.

The readings of physiological importance are:

- (1) sweatgland activity

- (2) total heat loss and production (including the dry fraction in the ventilatory circuit)
- (3) total cutaneous heat transfer for calculation of conductance.

If differentiation between the physical (not physiological) components of dry heat loss into the radiated and conducted-convected fractions is desired, four pi radiometry²⁶ may be contemplated. The four pi radiometer simultaneously affords a measurement of average radiant skin temperature without attachment of skin temperature measuring devices to the subject. At present, skin temperature is obtained by 10 properly distributed skin thermocouples in our calorimetric experiments on man. The physical differentiation between radiated and conducted losses from the skin is considered to be of secondary physiological importance when compared with the meaning of sweating and vasodilatation (conductance).

Calibration of The Calorimeter With Air Circuit

The precision of gradient calorimetry with an air circuit depends on a number of presuppositions: (a) nowhere must heat or air which would induce a change of enthalpy be added into or removed from the circulation; (b) the layers of plate meters P_1 and P_2 and the calorimeter proper must be adjusted to precisely matching response in volts per unit heat flow; (c) the exchange of heat must be practically complete at the ends of the air pathways; (d) air temperatures must be controlled with best possible precision to match between the entering and leaving points (T_1 between the saturator and P_1 and T_1 at the outlet of P_2 in Fig 7).

These presuppositions were checked individually in numerous tests. The ultimate performance characteristics could only be demonstrated by integrated experiments. All of the components were assembled and their combination was checked for leaks. Every unit was shunted to respond with 70 microvolt seconds per calorie at the particular temperature of its operation. The air circulation was established and air pressure gradients were adjusted to negligible levels. A wet source of heat was made of a flattened ribbon coil of constantan alloy and set up in the calorimeter. Its 1.7 m² of surface area were covered with blotting paper soaked with water. The coil was then supplied with

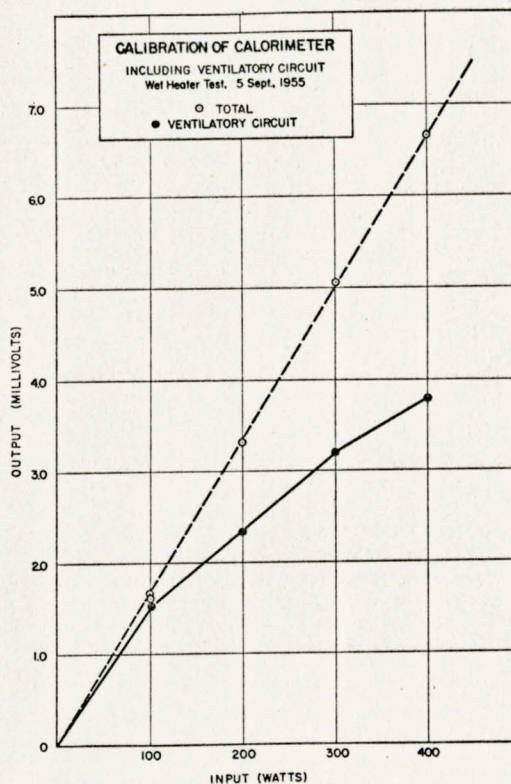


Fig. 10. Combined calibration of calorimeter with ventilatory air circuit. Response voltages from the calorimeter and from the circuit are added and plotted (broken line) against the energetic input. Solid line divides the total into "dry" and evaporative components. Note linearity of total response.

current at various constant levels, and the resulting constant layer responses were recorded.

In Fig. 10, total responses (calorimeter proper plus plate meters of the ventilatory air circuit) are plotted on the broken line against the various total levels of energetic input. On the solid line, the output from the plate system is plotted likewise. Vertical distances between the solid line and the dotted lines, therefore, represent the output of the calorimeter proper. It appears that the totals are correctly recorded regardless of the distribution between the calorimeter proper (recording mainly radiated and conducted losses) and the ventilatory circuit (recording mainly evaporative and a minor fraction of convected heat loss). It appears also that the ventilatory circuit carries the main load if the source of heat is wet. This will be the case with sweating human subjects. The major effort put into the design and construction of the

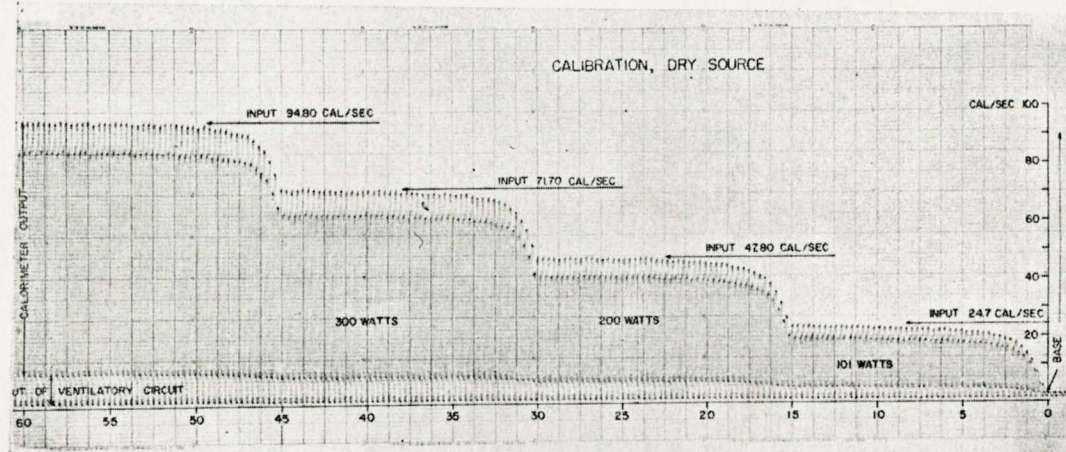


FIG. 11. Original recording of heat loss from dry source. With stepwise increases of energetic input (time running from right to left), the levels of response have been recorded for calibration. The pen recorder changes swiftly from the potential of the ventilatory circuit to the potential of the calorimeter and to the combined potentials of both components in series. Sequences of recorded points represent, therefore, from below to above: electrical zero base, output of the ventilatory circuit, output of the calorimeter proper, and combined outputs of the calorimeter and the ventilatory circuit.

ventilatory air circuit seems, therefore, justified. Both components contribute equally to the recording of total human heat output by means of the gradient principle.

Simultaneous and Continuous Recording of Calorimeter, Plate Circuit, and Total Output

In Fig. 11, an original recording has been reproduced to show the procedure of recording with a dry source of heat in the calorimeter. Time runs from right to left. A multiple point system makes the recording pen shift swiftly between electrical zero base, the output of the ventilatory circuit, the calorimeter output, and the total (ventilatory circuit plus calorimeter). The top recording is the total. Below it, the calorimeter output is shown. Lower yet, the ventilatory circuit output is recorded. The base of the heat measurement is elevated by one small scale division above the electrical zero. This shift is mainly caused by frictional generation of heat in both plates during air circulation. This input is additive in both plates, whereas the majority of stray energetic inputs cancel. The essential observation is the precise relationship between levels of input (horizontal arrows and figures) and the total responses at these levels. For final evaluation, the average is taken of level responses as observed with step by step increase and decrease of energetic input. In this

way, effects of lasting inertia of the heater are eliminated. In the test of Fig. 11, the calorimeter proper was operated at 27°C. The air entering the calorimeter had a relative humidity of 19.5% with 5.2 mm Hg vapor tension. Air flowrate was 800 l/min.

Precise response, regardless of widely different distribution of heat flow between radiation, convection-conduction, and evaporation, is best demonstrated in a test where the source of heat was first wet and then became dry under an air-flow of 800 l/min, as shown in Fig. 12. At the beginning of this recording (left), the heat input to the source was zero. The total heat loss was, therefore, recorded with a hardly perceptible error near the calorimeter base line. (The latter line was slightly higher than the electrical zero base because of the additive heats of friction in the ventilatory circuit.) Attention is called to the reproduction of the calorimeter base line at the end of the recording. The near zero heat output from the source was a result of two opposite heat changes: the wet source was *losing* heat by evaporative cooling. (This heat loss is represented by the corrected output from the ventilatory circuit.) It was *gaining* heat by radiation and conduction-convection. (This negative heat exchange, superimposed upon the corrected measurement of direct, evaporative exchange, made the total near zero.)

The difference between the uncorrected and

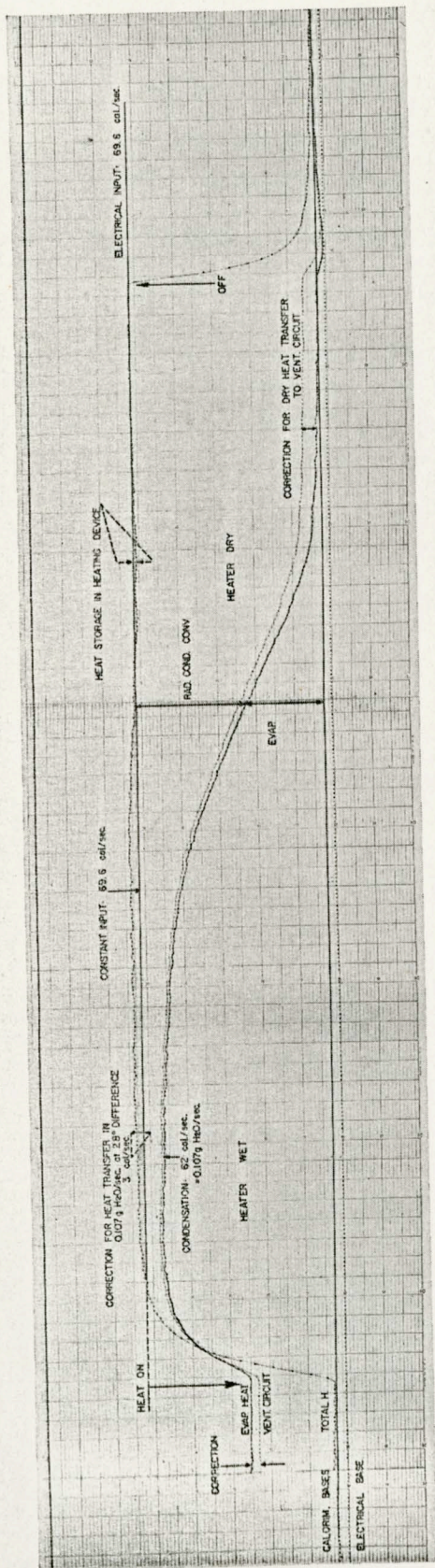


FIG. 12. Calibration of human layer calorimetry with source of heat, first wet, then drying. In this test of 140 minutes duration the source of heat, a resistance coil of ribbon was covered with wet blotting paper. From left to right the recordings show first the calorimeter base lines before addition of the water. Next the recording shows a total of zero heat loss from the nonenergized, wet source. This zero total is composed of a substantial evaporative *loss* and an equivalent radiated plus conducted-convected *gain*, as the air flow rate of 800 l/min leads to a steady evaporation of water. The inlet-outlet thermopile records a substantial correction between evaporative heat loss and ventilatory circuit-output, indicating that the air leaves cooler than it enters. At sign "heat on" the source is energized with 69.6 cal/sec. The total recorded is higher by 3 cal/sec, an additional enthalpy change due to deposition of water (0.107 g/sec) evaporated at a heater surface temperature of 33°C and deposited in the second plater meter at 5°C. (If this water were continuously brought back up to the temperature of the heating coil, the difference of 3 cal/sec would disappear.) As the source dries out, the coil becomes warmer and stores some heat which results in a minor transient deficit. At the end (right) voltage output checks directly with the input. The correction between evaporative heat loss and ventilatory circuit output is now inversed. The air leaves the calorimeter cavity warmer than it enters. On cutting off the electrical current, base-lines are restored after a transient deviation due to some inertia in the temperature control of the ventilatory plate meter.

corrected outputs from the ventilatory circuit was caused by the fact that, due to the evaporation, the air leaving the calorimeter was cooler than the air as it entered. This caused a negative "dry heat transfer" into the second plate meter of the ventilatory circuit measured with a thermopile shunted for correct response with 800 l/min air flowrate and added for continuous correction as shown. After the wet source was heated (heat on), an inversion of this correction was observed: the air was now leaving the calorimeter warmer than it had entered. The record of the total heat output rose to 72.6 cal/sec after beginning of the electrical input. This recorded heat loss exceeded the electrical input by 3 cal/sec. However, 3 cal/sec must be subtracted from the recorded heat output as a correction for the following reason: an additional enthalpy change of 3 cal/sec took place due to the continuous transfer of water (0.107 g/sec) from the surface of the source (where the water was evaporated at a temperature of 29.5°C) to the second plate meter of the ventilatory circuit (where the water was deposited at a temperature of 2°C). The deviation of the recording of the total from the electrical input is, therefore, proof of the reliable function of the calorimeter and the ventilatory circuit. After an hour, the source began to dry up as a consequence of the water loss from the blotting paper. The evaporative component of heat loss, therefore, decreased, and the radiated-conducted-convected fraction rose. The correction for water transfer now disappeared and the total was directly recorded as equivalent to the electrical input near the end of the recording. However, a transitory depression appeared due to some heat disappearing in the source as its temperature rose in consequence of drying. Attention is called to the increasing correction for dry heat transfer into the ventilatory circuit in the latter part of the test: with the dry heated source the air leaving the calorimeter becomes considerably warmer than the entering air. In later experiments with human subjects the uncorrected and corrected output from the ventilatory circuit were always recorded as shown with this record of calibration. The corrected line represents the evaporative heat or water loss of the subject. At the right end of the recording (with the source of heat completely dried out), the three recordings—total output, evaporative output, and uncorrected ventilatory circuit output—return as they should, to the

same zero calorimeter base which had been established at the beginning. The essential observation is that within the resolving power of the instruments the total and the components of heat loss from the source are correctly recorded with the methods of gradient layer calorimetry. It does not matter whether one of the two fractions represents by itself the total (dry output at the end of this test), or the other represents almost the total (evaporation at the beginning of this test, *after heating*), or whether the two exchanges occur in opposite directions, one of them representing a loss and the other a gain of heat (beginning of this test, *before heating*).

