NASA TECHNICAL MEMORANDUM   NASA TM-X-69354

REGULATORY BIOLOGY:
DEPRESSED METABOLIC STATES
MEETING OF OCTOBER 18 - 20, 1971
At:
NATIONAL AERONAUTICS
AND
SPACE ADMINISTRATION
WALLOPS STATION,
WALLOPS ISLAND, VIRGINIA

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

WALLOPS STATION

WALLOPS ISLAND, VIRGINIA

AUGUST 1973

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15. Supplementary Notes

Nine talks with discussion

16. Abstract

A group composed of NASA Life Scientists and NASA grantees concerned with regulatory biology (depressed metabolism) met at Wallops Station to discuss some aspects of space biology for future NASA missions. Each grantee presented his findings to date under NASA sponsorship. Questions addressed were: What have we done?; What are we doing?; and, Where are we going? This meeting marked the establishment of a consortium of life scientists dedicated to establishing Earth-based information for the definition and subsequent development of a space flight experiment utilizing small mammalian species.

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FOREWORD

This meeting marked the establishment of a consortium of life scientists
dedicated to establishing Earth-based information for the definition and
subsequent development of a space flight experiment utilizing small
mammalian species.

The group was convened at Wallops Station to discuss some aspects of space
biology for future NASA missions. Questions addressed were: What have we
done?; What are we doing?; and, Where are we going? The presentations and
discussions were transcribed from tapes and were not originally intended
as a formal publication. However, with the progress being made and the
solidification of the consortium, documentation of the meeting was deemed
important.

Since the Wallops' meeting, the consortium has been meeting semiannually
at various member laboratories to review and discuss progress as well as
to maintain program direction. Because of the cohesiveness, cooperation,
and interaction of all members of the consortium, considerable progress
toward definition of a small mammalian space experiment has been made.

It is in this context, then, that we believe a valid and scientifically
sound experiment will evolve in which answers will be forthcoming to
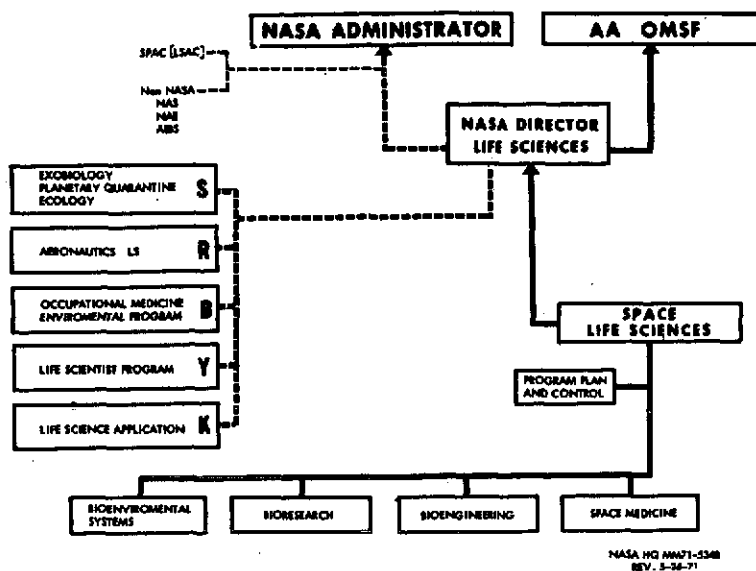questions that remain unanswerable on Earth.

Emily M. Holton, Ph.D.

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The Role of Depressed Metabolism in Space Biology: An Overview

Dr. Joseph Saunders
NASA Headquarters
Washington, D.C. 20546

I believe that most of you know why we called you together. NASA is funding an area of depressed metabolism consisting of a variety of technical approaches, (including hibernation and hypothermia, thermal regulation, and diluent gases) to learn how these might depress metabolism, particularly in the situation of 100% oxygen and reduced ambient pressures. With times being very critical we thought that we would call you together to review the program you are performing; and, to learn of your inputs, your ideas, your thoughts about what could be done for the future. This is particularly important, since I learned last week that the National Academy of Sciences is planning for a Committee on Space Biology as part of its Space Science Board. Over the past year or year and a half, most of you have heard of some of the derogatory comments that have been made about Space Biology: that there does not seem to be any future for it; vital questions are not being asked; is it really necessary to answer any of these questions in space; and, why can't the work be done in Earth-based laboratories? Most of us in the NASA Life Sciences organization believe that there is a need for space biology, that there are vital questions to be answered, and that these can be done honestly and scientifically in space using the space environment as a new dimension, an unique situation. We do not believe, however, that everything we support in the Earth-based laboratory is going to be a flyable or flight-type experiment. We feel that probably about 90% of the work that we do in Earth-based laboratories will stay as such - the basic research group for information and knowledge. The other 10% probably will evolve as experiments that should be flown in space because it is the only way the answer can be obtained. I leave you with these few thoughts to ponder for the next 2 1/2 days, because we are going to be "hitting" you over and over with these questions.