Because of the importance and delicacy of air circuit calorimetry, another recording (Fig. 13) is shown with a wet source of heat for a wider difference of temperatures between calorimeter and plate meter (calorimeter, +38°C, plate P_2 , +2°C). Under these conditions, the base is elevated by two small divisions above electrical zero. A rate of heat transfer of 33.6 cal/sec (more than a human basal metabolic rate) was recorded from the wet source at zero energetic input and correctly compensated to zero total response. At 100 watts input, the wet source still withdrew heat from the calorimeter. At 200 watts, the total is recorded in the ventilatory circuit alone with zero recorded in the calorimeter. At the 300- and 400-watt levels, contributions of 19 and 28.5%, respectively, were given off to the calorimeter walls. Here, the contributions from the enthalpy change of water during its transfer from the heater surface to the cold plate P_2 were substantial. They have been added to the input totals for comparison with voltage response levels. The degree of precision is evident from the relationship between the recordings and the horizontal arrows and figures.

Speed of Response

It seemed appropriate first to discuss precision and reproducibility of measurements in order to establish a connection with classical traditions of human calorimetry. Finally, we shall discuss speed of response, an additional characteristic, and one of the objectives for which this calorimeter has been designed and built. A different source of heat had to be chosen for studying the speed of response. Substantial heat capacity and limited energetic input make conventional heaters unsuitable for testing of

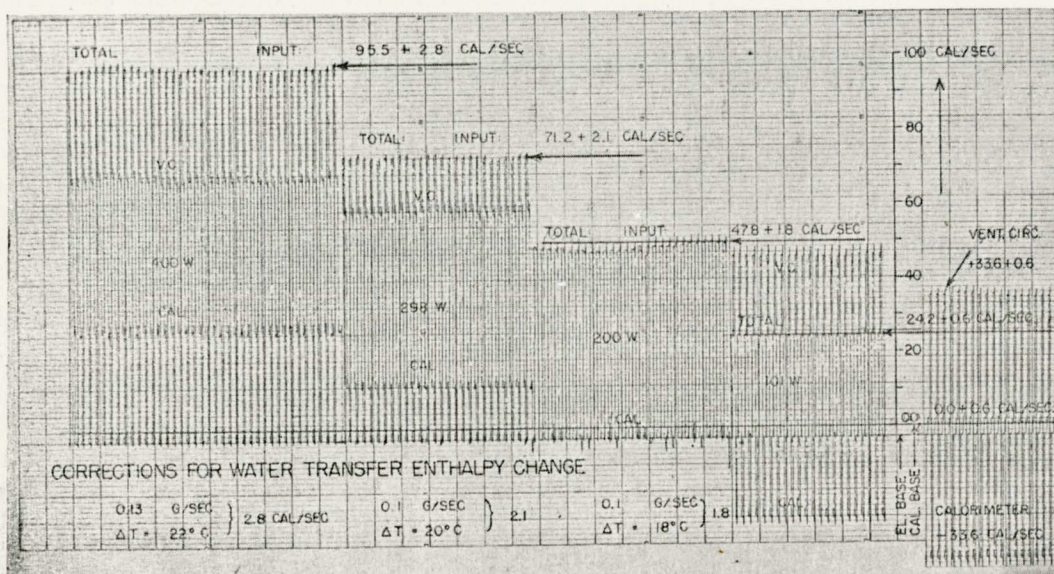


FIG. 13. Calibration with wet source of heat in warm environment and relatively dry air ventilation of 800 l/min. In the absence of electric current, a resistance coil covered with blotting paper absorbed 33.6 cal/sec during ventilation with air of $+38^{\circ}\text{C}$ saturated at $+2^{\circ}\text{C}$, in the calorimeter at $+38^{\circ}\text{C}$. Simultaneously, the ventilatory circuit recorded the same rate with opposite sign, indicating zero energetic output in the total. With various electrical inputs, totals were correctly recorded by the combined calorimeter and ventilatory circuit voltages.

speed of response. Such tests would merely reflect the inertia of the heater. Therefore, in lieu of observations of the rise and decline between steady states, "burst" measurements have been introduced. These do not require the presence of large quantities of water, nor a resistor capable of carrying a large current for long periods of time.

A soldered chain of a dozen pipe cleaners (steel wire with cotton wrapping) was soaked in water and supplied with a burst of electrical current in the calorimeter. The water evaporated almost instantaneously. Burning of the wire and cotton could be prevented. Two tests are shown in Fig. 14 with 7 and 12 seconds duration of the electrical input. The base lines exhibit the characteristics of recordings at 5 mv full-scale response, 800 l/min flowrate, and wet source of heat (see Fig. 13). From their respective bases, the recordings rise almost instantaneously, and then decline. The decay of the calorimeter response is almost exponential, with a $\frac{1}{2}$ period of 40 to 50 seconds. The ventilatory circuit is delayed in the beginning; however, it shows 95% response after 6 minutes.

It should be emphasized that the time lags thus shown are not introduced by any part of

the instrumentation, nor by the principle of measurement. Rather, they are caused by the system under study, namely the source of heat surrounded with air. The record of heat lost by conduction, convection, and evaporation reaches a steady level only after a final distribution of temperature has been attained throughout the cavity. For this reason, the size of the chamber was chosen as small as seemed permissible for a working subject.

With the knowledge of the response characteristics of Fig. 14, it will be possible to reconstruct, at increased resolution, the time course of physiological changes of heat loss. Even without such a refined analysis, responses as directly recorded seem sufficiently rapid for the observation of instantaneous changes of human energetic output.

EXAMPLES OF EXPERIMENTS WITH HUMAN SUBJECT

Although examples of human calorimetric experiments with the gradient layer method are documented elsewhere in this book,²⁷ some are shown here to illustrate other aspects of its per-

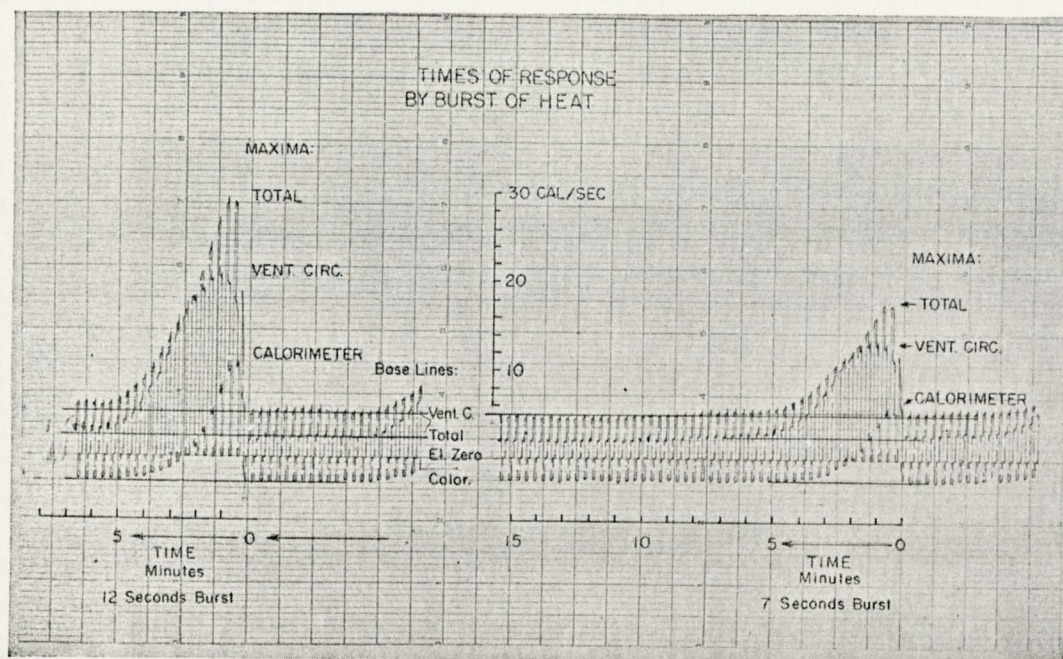


FIG. 14. Determination of speed of response. With bursts of heat from a wet source, response characteristics are demonstrated for the calorimeter, the ventilatory circuit, and their combined potentials.

formance. First, speed of response in physiological applications is shown.

Changes of Energetic Output with Changes in Radiating Body Surface

A sudden change in rate of heat loss from the human body is to be expected after an instantaneous alteration of body surface area (Fig. 15). A human subject was accommodated in the calorimeter at 25°C with a ventilation of 800 l/min and 30% relative humidity of the incoming air. The subject was fully extended in the supine position. He then flexed his back and extremities, holding the trunk and the upper and lower legs folded with the arms around them, lying on his back. After 5 minutes he unfolded and resumed the extended position. The recording reads from right to left: upon flexion the rate of heat loss dropped from 31.7 at rest to 27.6 cal/sec. Upon reextending it rose to a maximum of 37.2. A total of 1300 calories was stored in the body during the period of flexion. A total of 2200 calories, in excess of the resting level, was given off subsequently.

The difference between storage and loss may be partly due to the work effort required in maintaining the flexed position. The output of the ventilatory circuit did not change upon

flexion, indicating no appreciable reduction in vapor loss from the skin. A moderate increase occurs upon reextending, indicating excess water loss from surfaces on which moisture had been accumulated. Practically all of the physiological changes in this experiment are recorded by the calorimeter proper, which suggests changes in radiation as their main source.

Equally sudden, and more pronounced, changes of heat output were observed at a lower environmental temperature, +10°C, by removing a protecting cover of aluminum foil from the subject. In Fig. 16, a heat loss of 56.9 cal/sec was recorded with the naked body in still air, and 28.5 cal/sec from the subject covered with the foil.

Change of Heat Loss With The Onset of Convection

The experiment of Fig. 16 shows also the extent and suddenness of changes in heat loss with the onset and cessation of convection. The heat loss of the naked body, 55–57 cal/sec in still air, was 90 cal/sec during forced convection of approximately 1 m/sec induced by a 9-watt fan. The rate of 119 cal/sec, after removal of the covering aluminum foil and exposure to wind, was the maximum rate of heat loss thus

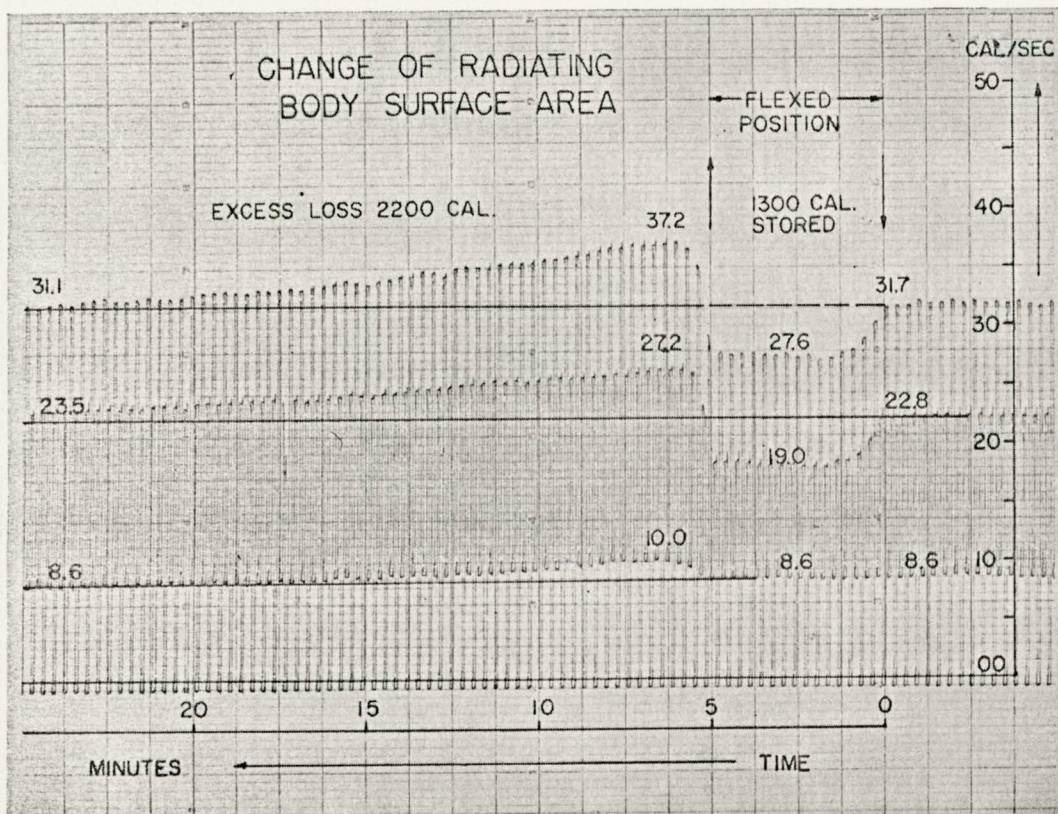


FIG. 15. Influence of posture on rate of human heat loss. From right to left, the changes in rate of heat loss are shown, as radiated body surface is reduced and re-extended by the subject going from the stretched supine into a flexed position and vice versa.

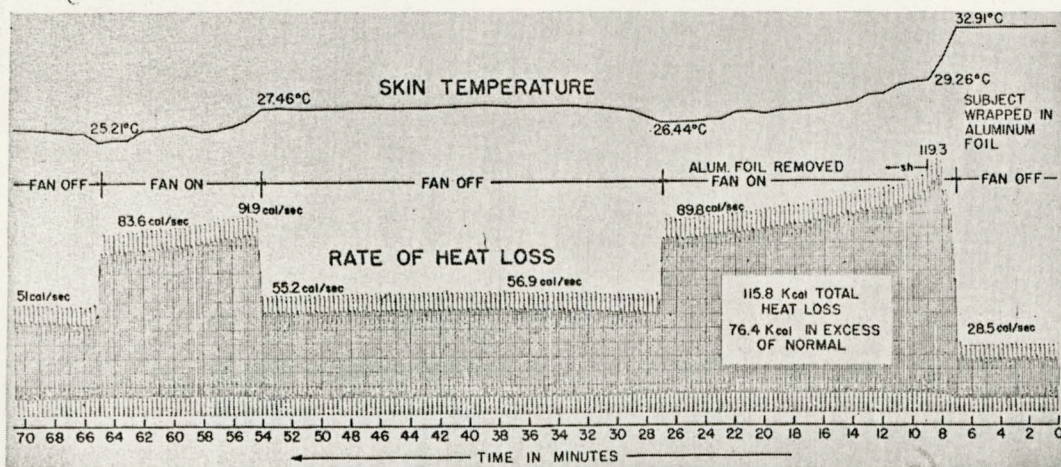


FIG. 16. Influence of convection on human heat loss and protective effect of a reflective foil. Speed of calorimeter response is shown by initiation and termination of forced air convection produced by a small fan, and (at right) removal of an aluminum foil which had covered the naked subject during the initial period, preventing excessive radiated heat loss in a cold environment ($+10^{\circ}\text{C}$). From the recorded levels to total energetic output, 2.15 cal/sec, the contribution of the 9-watt fan was subtracted to obtain the indicated figures for the human heat loss.

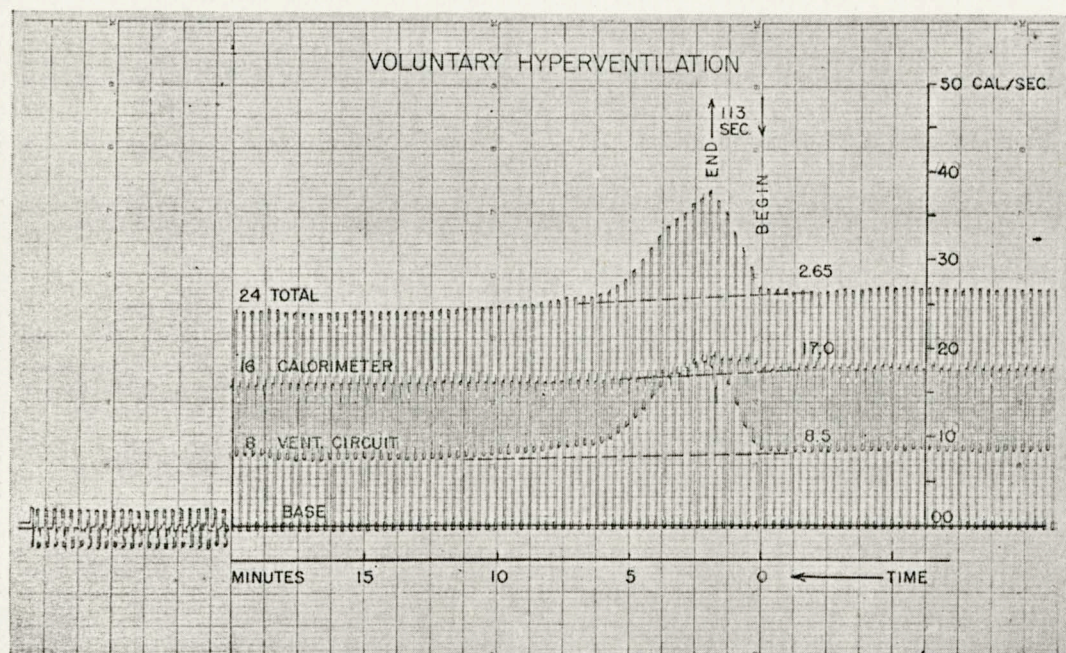


Fig. 17. Heat loss influenced by respiratory hyperventilation. Total heat loss rises sharply with voluntary increase of pulmonary ventilation. Since this heat loss is mainly evaporative, most of it is recorded by the ventilatory circuit.

far observed in our tests. The heat loss was corrected for the output of the fan (2.15 cal/sec).

It appears from the recordings of Figs. 15 and 16 that the calorimeter is capable of adequately recording changes in heat loss that are practically instantaneous. It seems possible, therefore, to observe in a quantitative manner the impact of sudden environmental stress upon the heat reserves of the human body.

Heat Loss During Hyperventilation

Changes in evaporative heat loss are known to occur with a panting dog. We expect these changes also when a human subject voluntarily hyperventilates. This type of heat loss should primarily appear in the ventilatory circuit (see the recording Fig. 17). In the output of the calorimeter proper, only a minor increase is observed. It may be partly caused by conduction-convection of respiratory air to the calorimeter walls in the absence of a respiratory circuit and closed system. The resting level of energetic output decreased during this recording because the body was slowly approaching thermal equilibrium with the environment, 29.5°C

calorimeter temperature. Air flowrate was 800 l/min, relative humidity of the air supplied, 17%. The total heat loss as a consequence of the hyperventilation was 2100 calories. 18.6 calories of excess heat were given off per second of the hyperventilation period, that is, the additional pulmonary loss was nearly equal to a basal human metabolic rate.

Heat Loss in Steady States of Rest or Exercise

In steady states, the advantages of rapid and continuous recording are not as decisive and obvious as they are for swiftly changing physiological responses. Nevertheless, it would seem more difficult to ascertain a steady state and to establish levels of evaporative and total heat losses by single measurements instead of continuous and swift recordings like Fig. 18. Whereas in cool environment, the rates of human heat loss are very steady (Figs. 15 and 16), considerable variation is seen when sweating adds an evaporative contribution. Figure 18 also illustrates the small correction for "dry" heat loss into the ventilatory circuit. (Calorimeter temperature was 30°C, skin temperature 34 to

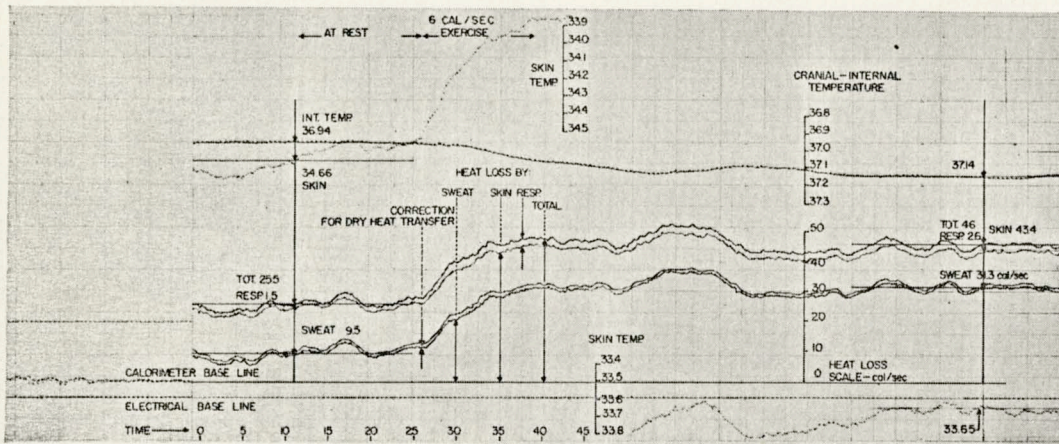


FIG. 18. Steady states of human heat loss at rest and during exercise. After establishing the base lines of zero heat loss (left), the resting rates of heat loss were recorded (from top to bottom): (1) total heat loss, (2) total minus respiratory heat loss, (3) uncorrected output of ventilatory circuit, and (4) rate of evaporative heat loss from the skin or sweating. Subsequently, these recordings were continued in a steady state of exercise with a total caloric output of 46 cal/sec. The increase of sweating from 9.5 to 31.3 cal/sec is commensurate with the increase of cranial internal temperature from 36.94 to 37.14°C (inversely recorded). Skin temperature (inversely recorded) cannot have caused the increase of sweating. It fell from 34.66 to 33.85. With an extended series of similar recordings, plotted on pages 651 to 656 of this book, it was proven that the response of sweating is elicited by central, not peripheral, warm-stimulation.

33°C.) It shows the small and steady contribution to the total by *pulmonary heat loss*, separately recorded with a respiratory circuit. In a sequence from top to bottom, pulmonary heat loss is represented by the narrow band between the uppermost and the second line, radiation-conduction-convection by the wider band between the second and the fourth, and sweating (evaporative) heat loss by the band between the fourth line and the base. Recordings like this were evaluated for Figs. 15 to 21 in the article on "The Human Thermostat," page 637, of this book. They demonstrated that internal, not skin, temperature is the source of the driving sensory impulses for sweating. Attention is, therefore, called in Fig. 18 to the meaningful relation between internal cranial temperature and sweating. Skin temperature is paradoxically related to the rate of sweating: sweating increased while skin temperature was falling from 34.6 to 33.9°C. The conditions for this experiment were the following: 30°C temperature of calorimeter and entering ventilatory air, 20% relative humidity of entering ventilatory air, 30°C temperature of inspired air, and 20% relative humidity of inspired air.

Reduced Heat Loss By Sweating After Ingestion of Ice

While the response of the gradient layer calorimeter to rapid changes in radiated heat loss was illustrated with Figs. 15 and 16, and to pulmonary heat loss with Fig. 17, a last illustration, Fig. 19, has been chosen to illustrate rapid changes in the rate of evaporative heat loss by sweating. After oral ingestion of ice in a hot environment, 45°C, total heat loss fell to zero and then changed sign. This change in total resulted from a decrease of evaporative heat loss from 55.7 to 21.2 cal/sec. The decrease of sweating was a consequence of the drop from 37.36 to 36.95°C in internal cranial temperature (inverted recording). Skin temperature, rising from 36.81 to 36.52, shows a paradoxical change with reference to sweating. The small respiratory contribution was separately measured only at the beginning and at the end. The small correction for "dry" heat loss into the ventilatory circuit is visible. The conditions for this experiment were set at 45°C calorimeter temperature and entering air temperature, 9% humidity in entering ventilatory air, 9% humidity in enter-

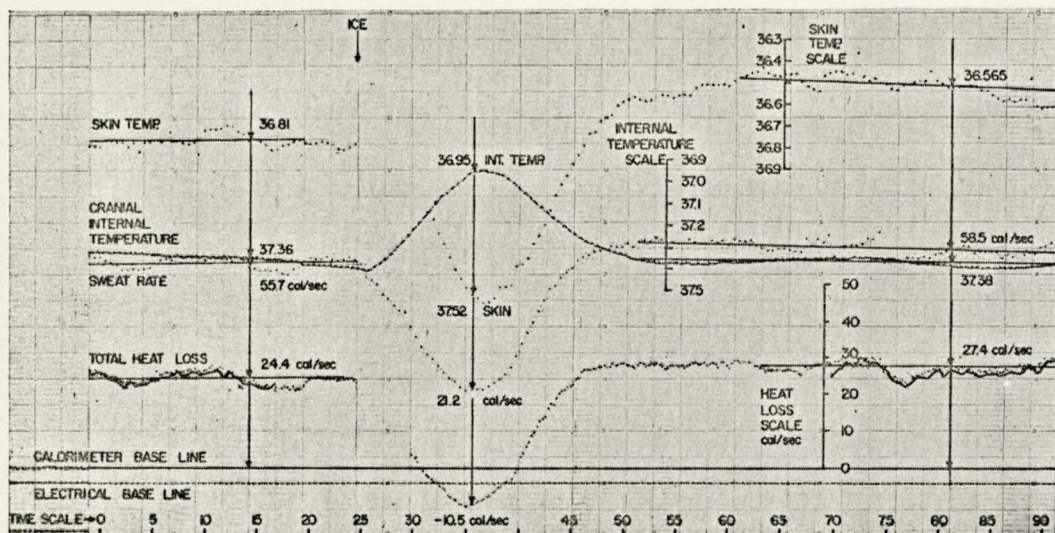


FIG. 19. Rapid changes of evaporative heat loss from the skin after eating of ice. Eating of 450 g of sherbet ice produces a drop of cranial internal temperature from 37.36 to 39.95° (inversely recorded). As a consequence, sweating rate falls from 55.7 to 21.2 cal/sec. Total heat loss, which had been leveled at 24.4 cal/sec before ice eating, falls rapidly, reaches zero, and becomes negative, a heat gain of 10.5 cal/sec. With the reduction of sweating, skin temperature in this hot environment (45°C) rises from 36.81 to 37.52° (inversely recorded). The gradient layer method permitted the recording of these transient changes and their interpretation: sweating rate is determined by cranial internal, not skin temperature. (At the beginning and end of this record, pulmonary heat loss was recorded as a small difference between the dotted and solid lines.)

ing respiratory air, and 45°C respiratory air temperature.

The experiment of Fig. 19 is one of many demonstrating the dominant role of the internal (not the cutaneous) thermoreceptive system for temperature regulation in warm environment. To clarify these quantitative relations would have been difficult without the gradient layer method. Additional examples of recordings are shown elsewhere in this book.

SUMMARY

The principle of gradient layer calorimetry permits the precise, direct, continuous, and rapidly responding measurement of heat loss in man or animals. It permits the partition of respiratory, cutaneous-evaporative, and dry (radiated and conducted-convected) heat losses. A wide range of physiological conditions may be covered in layer-calorimetric studies. The rapidity of response in gradient layer calorimetry is based upon the high thermal conductivity of thin gradient layers of solid matter. Sensitive voltage response is obtained by a large number

of thermoelectric junctions in serial wiring. The precision of the instrument rests upon the geometry of the blackbody, the uniform design and construction of the layer, and the complete and uniform distribution of a large number of individual gradient measuring sites. A description is given of the human layer calorimeter of the Naval Medical Research Institute at Bethesda. Its performance characteristics, particularly speed of response and precision, are documented with recordings. Examples of experiments with human subjects are presented. The response characteristics are illustrated by instances of thermal stress with rapid changes of radiated, convected, cutaneous evaporative, or respiratory loss of heat in man.

Acknowledgment

For Figures 2, 3, 6 and 9 and for major parts of the paragraphs "Calorimeter Shell and Subject Support" and "Temperature Control of the Calorimeter Shell" the authors are indebted to Mr. R. G. Huebscher, co-author of reference 22 and project engineer in charge of Human Calorimetry at the Research Laboratory of the American Society of Heating and Ventilation Engineers, 1949 to 1959.

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Income you didn't see this - thought you would be interested - here are things?

If They Can be Taught Hockey, Well—

Space-Flying Bears Make Good Sense to Mayo MD

From a Midwest Correspondent

ROCHESTER, Minn. — Send a bear, not a man, to Mars, suggests a Mayo Clinic specialist.

Why not, asks Ralph A. Nelson, MD, of the medical group's section of nutrition and department of physiology and biophysics? He explains:

"Room for food and water in the space capsule is not a problem for short-term flights to the moon. Space flights lasting two or three months, however, for which there is no hope of obtaining fuel and supplies from other spacecraft, will present a problem in the storage of supplies to meet food and water requirements.

"From what is now known about the bear, it seems that this beast would be best adapted for such a flight if this were performed during the winter months and if the ambient temperature in the capsule were similar to that of an ordinary cold room — five degrees (C) below zero, precautions being taken to maintain a certain level of air humidity."

Dr. Nelson regards the black bear, *Ursus americanus* as "truly ruler of the animal kingdom," able to defend itself against any foe except man, in either summer or winter. It is willing to eat almost anything, and has a "strange ability" to be independent of food and water intake for three to five months of the year while in a state of winter sleep, yet at the same time to arouse itself quickly into a mobile, reactive state, aware of its surroundings.

"Because the black bear can do all of this without urinating or defecating," Dr. Nelson writes in *Mayo Clinic Proceedings*, "it becomes a unique animal model for study and comparison with human beings in regard to problems of nutrition — such as medical management of chronic renal failure and long-term flights in space."

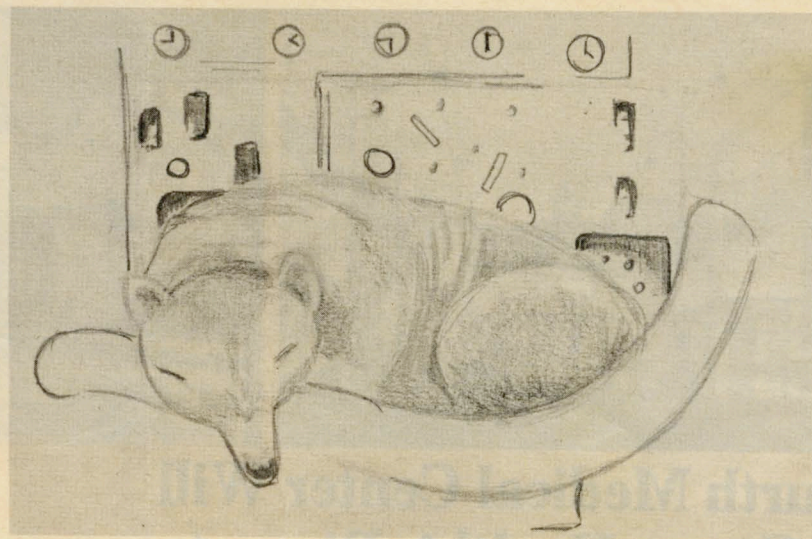
Heart rate during these sleeps drops to as low as eight beats per minute, compared with 40 bpm recorded in its sleep during summer. These and related findings already have led to refinements in hemodialysis for humans:

"Control of human water balance was achieved by matching water in food and water of metabolism with the ability of the anephric subject to lose water through insensible pathways. To satisfy daily protein requirements, sufficient protein was fed to meet obligatory needs while Kcalorie requirements were met by ingestion of fat and carbohydrate.

"With this method, the need for hemodialysis was postponed for 10 days. At the end of this period — equivalent to a period with urination only once in 10 days — the patients had remained ambulatory, had drunk no water except for small sips for medication, and were in good clinical condition."

That bear-inspired approach, says Dr. Nelson, offers a realistic and economical program for patients taking short vacations

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RESPONSES OF SOME HEMATOLOGIC PARAMETERS OF ACTIVE AND HIBERNATING SQUIRRELS (*SPERMOPHILUS MEXICANUS*) UPON EXPOSURE TO HYPOBARIC AND ISOBARIC HYPEROXIA*

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(Received 13 March 1972)

Abstract—1. Hibernation in ground squirrels (*Spermophilus mexicanus*) results in: decreased RBC count, decreased WBC count, increased plasma LDH levels and increased RBC LDH levels.

2. Hibernation in ground squirrels along with hyperoxia compared to hibernation alone results in: decreased hematocrit, decreased hemoglobin, decreased total RBC LDH levels, increased RBC G-3-P-DH levels and increased RBC GSH levels.

INTRODUCTION

THE ONLY known physiologic situation wherein the life span of the red blood cell (RBC) increases is in hibernating animals. In some of these animals, the increase is related to the length of hibernation. In the American marmot with a mean RBC life span of 36 days, hibernation of 35 or 82 days resulted in a mean RBC life span of 65 or 112 days respectively (Brace, 1953). It is unclear to what extent this prolongation of RBC life span is dependent upon temperature and alteration of erythrocyte metabolism (Brock, 1960). It has been noted that cooling is protective of mammals in hypoxic environments although there is marked species variation (Bullard *et al.*, 1960). No information is available on the interesting effects of cooling and hyperoxia on hematopoietic tissue.

The work of Mengel and others has shown the marked effects of hyperoxia upon mammalian erythrocytes and the modification of such effects by antioxidants (Mengel, 1968). Little information is available about the effects of hyperoxia upon hibernators and their hematopoiesis. Further, the cumulative effects of the temperature changes in hibernation along with effects of hyperoxia and hypobaria upon red cell count, the red cell population, the levels of protective antioxidants such as RBC glutathione and upon RBC glycolytic enzymes (especially those containing sulfhydryl (SH) groups such as lactic dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase), are unknown.

* This study was supported by the Laboratory Director's Fund, United States Air Force, School of Aerospace Medicine, Aerospace Medical Division, Brooks Air Force Base, Texas.

Because of its inability to dedifferentiate, its limited metabolic capacities, and its finite life span, the mature erythrocyte offers a unique opportunity to examine the effects of hyperoxia and hypobaria upon cellular function (Danon, 1966; Bernstein, 1959). For these reasons, the following studies were done.

MATERIALS AND METHODS

Animals and environmental conditions

Mature Mexican ground squirrels (*Spermophilus mexicanus*) were used in this study. Active animals were housed in a vivarium at ambient temperatures of $22 \pm 2^\circ\text{C}$ and relative humidity of $50 \pm 10\%$. All animals in which hibernation was induced were placed in an environmentally controlled hibernaculum and exposed to ambient temperatures of $4 \pm 1^\circ\text{C}$ and a relative humidity of $50 \pm 10\%$. The light to dark cycle in the vivarium was 9 and 15 hr respectively and in the hibernaculum, 6 and 18 hr respectively. Ground squirrels in the hibernation groups were allowed to hibernate for at least 14 days before any studies were done. Data from those animals either entering or arousing from hibernation were not included in the results.

Animals to be exposed to hyperoxic environments were removed from the vivarium or the hibernaculum to a hypo-hyperbaric chamber (Model 1836, Bethlehem Corporation, Bethlehem, Penn.). Humidity in this chamber was maintained at about 50 per cent. Duration of exposure for all groups of animals was 96 hr. The atmospheric conditions used are noted below (Table 1).

TABLE 1—ENVIRONMENTAL CONDITIONS

No. of animals	State	Temperature ($^\circ\text{C}$)	$p\text{O}_2$ (mm Hg)	$p\text{B}$ (mm Hg)
22	Active	22	152 ± 5	760
15	Active	22	760 ± 5	760
6	Active	22	460 ± 5	460
6	Active	22	152 ± 5	460
15	Hibernating	4	152 ± 5	760
15	Hibernating	4	760 ± 5	760
6	Hibernating	4	460 ± 5	460

The mean values for the environmental parameters, including the atmospheric pressure ($p\text{B}$), which were maintained in the chamber during the studies are shown. The partial pressures of oxygen ($p\text{O}_2$) in mm of mercury (mm Hg) are shown with their standard deviations.

O_2 levels were determined at least four times daily on gas samples extracted from the exposure chamber.

Blood count and glutathione

Blood was taken by cardiac puncture from unanesthetized animals. Hematocrit, hemoglobin and white blood cell count were performed using standard laboratory procedures. Total red blood cell counts were done in duplicate with a Model B Coulter counter. Reduced glutathione levels of whole blood and red blood cells and glutathione stabilities were measured by a modification of the method of Buetler *et al.* (1963).

Lactic dehydrogenase

Total lactic dehydrogenase (LDH) and LDH isoenzyme activity were measured in the plasma and red blood cell hemolysate of each animal respectively. Total LDH activity was determined by utilizing the Lac-dehystrate kit (General Diagnostics, Morris Plains, N.J.). LDH units are expressed in international units. The isoenzymes of LDH were measured utilizing the Microenzyme Cellulose Acetate System (Fisher *et al.*, 1967; Beals, 1969).

LDH assays were not done upon animals at 460 mm Hg.

Glyceraldehyde-3-phosphate dehydrogenase

Total glyceraldehyde-3-phosphate dehydrogenase (G-3-P-DH) activity was measured from extracts of red cell hemolysates by a modification of the method of Schrier (1966). The assay method depends upon the spectrophotometric change noted at 340 m μ as nicotinamide adenine dinucleotide (NAD) is converted to reduced nicotinamide adenine dinucleotide (NADH) and as D-glyceraldehyde-3-PO₄ is converted to 3-phosphoglycerate.

Density distribution

Density distribution of the red blood cell populations was performed by a modification of the method of Danon & Marikovsky in which the cells are separated according to their density using mixtures of methyl phthalate and di-*n*-butyl phthalate as separating fluids (Danon & Marikovsky, 1964).

Statistics

The study was primarily a two factorial study: (1) the effects of hibernation; (2) the effects of O₂; and (3) the interactions of O₂ and hibernation.

A paired *t*-test was performed for each parameter measured on the mean values between animal groups. In addition, an analysis of variance was performed on the data for the following parameters: sex, temperature, atmosphere, sex vs. temperature, sex vs. atmosphere, atmosphere vs. temperature, sex vs. temperature and atmosphere.

RESULTS

Values for hematocrit (Hct.), hemoglobin (H) and red blood cell (RBC) count with their standard deviations are shown in Table 2. No statistically significant

TABLE 2—EFFECTS OF HYPOBARIC AND ISOBARIC HYPEROXIA

	pB	pO_2	Hct.	H	RBC
Active animals	760	152	44.3 $\pm$ 7.93	14.5 $\pm$ 2.34	8.69 $\pm$ 1.61
Hibernators	760	152	44.0 $\pm$ 7.39	14.0 $\pm$ 2.00	6.66 $\pm$ 3.42
Active animals	460	460	44.7 $\pm$ 7.40	13.7 $\pm$ 2.21	8.90 $\pm$ 1.5
Hibernators	460	460	40.9 $\pm$ 5.57	12.9 $\pm$ 1.59	6.26 $\pm$ 3.19
Active animals	760	760	45.8 $\pm$ 6.61	14.8 $\pm$ 1.33	7.86 $\pm$ 2.42
Hibernators	760	760	42.2 $\pm$ 7.14	13.1 $\pm$ 1.91	6.28 $\pm$ 1.72
Active animals	460	152	47.3 $\pm$ 4.33	14.9 $\pm$ 1.46	8.35 $\pm$ 0.83

The mean values for the percentage hematocrit (Hct.), hemoglobin (H), and red blood cell count (RBC) with their standard deviations are shown. Hemoglobin is expressed in g per 100 ml of whole blood and the red blood cell count in millions of red cells per mm³ of whole blood. pB , total atmospheric pressure; pO_2 = partial pressure of oxygen.

differences in hematocrit or hemoglobin appear between active animals and hibernators at 760 mm Hg with 152 mm Hg O₂. There is a slight decrease in the hematocrit and hemoglobin of the hibernators compared to normal animals under 100% oxygen at 760 and 460 mm Hg ($P < 0.05$). Hibernators have a slightly decreased RBC count at 760 mm Hg with 152 mm Hg O₂ compared to their normal controls ($P < 0.05$). The hibernators at 460 and at 760 mm Hg with 100% O₂ have a decreased RBC count ($P < 0.05$) compared to the normal animals at 760 mm Hg with 100% O₂, 760 mm Hg with 152 mm Hg O₂, and 460 mm Hg with 152 mm Hg O₂. Table 3 lists the mean RBC indices and reticulocyte counts with their standard deviations for each group of animals.

TABLE 3—EFFECTS OF HYPOBARIC AND ISOBARIC HYPEROXIA

	pB	pO_2	MCV	MCHC	Reticulocytes (%)
Active animals	760	152	51.6 ± 8.10	33.0 ± 2.58	0.50 ± 0.22
Hibernators	760	152	66.0 ± 8.71	32.6 ± 2.93	0.43 ± 0.20
Active animals	460	460	55.5 ± 8.00	31.6 ± 1.67	0.59 ± 0.31
Hibernators	460	460	50.2 ± 2.64	30.6 ± 1.10	0.43 ± 0.20
Active animals	760	760	62.6 ± 16.5	32.5 ± 3.23	0.59 ± 0.24
Hibernators	760	760	52.2 ± 8.30	31.1 ± 1.49	0.32 ± 0.16
Active animals	460	152	56.7 ± 3.50	31.6 ± 0.87	0.56 ± 0.15

The mean values for red cell mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and reticulocyte count with their standard deviations are shown. The MCV is expressed in μ^3 . The MCHC and the reticulocyte count are expressed as a percentage. pB , total atmospheric pressure; pO_2 , partial pressure of oxygen.

The mean corpuscular volume (MCV) of hibernators at 760 mm Hg with 152 mm Hg O₂ is different from all other hibernating groups ($P < 0.05$) and from normal animals at 760 mm Hg with 152 mm Hg O₂ ($P < 0.05$).

The white blood cell (WBC) count of the hibernating animals fell dramatically to about 10 per cent of the active animal count as shown in Table 4. This dramatic decrease ($P < 0.001$) was an accurate index of the animals' depth of hibernation. Ground squirrels either entering or arousing from hibernation had significantly higher values than those in deep hibernation. Active animals exposed to hyperoxia had a significantly lower WBC than their controls ($P < 0.05$). Active animals exposed to normoxic hypobaria also had lower WBC than their controls.

No sex differences were noted in any of the RBC or WBC parameters measured.

The mean values with their standard deviations for plasma and RBC total LDH levels are shown in Table 5.

The mean values with their standard deviations for plasma LDH isoenzyme and RBC LDH isoenzyme levels are shown in Tables 6 and 7 respectively.

TABLE 4—EFFECTS OF HYPOBARIC AND ISOBARIC HYPEROXIA UPON THE WHITE BLOOD CELL COUNT

	pB	pO ₂	WBC
Active animals	760	152	7605 ± 1749
Hibernators	760	152	500 ± 205
Active animals	460	460	3867 ± 726
Hibernators	460	460	886 ± 434
Active animals	760	760	5566 ± 902
Hibernators	760	760	410 ± 179
Active	460	152	5740 ± 1479

The mean values for the white blood cell count (WBC) with their standard deviations are shown. The white blood cell count is expressed in thousands of cells per mm³ of whole blood.

TABLE 5—HYPEROXIC EFFECT UPON TOTAL PLASMA LDH LEVELS

	Active		Hibernating	
	Normoxic	Hyperoxic	Normoxic	Hyperoxic
Group mean	115.14	101.20	213.94	238.25
S.D.	53.68	13.60	59.34	67.61

HYPEROXIC EFFECT UPON TOTAL RBC HEMOLYSATE LDH LEVELS

	Active		Hibernating	
	Normoxic	Hyperoxic	Normoxic	Hyperoxic
Group mean	1815	2452	4983	2413
S.D.	719	999	1547	664

The mean values for total plasma and RBC hemolysate LDH levels are shown with their standard deviations (S.D.). The LDH values are expressed in international units (one unit equals that amount of enzyme that will convert 1 μ mole of substrate per min at 25°C) per milliliter (ml) of plasma and packed red blood cells respectively.

The statistical analysis with the paired *t*-test of the LDH data showed the following:

1. Plasma total LDH. The plasma from hibernators had more enzyme activity than plasma from active animals ($P < 0.01$). Oxygen exposure did not result in any appreciable change.

2. Plasma LDH-1. Active animals exposed to O₂ for 96 hr had a greater percentage of LDH-1 than hibernating animals with like exposure ($P < 0.02$).

TABLE 6—HYPEROXIC EFFECT UPON PLASMA LDH ISOENZYME LEVELS

	Active animals									
	Normoxic					Hyperoxic				
	1	2	3	4	5	1	2	3	4	5
Isoenzyme										
Group mean	29.00	13.29	18.86	23.29	15.43	36.80	16.80	14.20	22.00	11.20
S.D.	7.14	5.62	7.86	6.97	12.38	10.71	3.70	2.77	9.62	5.89

	Hibernating animals									
	Normoxic					Hyperoxic				
	1	2	3	4	5	1	2	3	4	5
Isoenzyme										
Group mean	23.77	19.22	21.22	21.56	14.56	22.78	19.75	22.88	20.75	13.25
S.D.	4.92	3.38	4.47	5.10	6.06	3.11	2.77	2.85	3.96	5.29

The mean values for plasma LDH isoenzyme levels are shown with their standard deviations (S.D.). The isoenzyme values are expressed as a relative percentage.

TABLE 7—HYPEROXIC EFFECT UPON RBC HEMOLYSATE LDH ISOENZYME LEVELS

	Active animals									
	Normoxic					Hyperoxic				
	1	2	3	4	5	1	2	3	4	5
Isoenzyme										
Group mean	5.57	14.57	31.71	37.29	10.29	7.20	18.40	34.60	35.60	4.80
S.D.	7.28	7.41	3.73	8.94	8.56	4.32	6.03	2.70	7.67	5.07

	Hibernating animals									
	Normoxic					Hyperoxic				
	1	2	3	4	5	1	2	3	4	5
Isoenzyme										
Group mean	5.73	13.00	27.91	39.64	13.91	6.17	14.33	28.33	34.67	17.00
S.D.	2.49	4.17	3.62	3.29	7.67	5.04	4.76	2.66	2.25	8.70

The mean values for RBC hemolysate isoenzyme levels are shown with their standard deviations (S.D.). The isoenzyme values are expressed as a relative percentage.

3. Plasma LDH-2. Hibernating controls had a higher percentage of LDH-2 than did the active controls ($P < 0.05$).

4. Plasma LDH-3. Hibernating animals exposed to O_2 for 96 hr had a greater percentage of LDH-3 than did active animals with like exposure ($P < 0.01$).

5. Hemolysate total LDH. Hibernating controls had greater enzyme activity than did either active controls ($P < 0.01$) or hibernators with 96 hr O_2 exposure ($P < 0.01$).

6. Hemolysate LDH-3. Active animals had higher percentages of LDH-3 than did hibernating squirrels ($P < 0.05$).

7. Hemolysate LDH-4. The percentage of LDH-4 was greater in the hibernating controls than in the hibernators exposed to 96 hr of O_2 ($P < 0.01$).

8. Hemolysate LDH-5. Hibernating animals exposed to 96 hr of O_2 had a greater percentage of LDH-5 than did active squirrels with like exposure ($P < 0.02$).

Multifactorial analysis of variance confirmed the t -test results and, in addition, indicated that some sex differences existed.

1. Plasma LDH-1. Active females (F) with 96 hr O_2 exposure had a higher percentage of LDH-1 than did males (M) with like exposure ($P < 0.05$).

2. Plasma LDH-2. F had higher percentage LDH-2 than M ($P < 0.025$).

3. Plasma LDH-4. M had higher percentage LDH-4 than F ($P < 0.01$).

4. Hemolysate LDH-1. Active F had greater percentage LDH-1 than did active M ($P < 0.01$).

5. Hemolysate LDH-4. Active M controls had a greater percentage of LDH-4 than did active F controls ($P < 0.005$).

6. Plasma LDH-1. Active F with 96 hr O_2 exposure had a higher percentage of LDH-1 than did hibernating F ($P < 0.05$) or active controls ($P < 0.05$).

7. Hemolysate LDH-3. Active F with 96 hr O_2 exposure had a greater percentage of LDH-3 than did either active F controls ($P < 0.05$) or hibernating O_2 exposed F ($P < 0.05$). Also, active M controls had higher values than did hibernating M controls ($P < 0.05$).

The results of the red blood cell glyceraldehyde-3-phosphate dehydrogenase content in active and hibernating squirrels is shown in Table 8. Active animals and hibernating animals at normal atmospheric conditions have a lower level of

TABLE 8—HYPEROXIC EFFECTS UPON RBC GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (G-3-P-DH) LEVELS

Animal state	pB	pO ₂	G-3-P-DH
Active	760	152	1868 ± 357
Hibernating	760	152	1732 ± 201
Active	460	460	2708 ± 402
Hibernating	460	460	1791 ± 320
Active	760	760	2088 ± 445
Hibernating	760	760	2286 ± 138

The mean values for total RBC hemolysate glyceraldehyde-3-phosphate dehydrogenase levels are shown with their standard deviations. The G-3-P-DH levels are expressed in international units per ml of packed red blood cells with their standard deviation (S.D.).

glyceraldehyde-3-phosphate dehydrogenase than do hibernating animals exposed to 100% O₂ at 760 mm Hg ($P < 0.05$ and $P < 0.01$ respectively). Active animals exposed to 100% O₂ at 460 mm Hg have higher enzyme levels than hibernators so exposed ($P < 0.05$).

The glutathione levels (both pre and post incubation) obtained for all groups are shown in Table 9. Statistical evaluation indicates no effect of 96 hr of hyperoxia on normal animals, but there is a significant effect upon hibernators ($P < 0.05$).

TABLE 9—EFFECT OF HYPOBARIC AND ISOBARIC HYPEROXIA UPON GROUND SQUIRRELS

		Glutathione levels					
		Pre-incubation			Post-incubation		
		mg GSH		mg GSH		mg GSH	
Animal state	pB	pO ₂	100 ml whole blood	100% RBC	100 ml whole blood	100% RBC	
Hibernating (4°C)	760	152	33.28 ± 11.51	81.39 ± 23.74	34.98 ± 5.92	87.11 ± 16.75	
Hibernating (4°C)	760	760	44.20 ± 12.21	103.58 ± 21.07	47.12 ± 12.71	108.57 ± 19.47	
Hibernating (4°C)	460	460	39.81 ± 4.39	101.19 ± 14.06	42.74 ± 5.59	107.44 ± 11.86	
Active (22°C)	760	152	65.15 ± 9.71	146.95 ± 12.69	65.30 ± 10.10	148.10 ± 13.51	
Active (22°C)	760	760	63.07 ± 8.21	137.03 ± 21.18	65.46 ± 6.94	141.83 ± 16.21	
Active (22°C)	460	460	71.33 ± 5.07	162.63 ± 24	67.29 ± 5.75	153.33 ± 23.33	
Active (22°C)	460	152	61.33 ± 12.30	129.45 ± 21.07	63.23 ± 12.25	134.31 ± 22.19	

The mean values for reduced glutathione (GSH) with their standard deviations are shown. The values are expressed as mg of reduced glutathione (mg GSH) per 100 ml (ml) of whole blood and packed red blood cells.

There is an atmospheric pressure effect upon normal animals but not upon hibernators ($P < 0.05$). There is also a temperature effect ($P < 0.01$) noted for all animals. No sex group differences were noted in any glutathione levels measured.

Results of the RBC density distribution measurements are summarized in Table 10. The percentages are mean values for all animals in a given group. Percentages of the RBC populations are given at four of the twenty density levels measured: 1.114 specific gravity (S.G.), 1.106 S.G., 1.098 S.G. and 1.090 S.G. In all cases the mean percentage of cells appearing in the "oldest" fraction (density greater than 1.114 S.G.) was less than 10 per cent. Eighty-four to 97 per cent of all mean populations fell within these limits (S.G. 1.090–1.114). With two exceptions, all comparisons show the RBC population of hibernators to be significantly

TABLE 10—EFFECTS OF HYPOBARIC AND ISOBARIC HYPEROXIA ON RBC-DENSITY DISTRIBUTIONS

Animal state	pB	pO ₂	Density levels			
			1.114 S.G.	1.106 S.G.	1.098 S.G.	1.090 S.G.
			% ≥ S.D.	% ≥ S.D.	% ≥ S.D.	% ≥ S.D.
Active	760	152	5.90 ± 8.89	40.12 ± 27.52	83.44 ± 16.27	96.46 ± 2.46
Hibernating	760	152	6.74 ± 1.10	27.91 ± 24.50	68.73 ± 13.77	90.94 ± 4.28
Active	460	152	2.87 ± 1.06	54.70 ± 14.60	94.00 ± 36.51	99.36 ± 0.27
Active	460	460	3.42 ± 1.83	49.48 ± 22.66	91.80 ± 8.59	99.07 ± 0.38
Hibernating	460	460	9.30 ± 5.47	47.16 ± 13.06	83.39 ± 7.50	95.43 ± 2.92
Active	760	760	3.15 ± 2.13	31.54 ± 24.98	82.80 ± 14.44	97.36 ± 2.11
Hibernating	760	760	2.24 ± 1.33	30.13 ± 14.19	71.46 ± 18.09	91.52 ± 2.34

The mean percentages of the RBC populations with their standard deviation (S.D.) are given at four of the density levels measured: 1.114 specific gravity (S.G.), 1.106 S.G., 1.098 S.G. and 1.090 S.G. The mean percentage represents those cells of greater density than or density equal to ($\geq$) the density of a given phthalate oil.

less dense than those of active animals ($P < 0.05$). The two exceptions are seen at 1.114 S.G. under exposure to 760 mm Hg with 152 mm Hg O₂ and at 1.114 S.G. under exposure to 460 mm Hg with 100% O₂. Under these two conditions, hibernators have a percentage of dense cells which is greater than those of corresponding active animals.

The overall effect of all abnormal atmospheres on RBC density distributions with one exception was a shift toward higher densities when compared with the distribution in corresponding control groups. In active animals at 760 mm Hg with 100% O₂, there was a slight shift of the RBC population toward lower densities than that shown by the active control mean population.

Differences between experimental and control groups were most striking under hypobaric conditions at 460 mm Hg. The largest differences appear when the mean density distribution of hibernators at 460 mm Hg with 100% O₂ is compared with the control group of hibernators at 760 mm Hg with 152 mm Hg O₂.

DISCUSSION

The most striking change in standard RBC parameters measured was the decreased RBC count of hibernating animals compared to the active animals. This is in contrast to data presented for other hibernating species (Brock, 1960). Hyperoxia resulted in a decrease in hematocrit, hemoglobin and RBC of hibernators.

However, as no plasma volume or RBC mass measurements were made, no statement can be made regarding the total red cell mass.

Different tissues have different LDH isoenzyme composition and within any one species, the isoenzyme pattern for a given tissue is characteristic. In most mammalian species, erythrocytes contain all five isoenzymes on maturation. Members of the ground squirrel family (*Spermophilus*) have multiple LDH isoenzymes in the RBC hemolysates (Baur *et al.*, 1968). *S. mexicanus* has five LDH isoenzymes which act electrophoretically similarly to humans under the conditions cited.

Plasma elevation of LDH levels is associated with injury of organs such as RBC hemolysis. A rise in LDH plasma levels may be characterized frequently by tissue-specific isoenzyme patterns. Hibernation resulted in a significant increase in total LDH plasma levels in Mexican ground squirrels. The hibernation state also resulted in the relative elevation of plasma LDH-2 levels compared to other isoenzymes. The controlling mechanisms for the plasma LDH levels during hibernation are unknown and only limited information is available. In thirteen lined ground squirrels (*S. tridecemlineatus*), hibernation causes increased plasma total LDH and increased percentage of LDH-5 in cardiac muscle, liver and brain. The total LDH in skeletal muscle decreases with hibernation. Burlington (1968) suggests that these changes are not due to increased enzyme production, but rather to "conformation changes in the enzyme molecule resulting from fluctuation of activators or inhibitors (effectors) during the hibernation cycle."

Some studies of lactate metabolism indicate that hibernation has little or no effect on the enzyme system as a whole. Plasma, liver, brain, cardiac muscle and skeletal muscle lactate concentrations did not change significantly during hibernation in hedgehogs (*Marmota* species) or in golden mantled ground squirrels (*S. lateralis*) (Hanson *et al.*, 1961; Twente *et al.*, 1968). These studies were interpreted to mean that the hibernating animal was not in a hypoxic state. Zimny (1955), however, reports that in hibernating thirteen lined ground squirrels tissue lactate and pyruvate decreased 80 and 50 per cent respectively, suggesting significant changes in the enzyme system.

Elevated plasma LDH values in the Mexican ground squirrel could be due to: (1) increased LDH release from various tissues as a result of the hibernation state and/or of tissue injury, (2) decreased clearance rate of one or more LDH isoenzymes from the plasma or (3) the amount of free enzyme available for analysis. Clearly, some of the plasma LDH of the animals exposed to hyperoxia must have been due to tissue damage. Histological examination of the tissues showed damage to lung tissue of both hibernating and active animals under all hyperoxic conditions (E. C. Larkin & R. Simmonds, unpublished observations). A decreased clearance rate of LDH from the plasma of hibernators at 4°C compared to the active animals may also contribute to the higher levels of total LDH in the plasma of hibernators. Clearance rates for human LDH are progressively faster for the more cathodal LDH isoenzymes such as LDH-5, and parallel findings have been noted for animals (Boyd, 1967a, b). There was no evidence of electrophoretic polymorphism analogous to humans in any of the animals examined (Vesell, 1965).

In normoxic hibernators, the total LDH activity of the RBC hemolysates was greater than that of normoxic active animals. LDH-3 levels in normoxic hibernators were slightly lower than in normoxic active animals.

In human RBC, the total LDH levels drop slightly with aging of the cell population, the percentages of LDH-5 (4M) and LDH-4 decreases with age while the percentages of LDH-1 (4H) and LDH-2 increases in the red cell (Vesell, 1962; Starkweather, 1965). There is an increased mean age of the red cell with hibernation, but the concomitant drop in temperature with this state may result in decreased catabolism of LDH enzymes. The result of a decreased loss of RBC LDH enzyme in hibernators compared to loss of the enzyme in red cell at normal temperature could account for the increased total LDH activity.

It has been speculated by Kaplan and others that the variety of LDH isoenzymes suggests that given enzyme species arise in different tissues or organisms which are most advantageous with a given set of metabolic conditions (Cahn, 1962). A change in the metabolic parameters induced by hibernation in a tissue or tissues (e.g. pH, ionic strength, substrate levels, product inhibition or the rate of synthesis of M-type or H-type polypeptide chains) could account for decreased levels of RBC LDH-3, decreased plasma LDH-2 in normoxic hibernators and some of the differences in LDH isoenzymes found between the sexes (e.g. the fact that active female squirrels had higher LDH-1 percentages than active male squirrels) (Stein, 1970).

Hibernators exposed to oxygen had a decrease of their total RBC LDH activity compared to hibernators not so exposed; active animals exposed to oxygen had no such decrease. Hyperoxia had no effect upon the total RBC glyceraldehyde-3- PO_4 -dehydrogenase levels in active squirrels. The levels of isobaric and hypobaric hyperoxia used may not have been enough to cause a significant depression in the enzyme activity. If hyperoxic effects at 460 and 760 mm Hg upon the flavoprotein enzymes examined are reversible, such effects could be either blunted or destroyed in the *in vitro* assays which are conducted under normoxic conditions. Hypobaric hyperoxia resulted in active animals with a higher RBC glyceraldehyde-3- PO_4 -dehydrogenase level compared to hibernators so exposed. The differences in the effects of hypobaric hyperoxia (460 mm Hg at 100% O_2) on the RBC glyceraldehyde-3- PO_4 -dehydrogenase of active and hibernating animals may be a reflection of the greater oxygen consumption of the active animals. Increased hyperoxic exposure (760 mm Hg at 100% O_2) results in increased RBC glyceraldehyde-3- PO_4 -dehydrogenase activity in hibernators compared to hibernators at normal atmosphere. The differences noted at 460 mm Hg at 100% O_2 between hibernators and active animal enzyme levels are ablated.

It has been shown that the effect of oxygen on several important enzymes containing thiol groups differ considerably (Dickens, 1962). In the intact animal, the effects of hyperbaric hyperoxia have been shown to be reversible (Riggs, 1945). The levels of intracellular heavy metals such as iron or copper can also influence the rate at which SH containing enzymes as well as unsaturated fatty acids are oxidized (Michaelis, 1929; Gerschman *et al.*, 1958; Mengel, 1968). Compounds

such as glutathione have been shown to reverse the depression of glyceraldehyde-3- PO_4 -dehydrogenase activity in tissue homogenates (Horn *et al.*, 1965; Horn *et al.*, 1967). It has been pointed out by Webb among others that the degree to which different SH groups, even on the same protein, react to oxidizing agents varies markedly (Webb, 1966).

It has been suggested that the bulk of glutathione oxidized in the human red cell is oxidized mainly by reacting directly with oxygen itself (Jocelyn, 1960). Further, it appears that reduced glutathione (GSH) protects the SH groups of red cell enzymes and hemoglobin from damage resulting from the generation of intracellular hydrogen peroxide (Jacob & Jandl, 1966). There was a significantly reduced level of glutathione in hibernators. This could be a result of increased oxidative assault upon the RBC of the hibernators or due to decreased activity of the enzymes present in the erythrocytes responsible for the synthesis of GSH (possibly secondary to a temperature effect).

That the temperature effect alone or along with some other influences on the activity of enzymes synthesizing GSH could account for low GSH levels is supported by the fact that there was no effect of hyperoxia upon the RBC GSH levels of active animals. There was no evidence in RBC or whole blood of any GSH instability upon incubation in any group of animals. Further, *in vitro* studies have shown that the rate of glutathione oxidation is reduced with a lowering of the temperature (Jocelyn, 1960). But the RBC GSH levels of hibernators were significantly raised in response to hyperoxia which suggests that the cells may have been responding to oxidant stress.

Normoxic active animals at 760 mm Hg had a significantly higher level of RBC GSH compared to normoxic active animals at 460 mm Hg. This difference could indicate an effect of total atmospheric pressure alone on glutathione metabolism. A significant difference of RBC density populations were also noted between these two animal groups; therefore, the differences in RBC GSH levels could also reflect these differences in the RBC population.

SUMMARY

1. Hibernating ground squirrels (*S. mexicanus*) showed a significant decrease in hematocrit, hemoglobin and RBC count upon exposure to hypobaric and isobaric hyperoxia.
2. Hibernators showed a significant decrease in RBC count compared to active animals in a normoxic environment.
3. The WBC count of hibernators was about 10 per cent of that of the active animals. Active animals exposed to hyperoxia had a significantly lower WBC count than active animals under normoxic conditions.
4. RBC and plasma total LDH activity increases with hibernation.
5. RBC and plasma total LDH activity is unaffected in active Mexican ground squirrels exposed to hyperoxia.
6. RBC total LDH activity was decreased in hibernating Mexican ground squirrels exposed to hyperoxia compared to hibernators not so exposed.

7. Isobaric hyperoxia results in an increase in the RBC glyceraldehyde-3-phosphate-dehydrogenase activity of hibernators compared to hibernators at a normal atmosphere.

8. RBC GSH levels were increased in hibernators exposed to hyperoxia compared to unexposed hibernators.

9. RBC GSH levels were increased in active animals exposed to normoxic hypobaria (460 mm Hg total atmospheric pressure, 152 mm Hg oxygen partial pressure).

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Key Word Index—Ground squirrels; hibernation; hypobaric hyperoxia; isobaric hyperoxia; *Spermophilus mexicanus*; LDH in blood; G-3-P-DH; GSH.

Hypersensitivity Angiitis in a Dormant Black Bear (*Ursus americanus*)

Richard C. Simmonds, D.V.M., M.S., and Charles L. Wells, M.D.

SUMMARY

A captive, 1-year-old female black bear (*Ursus americanus*) developed severe necrotizing lesions on all 4 paws, nose, and both ears. The bear was being used as a subject in a winter dormancy study and had been given multiple drug injections. The gross lesions resembled those found in some human angiotides, especially Wegener's or lethal midline granulomas and allergic vasculitis. Comparison of the pathologic findings of this case with those reported for angiotides in man and lower animals indicated that the disease in this bear was a hypersensitivity angiitis, with penicillin being the most likely etiologic agent.

THE BEAR has not been commonly employed in laboratory experimentation in the past because of its size, expense of maintenance, and potentially dangerous nature. Nevertheless, in recent years the use of bears as experimental models has increased due to similarities in their physiology and anatomy to that of man and because of their ability to adapt to extremely cold temperatures. The following report describes a case of angiitis that developed in a bear while the animal was in winter dormancy.

The occurrence of necrotizing angiitis in this bear after the parenteral administration of drugs, particularly penicillin, suggests an acquired capacity to react to such stimuli.

History and Clinical Signs

A yearling female black bear (*U. americanus*) was obtained from the "wilds" of Alaska when approximately 4 weeks old and raised at the USAF Arctic Aeromedical Laboratory near Fairbanks, Alaska. It was confined in an outdoor cage consisting of a steel-barred open portion and a metal lined, insulated, unheated, wooden den. Entry was via an L-shaped tunnel.

The experiment was designed to determine cardiac function and hematologic changes during winter dormancy (physiologic condition characteristic of denning bears often referred to as hibernation). An implantable ECG telemetry capsule⁵ was surgically placed in the bear's abdominal cavity and recovery was complete and uncomplicated. On Dec. 27, 1969 (2nd month after surgery), all food and water were withdrawn and the bear entered dormancy 4 days later. Approximately every 14 days the animal was awakened, immediately immobilized with phencyclidine hydrochloride,^a and 40 ml. of blood

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The authors acknowledge the assistance of Dr. Harold W. Casey and his staff of the Cytology-Pathology Branch and Mr. Orville V. Anderson of the Photography and Television Branch, U.S. Air Force School of Aerospace Medicine, in preparing the histologic sections and photomicrographs.

^a Sernylan, Bio-Ceutic Laboratories, Inc., St. Joseph, Mo.

was collected via venipuncture utilizing the jugular vein. Following the blood collection, 1,000,000 units of aqueous penicillin G was administered and the animal returned to its den. Complete recovery from the phencyclidine was achieved after each use. Ambient exterior temperatures as low as -45°C . (-50°F .) were reported during the study period. Den temperatures were not obtained because the temperature probes were destroyed by the bear.

On day 41 after the bear entered dormancy (February 10), a blood sample was collected and 1,000,000 units aqueous penicillin G was administered intramuscularly. On days 58 and 59 (17 days after last penicillin injection) the bear was observed out of her den. It was decided that another blood sample would be collected should she be seen again the following day. Next morning, when the cage was approached, the bear was observed walking on her elbows, with a large necrotic lesion visible on her nose. The bear was immobilized, and close examination revealed extensive necrotic lesions on all 4 paws, the nose, and both ears. Other clinical signs were not noticed. Euthanasia was performed with intravenous sodium pentobarbital following collection of a blood sample. During the period between the last penicillin injection (41st day of dormancy) and euthanasia (59th day of dormancy), ambient temperatures ranged between -40 and -3°C . (-42 and $+27^{\circ}\text{F}$.).

Diagnosis

Based on the gross lesions, a presumptive diagnosis of necrotizing vasculitis was made. Cold injury was not considered plausible because ambient temperatures were not significantly lower than those the bear had experienced previously, the tissues were not frozen, and the peripheral lesions were not located on the most distal part of the extremities.

Histologic studies confirmed the diagnosis and indicated that hypersensitivity angitis was a more exacting description of the condition. Treatment was not at-



Fig. 1—Bear's head with centrally located, granulomatous nasal lesion.

tempted because of the animal's extreme debilitation.

Necropsy Findings

The lesion on the nose was centrally located, involved both nostrils, and the tissues were eroded sufficiently to expose the septal cartilage (Fig. 1). The lesion was free of exudate and appeared to consist of red granulation tissue. A line of demarcation, similar to that found with peripheral gangrene, was noticed on the bear's ears. The distal third of both ears was necrotic and had nearly sloughed off.

The gross lesions were similar on all 4 paws (Fig. 2). There was a definite line



Fig. 2—Bear's paw with necrosis and erosion of the soft tissue.

Fig. 3—Photomicrograph of subcutis showing fibrinoid degeneration and mixture of inflammatory cells invading (1) a medium size transport artery and (2) small arterioles. H&E stain; $\times 100$; line indicates $200\ \mu$.



of demarcation at the level of the metacarpophalangeal and metatarsophalangeal articulations. The overlying tissues on both the dorsal and ventral surfaces had eroded to the extent that 18 of the 20 toes had some degree of disarticulation. The lesions were moist and in several isolated areas a purulent exudate was noticed. The tissue proximal to the line of demarcation was red and macerated, with some granulation occurring; tissue distal to the line of demarcation was white and obviously nonviable.

Gross visceral lesions were numerous although not diagnostic. The lungs were generally hemorrhagic with some lobar hepatization. Most lymph nodes were enlarged and hyperemic. The capsules of the kidneys were easily removed and the underlying surfaces resembled a "hob-nail" liver with diffuse spotting not found in other bears. The renal cortices were yellow and there was very little bleeding from the cut surface. Marked mucosal congestion was noticed in the duodenum, jejunum, and colon.

Cultures of all lesions failed to yield any pathogenic organism.

Microscopically, the skin lesions appeared as shallow, nonspecific ulcers, beneath which lay mixed inflammatory

exudate. The subcutis was superficially infiltrated with a mixture of inflammatory cells, but the underlying musculature was spared. The arterioles apparently leading to these sites, but distant from the inflammatory process, had thickened eosinophilic hyalinized (fibrinoid) necrotic walls that in some instances were penetrated by polymorphonuclear leukocytes and a few eosinophils (Fig. 3 and 4). Capillaries and venules were also involved. Similar but less striking lesions were found in muscle tissue from the thighs and the heart. Vascular lesions in the lungs were similar to those in the skin but were of a more chronic nature and were relatively mild in severity. In some areas of the pulmonary parenchyma, frank hemorrhage was noticed. The nasal lesion consisted of abundant proliferating ulcerated granulation tissue and apparently viable exposed cartilage.

Discussion

Classification of the many diseases manifesting necrotizing lesions of blood vessels in man lacks uniformity. One author differentiates 5 specific necrotizing angitides. Her description of "hypersensitivity angitis" includes a course

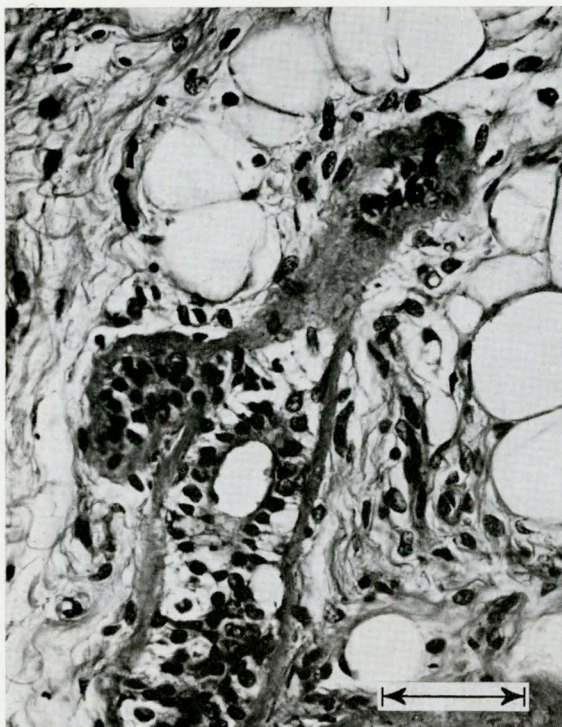


Fig. 4—Photomicrograph, higher magnification of small arterioles in Figure 3 showing hyalin-like degeneration of vessel wall. H&E stain; x 400; line indicates 50 μ .

“usually less than 1 month”; involvement of arterioles and venules; predilection for lesions to appear in the kidneys, heart, lungs, spleen, and dermis; lesions (perhaps exudative) of approximately the same age; and frequent association with necrotizing glomerular nephritis.^{18,19}

Another investigator describes “systemic allergic vasculitis” in similar terms, with the addition that the disease is usually “self-limiting” and not fatal. He goes on, however, to say that 10 of 40 patients died, usually because of renal failure.⁸

Several other investigators merely differentiate periarteritis nodosa, allergic angiitis and the various local angiitides such as Wegener's or lethal midline granuloma and temporal arteritis.^{1,3,11-13,15,16}

Although periarteritis nodosa^{2,14} and necrotizing angiitis of aged mice^{4,17} are the “naturally occurring” necrotizing angiitides most frequently reported in animals, the case described here would

not seem to qualify because of lack of grossly visible nodular lesions and the occurrence of venous and pulmonary lesions. The generalized involvement rules against localized angiitides.

The eroded nasal lesion grossly resembled Wegener's and lethal midline granulomatosis as seen in man. Histologically, the lesion resembled a “pyogenic granuloma.”^{13,15}

This case most closely resembles the previously described hypersensitivity angiitis, lacking only renal lesions. The lack of renal changes is perhaps explained by the fact that the bear was in winter dormancy when the disease developed. Studies have indicated that there is a significant decrease in urine production during dormancy and probably a relatively complete shutdown of kidney function during this period.⁶ Perhaps the absence of expected renal lesions is the result of the altered physiologic state of the organ.

The cause of the allergic reaction and subsequent vascular inflammation was most likely the aqueous penicillin G administered to the animal. Penicillin and sulfonamides are the drugs most often incriminated in cases of allergic angiitides in man.^{9,12} The mechanism of injury is reputed to be an antigen-antibody reaction within the vessel wall, with resulting inflammation and necrosis.¹⁰ For this reason, any material capable of being an antigen may cause hypersensitivity angiitis. Whether phencyclidine could also act in this capacity is not known.

On the basis of laboratory cultures of the lesions, it is considered unlikely that a bacterial cause existed. Because of lack of facilities, viral studies could not be accomplished. For this reason, the possibility of a viral cause, such as is found in equine viral arteritis, cannot be completely disregarded.⁷

A specific attempt was not made to recreate the disorder; however, at least 12 other bears were given the same agents (phencyclidine and penicillin) and were maintained under nearly identical environmental conditions without developing similar lesions.

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Animal Maintenance for Biomedical Evaluation of Lunar Material

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SUMMARY

Fifteen species of animals, representing 5 phyla, were used in the Apollo 11, 12, and 14 quarantine test programs. These animals were maintained successfully under the constraints imposed by biological containment cabinets. Invertebrate species and fish species were maintained together within a single negative-pressure cabinet system, thereby causing problems of husbandry. Positive-pressure germfree isolators were installed in the negative-pressure cabinets for housing gnotobiotic mice. The other species were housed in separate cabinets without special gnotobiotic provisions. Careful arrangement of species and strict adherence to a determined movement of all materials in and out of the cabinets resulted in completion of the test protocols with minimal problems. The generally negative test results obtained with these animal systems are also presented.

THE APOLLO 11 flight to the Moon (July, 1969) brought directly to Earth, for the first time, extraterrestrial materials. Because of the remote chance that life could exist on the Moon, strict containment of the potentially dangerous lunar material was necessary until reasonable assurances could be given that the material was harmless.

The Lunar Receiving Laboratory (LRL), located at the NASA Manned Spacecraft Center (MSC), Houston,

Texas, was built to provide a quarantine facility for the astronauts and the lunar material while certain biomedical investigations and time-critical physical-science experiments were conducted.

An exhaustive quarantine and biomedical test protocol was established that included isolation of the astronauts and isolation and observation of the lunar material for the detection of viable or replicating life forms, if any. A broad spectrum of terrestrial fauna and flora was exposed to the lunar soil in an effort to detect "infectious" components of lunar material not detectable *in vitro*. Cooperating in the quarantine program with the National Aeronautics and Space Administration were the U.S. Department of Agriculture, the U.S. Department of Interior, the National Academy of Science, and the U.S. Public Health Service.³ The lunar-sample quarantine

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Much of the information contained in this report was presented at the 22nd Annual Meeting of the American Association for Laboratory Animal Science, Oct. 11-15, 1971, New York, N.Y.

The authors gratefully acknowledge the efforts of Mr. Charles Neal and the Manned Spacecraft Center Animal Care Staff who greatly contributed to the successful completion of the Lunar Quarantine Program.

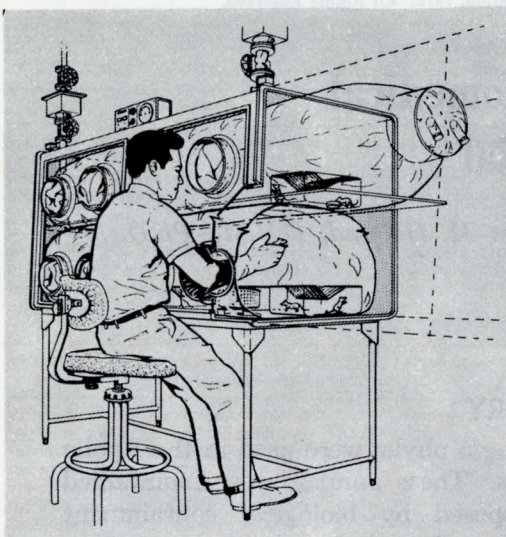


Fig. 1—Diagrammatic representation of the arrangement of positive-pressure isolators within the negative-pressure containment cabinets.

and preliminary examination program began with the Apollo 11 mission and continued through the Apollo 14 mission. The methods used to maintain the animals used in the biomedical testing, the problems encountered with these species, and an abbreviated summary of the results obtained from exposure of the animals to lunar material are contained in this report.

Materials and Methods

Twelve species of animals were selected for the original Apollo 11 quarantine protocol; 3 species were deleted and 3 were added during successive missions (Table 1). The type of containers used to house the animals, the ambient environment conditions to which the animals were exposed, and the lunar-sample inoculation data for each of these species are listed (Table 2).

All animals, except the planaria, were acclimated to the containment cabinets for 2 weeks before being exposed to the lunar material. The planaria were introduced immediately before their exposure. The gnotobiotic mice were maintained inside custom-made, flexible, positive-pressure isolators installed within the negative-pressure cabinets. This arrangement required that the mice be manipulated through 2 pairs of rubber gloves (Fig. 1). The quail were

housed (singly or in pairs) in standard quail pens 6 by 6 by 7 inches (15.2 by 15.2 by 17.8 cm.), arranged in 3 tiers within a cabinet with a volume of approximately 150 cu. ft. To prevent lacerations of the cabinet gloves, the cages were dip coated with rubber before use. The oysters were maintained in individual pint jars and monitored for activity by visual observation during the Apollo 11 and 12 missions. During the Apollo 14 mission, the oysters were set up for individual automatic monitoring, using myographs and a pen recorder (Fig. 2).^a This oyster monitoring technique has been reported in detail elsewhere.² The animals stayed in the cabinet for 30 to 44 days.

Because of the differences in cultural methods for the aquatic and terrestrial species, the methods of providing exposure to the lunar samples differed. The aquatic species were exposed by adding lunar material to the medium in which the animals were living. Only during the

^a NARCO BioSystems, Inc., Houston, Texas.

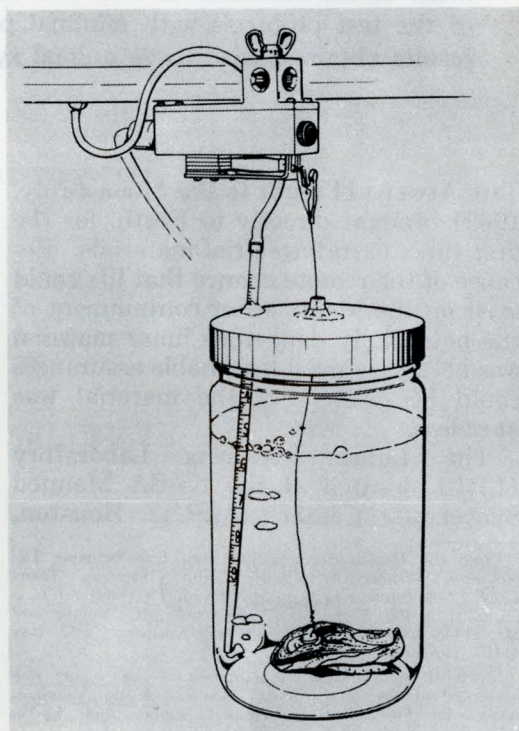


Fig. 2—Diagrammatic representation of the use of myograph for automatic monitoring of oyster valve movement.

TABLE 1—Animals Used for Lunar Quarantine Testing and Results of Exposure to Lunar Soil*

Phylum	Class	Genus, species, and common name	Results**
Protozoa	Mastigophora	<i>Euglena gracilis</i> —Euglena (Apollo 11 only)	Slight reduction in locomotive ability after exposure and a return to normal activity by the 4th day. All groups had normal morphologic features.
	Ciliata	<i>Paramecium aurelia</i> —paramecium	Initial reduction in fission rates after exposure, rapidly increasing to normal after 4 to 5 days. All groups had normal morphologic features.
Platyhelminthes (flatworms)	Turbellaria	<i>Dugesia dorotocephala</i> —planaria	No significant gross or histopathologic changes.
Mollusca	Bivalvia	<i>Crassostrea virginica</i> —commercial oyster	During missions 11 and 14 large numbers of deaths were encountered in all groups, but correlation could not be shown between the deaths and exposure to lunar material. During mission 12, all oysters remained in excellent health.
Arthropoda	Crustacea	<i>Penaeus aztecus</i> —brown shrimp (Apollo 11 and 14)	No abnormal behavior or significant gross or histopathologic changes.
		<i>Penaeus duorarum</i> —pink shrimp (Apollo 12 only)	Considerable fighting in all groups early in test. No significant gross or histopathologic changes.
	Insecta	<i>Blattella germanica</i> —German cockroach (gnotobiotic)	No unaccountable gross or histopathologic changes.
		<i>Musca domestica</i> —housefly	No unaccountable gross or histopathologic changes.
		<i>Galleria mellonella</i> —greater wax moth	No unaccountable gross or histopathologic changes.
Chordata	Osteichthyes	<i>Lebistes reticulatus</i> —guppy (Apollo 12 and 14)	No unaccountable gross or histopathologic changes.
		<i>Fundulus heteroclitus</i> —mummichog minnow	With the exception of a few fish in each group during mission 12 (lost because of gill congestion from exposure to hypochlorite), all mummichogs remained in excellent health and no unaccountable gross or histopathologic changes were found.
		<i>Pimephales promelas</i> —fathead minnow (Apollo 11 only)	Sporadic deaths in all groups because of sodium hypochlorite spill. No unaccountable gross or histopathologic changes.
	Aves	<i>Coturnix coturnix</i> —Japanese quail (Apollo 11 and 12 only)	No unaccountable gross or histopathologic changes. Several deaths attributed to inoculation laceration of internal organs or self-inflicted trauma.
	Mammalia	<i>Mus musculus</i> —gnotobiotic CD-1 mice†	No indication of any infectious disease-producing agent or acute toxic component in lunar material. Some evidence of long-term irritative effect; however, resolution of this point must await complete analysis of data obtained from long-term test groups.
		<i>Cavia porcellus</i> —guinea pig (Apollo 14 only)	No unaccountable gross or histopathologic changes.

* Unless otherwise stated, animals were used in Apollo missions 11, 12, and 14 quarantine protocols. ** Dosages of lunar soil in Table 2. † Charles River Breeding Laboratories, Inc., Wilmington, Mass.

TABLE 2—Apollo Quarantine—Animal Protocol Maintenance Containers, Environmental Conditions, and Lunar Sample Inoculation Data

Animal	Container	No. of animals/container	Diet	Medium	Hours of light/dark photoperiod	Temperature (C.)	0.220 Gm. of lunar material/unit
Paramecium	125-ml. flask	1.0 X 10 ³ to 2.0 X 10 ⁶	Aerobacter aerogenes Medium	Lettuce-leaf infusion	16/8	22	Flask
Euglena	250-ml. flask	2.0 X 10 ⁴ to 5.5 X 10 ⁶	None	Synthetic inorganic medium	16/8	22	Flask
Planaria	300-ml. fingerbowl	10-50	None	Aged tapwater	16/8	22	Bowl ^a
Shrimp	20-L. aquarium	20	Live brine shrimp	Sea water	16/8	27	Shrimp
Oyster	1-L. jar	1	None	Artificial sea water ^c 21 parts/thousand	16/8	22	Oyster
German cockroach	500-ml. jar	20/15 ^{a,b} — 10/20	Mouse diet ^{c,d,e}	NA	16/8	25	Jar
Housefly	500-ml. jar	25/25 ^f	Compounded diet ^g	NA	16/8	25	Jar
Greater wax moth	500-ml. jar	20-25	Compounded diet ^h	NA	Total darkness	25	Jar
Guppy and fat-head minnow	20-L. aquarium	20	Live brine shrimp	Aged tapwater	16/8	22	Fish
Mummichog minnow	20-L. aquarium	20	Live brine shrimp	Artificial sea water ^c 21 parts/thousand	16/8	22	Fish
Japanese quail	Rubber-coated wire cage 6 by 6 by 7 inches	1-2		NA	16/8	22	Bird (intra-peritoneally)
Mice	Polycarbonate shoebox cage 8½ by 14 by 5½ inches	6	Mouse diet	NA	16/8	22	Mouse (intra-peritoneally, subcutaneously)
Guinea pig	Polycarbonate shoebox cage 13 by 15 by 6½ inches	3	Guinea pig chow ⁱ	NA	16/8	22	Guinea pig #

^a 1.10 Gm./bowl. ^b Nymphs/adults. ^c Charles River prefortified rat/mouse diet, Charles River Laboratories, Wilmington, Mass. ^d Larvae/adults. ^e Sugar, albumen, sodium oleate, Weeson salts (Nutritional Biochemical Company, Cleveland, Ohio), B vitamins. ^f Pabulum (Gerber Products Company, Fremont, Mich.), sugar, glycerol, water, B vitamins. ^g Purina Guinea Pig Chow, Ralston Purina Company, St. Louis, Mo. ^h Guinea pigs exposed by tracheal insufflation of 2 ml. of a 1% suspension of the lunar material in 0.9% saline. ⁱ Instant Ocean, Aquarium Systems, Eastlake, Ohio.

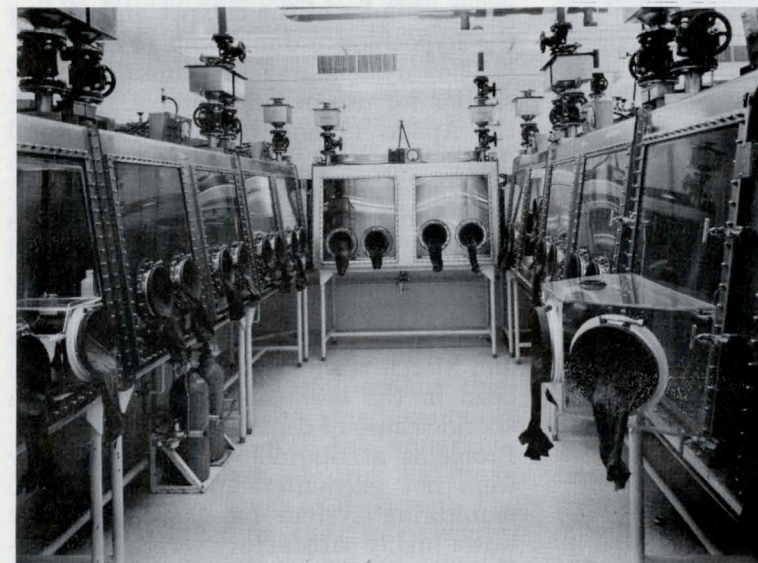


Fig. 3—Biological containment cabinets used for lunar-sample quarantine program.

Apollo 14 mission were the oysters exposed by introduction of the test material into the shell cavity through a 1/8-inch (0.3-cm.) hole drilled in the shell. The insect species were exposed by mixing the lunar samples with their food. The mice and rats were exposed by parenteral inoculation (intravenously, subcutaneously, or intraperitoneally). The guinea pigs were used to evaluate pulmonary response to the lunar material. Exposure was accomplished by tracheal insufflation with 2 ml. of a 1% suspension of the material in 0.9% saline. The quail were exposed by intraperitoneal inoculation. The amount of lunar material used per animal or culture is given (Table 2).

Following the initial unpacking, cataloging, and observing of the lunar samples, representative portions were selected for the biological test program. The selected samples were first screened using a 104-μ sieve; the material passing through the sieve was set aside at this point. All remaining material was crushed or ground until the particles were reduced sufficiently in size to pass through the sieve. Then, the crushed sample was recombined and mixed with the untreated sample. The particle size of the lunar material, as used in the biological testing, ranged from 13 to 30 μ for the 3 missions.

Before certification of the lunar material as being biologically safe, all work performed with the rocks and soil was ac-

complished within biological containment cabinets (Fig. 3).^b The air for the cabinets was drawn through a high-efficiency particulate air (HEPA) filter from the laboratory in which the cabinet was located. The exhaust air was first passed through a HEPA filter, then heated to 500 F. (110 C.), and finally passed through a 2nd HEPA filter before being released into the atmosphere. The HEPA filters removed all material greater than 0.2 μ in size. Approximately 6 to 10 air changes per hour were achieved within the cabinets while, at the same time, a negative pressure of 1 inch (2.5 cm.) of water was maintained relative to the room. The cabinetry available for the animal-testing program provided approximately 348 sq. ft. of floor area and 912 cu. ft. of volume.

Before introduction of the lunar material, the cabinet lines were decontaminated with vaporized paraformaldehyde followed by a 72-hour air wash. Once the lunar samples had been introduced into the cabinets, all material brought out of the cabinet line had to be sterilized by steam autoclaving, ethylene oxide, or passage through a hypochlorite dunk tank (5,000 p.p.m. chloride), with a holding period of 30 minutes in the hypochlorite. The method of choice was determined by the nature of the sample to be removed.

^b Blickman, Weehawken, N.J.

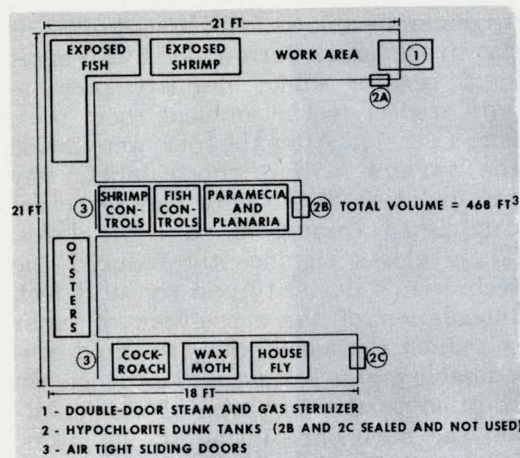


Fig. 4—The arrangement of invertebrate and fish species in Apollo 14 biological containment cabinets.

Results and Discussion

The quarantine mission of the LRL was to isolate, characterize, and biologically contain any infectious components of the lunar material. To accomplish this goal with reasonable assurances of finding any alien life form, it was decided to expose a representative selection of terrestrial fauna and flora to virgin lunar soil and to conduct a direct, *in vitro* search for microbial agents. The constraints of the biological containment cabinetry severely limited the size and number of individual animals that could be used for such testing. In addition, the stresses imposed by the close confinement within the cabinets further limited the choice of species.

A brief summary of the results from the exposure of the test animals to the lunar soil is presented (Table 1). The results obtained with the invertebrates and fish during the Apollo 11 quarantine have been reported in detail.¹

Maintenance of the protozoan and planaria species within the containment cabinets was easily accomplished. The major problem encountered with these groups was a sensitivity to chlorine fumes from the dunk tanks. This problem was solved by locating these species in an area that could be sealed off from

the remainder of the cabinet and sealing the hypochlorite dunk tank (Fig. 4).

The oyster was an unsatisfactory species for use in our protocol. In both the Apollo 11 and 14 missions, several deaths occurred at random among the treatment groups, including the controls. The Apollo 11 postflight investigations occurred in July and August, the normal spawning period for oysters, and this may have explained many of the mortalities. The Apollo 14 mission was in February and March when the oysters should have been in the best condition; however, a specific cause of death was not identified. Problems attributable to the cabinetry were not encountered. The automatic monitoring system for shell movement was a highly satisfactory way of monitoring oysters because they were very sensitive to activity within the laboratory. The automated system permitted accurate monitoring during the quiet periods.

The 2 species of shrimp that were used in our testing were satisfactory subjects. A 1½-inch (3.8-cm.) layer of sand was placed in each aquarium, permitting the shrimp to pursue their normal burrowing activity. At times, only their eyes could be seen above the sand. The only problem encountered related to the cabinetry was the weight of the filled aquarium (approximately 40 lb. (18.2 kg.)). Passing the filled aquarium along from glove to glove was impossible. This problem was solved by fabricating small plexiglass-wheeled dollies. The filled tanks sat on the dollies and could be pushed easily along the floor of the cabinet.

All 3 species of insects, both adults and pupae, were excellent test systems. Their small size, rapid development, and minimal-care requirements were characteristics that were highly desirable in our program. Relatively large numbers could be housed in the small space available. However, these species also were sensitive to the chlorine fumes from the dunk tanks. With insects, as with the protozoa and planaria, this problem was solved by isolating the subjects within the cabinetry and sealing the associated dunk tank.

The fathead minnows also were sensitive to the chlorine fumes; therefore, it was decided to substitute the guppy for this species in the Apollo 12 and 14 missions. The mummichog minnow, an estuarine species, was used throughout the quarantine program. To further reduce the possibility of accidental contamination of the aquariums with hypochlorite, anything going into the cabinet lines through the dunk tanks was double packaged. Once inside the cabinet, the outer container was removed over a pan of vermiculite treated with sodium thiosulfate. Then the dry inner container was passed along over the aquariums without the danger of dripping hypochlorite into the tanks as it passed. The problem of handling the heavy water-filled aquariums was solved with dollies, as described previously for the shrimp.

The Japanese quail, although able to live in the confinement of the cabinets and despite their size advantage, were unsatisfactory as a test species. Their extremely nervous and excitable nature resulted in numerous self-inflicted injuries because of frantic attempts to escape whenever a technician entered the laboratory. Egg production varied greatly from day to day. The quail were deleted from the Apollo 14 protocol because of these problems and because the class Aves was well represented by embryonating chicken eggs and duck embryo tissue cultures used in the virology protocol.

Maintenance of the gnotobiotic mice was one of the greatest logistic problems; however, the mice thrived. The extreme complexity of the caging and biological isolator systems aggravated a problem inherent in gnotobiotic technique (that of maintaining a stable, defined environment). Only during the Apollo 11 mission did the mice remain uncontaminated; in the succeeding missions, 1 or 2 contaminants were encountered. Rigid biological isolators and heavy rubber gloves were an added requirement to ensure adequate protection of operator personnel against exposure to potentially dangerous lunar material. This system resulted in a dual isolator configuration:

an inner flexible isolator maintained at positive pressure, surrounded by an outer rigid isolator under negative pressure with respect to the ambient room pressure (Fig. 1). After the mice were inside the isolator within the cabinet, any manipulation of the mice had to be accomplished through 2 pairs of gloves. These gloves significantly reduced the technician's dexterity and sense of feel. Inoculation of the suspension of lunar soil under these conditions required considerable practice, time, and patience. In addition to the problems of manipulating the mice once inside the isolators, getting the mice from the germfree transporter into the isolator was a time-consuming job. During the Apollo 11 mission, a connecting flexible vinyl tunnel (approximately 10 ft. (3.1 m.) in length), was passed from the transporter through the hypochlorite dunk tank to the test isolator. Passage of the mice through this tunnel was extremely difficult. During the Apollo 12 and 14 missions, transporters small enough to pass through the autoclave chamber (20 by 20 inches (51 by 51 cm.)) were used and transfer of the mice was less difficult. After all logistics and handling problems were solved, the mouse was one of the most carefree species.

The guinea pigs were housed in part of the cabinet line being used for the clinicopathologic support of the animal protocols. With the exception of the large amount of bedding and water required, problems of any magnitude were not encountered with this species.

The oysters, shrimp, and mummichog minnows required salt water for normal maintenance. This requirement was satisfied by using water obtained from the Gulf of Mexico or by using a commercial substitute.^c The salt water did cause some housekeeping problems within the confinement cabinets. Large quantities of precipitated salt would accumulate on the aquarium filters and subsequently on the cabinet floor. Because of the difficulty of moving large

^c "Instant Ocean," Aquarium Systems, Inc., Eastlake, Ohio.

quantities of water in and, particularly, out of the cabinets, the precipitated salt was allowed to accumulate during the missions. After the quarantine was completed, one of the technicians would crawl through the autoclave and clean the cabinets.

Another general problem encountered was the introduction of steam into the cabinets from the pass-through autoclaves. If the inner door of the autoclave was opened too soon following a sterilization cycle, residual steam would enter the cabinet and cause heat stress to the animals. To overcome this potential hazard, technicians were instructed not to open the inner doors until a full exhaust cycle had been completed (as recorded in a written log) and the exterior of the autoclave was cool to the touch.

The mice, quail, and conventional guinea pigs were each housed in different cabinet lines, and, therefore, special planning for placement was not required. A specific preplanned location arrangement was necessary for the invertebrate and fish species because all these animals were housed within a single cabinet line (Fig. 4). The insects were introduced first, followed in turn by the protozoa, oysters, shrimp, and fish. The shrimp and fish were introduced last because this required moving the filled aquariums the least distance. The planaria were passed into the cabinet line immediately

before exposure and passed along above the aquariums to their position with the protozoa. Once all species were in place in the cabinet line, strict control was maintained on introduction and removal of materials.

A large number of species could have been selected for inclusion in the Lunar Sample Quarantine Program; however, space limitations greatly reduced the number of choices. The species selected, with other systems being utilized in other parts of the quarantine protocol (e.g., cell cultures), provided exposure to a broad spectrum of terrestrial fauna. The investigations of lunar material did not reveal the presence of living organisms; however, the search for extraterrestrial life may be successful following some future planetary mission.

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