This is a critical year, as I mentioned. We are reviewing the entire biology program. We have to have something very firm upon which we can mold a future. This has evolved because of what has happened within the past year. As you recall, there have been three distinct organizations in NASA dealing with the life sciences. I am from the organization that was known as the Bioscience Programs in the Office of Space Science and Applications. Dr. Oyama, our conferee from Ames, was involved primarily with the second organization in NASA known as Human Research and Bio-Technology of the Office of Advanced Research and Technology under Dr. Walton Jones. The third segment was Manned Medicine. Last year (1970), NASA asked the National Academy of Sciences to review its entire life sciences programs and to render recommendations as to how the program could be the most effective. The Academy convened a committee referred to as the Glass Committee. The committee visited our NASA centers, made their deliberations, and concluded at Woods Hole with a strong recommendation to reorganize the life sciences under one leader. In December of 1970, the new Office of Life Sciences was formed and its first director was Dr. James Humphreys. Dr. Humphreys resigned in May and has been replaced recently by Dr. Charles Berry. I believe that it is appropriate for you to know how this organization functions. With the first viewgraph, I will try to provide you with a summary as to how the organization is structured

NASA ADMINISTRATOR   AA OMSF

SPAC (LSAC)

New NASA
NAS
NAE
ABS

NASA DIRECTOR
LIFE SCIENCES

EXOBIOLOGY
PLANETARY QUARANTINE   S
ECOLOGY

AERONAUTICS LS   R

OCCUPATIONAL MEDICINE
ENVIRONMENTAL PROGRAM   B

LIFE SCIENTIST PROGRAM   Y

LIFE SCIENCE APPLICATION   K

SPACE
LIFE SCIENCES

PROGRAM PLAN
AND CONTROL

BIOENVIRONMENTAL SYSTEMS   BIORESEARCH   BIOENGINEERING   SPACE MEDICINE
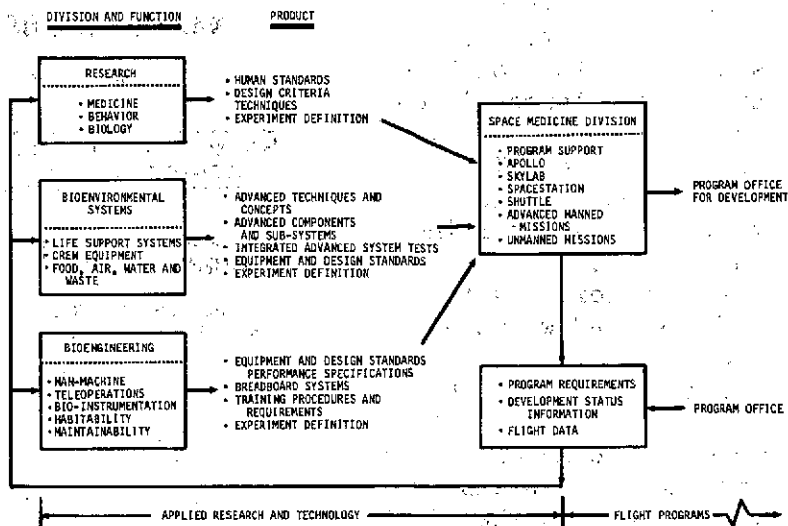
NASA HQ MM71-5348
REV. 5-36-71

VIEWGRAPH 1

and what its functions are. The NASA Director for Life Sciences is the new directorate, a new office. As I mentioned, formerly we had a Bioscience Programs in Space Science and Applications, a program in the Office of Advanced Research and Technology, and, of course, Manned Medicine. Now, the Director for Life Sciences, in charge of the Office of Life Sciences, deals with four principal functions: a program in Bioenvironmental Systems, a Bioresearch Division (which I represent), a Bioengineering Division, and the Space Medicine Division. These are his prime responsibilities for which he reports to the Associate Administrator for Manned Space Flight as well as to the NASA Administrator. In addition, he is responsible for coordinating all other activities. Exobiology and Planetary Quarantine, as you see, still remain in Space Science and Applications, principally because they are directly related to Project Viking which will orbit and land on Mars in 1975, if things go well. We also have an Ecology Program which is part of the Earth Resources Organization in Space Science and Applications. Dr. Berry is responsible for these programs as well. He is also responsible for the Aeronautic Medical Program which deals primarily with vibration, noise, comfort, habitability, etc., of airplanes for passenger comfort, etc. Occupational Medicine has a program in every NASA center and is coordinated by Dr. Louis Arnoldi at NASA Headquarters. Dr. Arnoldi also reports to Dr. Berry as coordinator of all Life Sciences. We have a Life Scientist Program which was initiated this year, again a recommendation of the Glass Committee. It is handled by the Office of University Affairs, however, we have the final decision making function as to who might be the Life Scientist Program awardees. Finally, Life Sciences Applications. This, then, gives you the overall picture of NASA Life Sciences, what the role and responsibilities are of the NASA Director for Life Sciences, and so on. Now let us look at Bioresearch. The Bioresearch Division entails 3 programs: the Biology Program (for which I am responsible), the Medical Program under Dr. Sherman Vinograd, and the Behavioral Biology Program under Dr. Richard Belleville. In the next viewgraph (2) are the four divisions that you saw in the bottom of the previous viewgraph. In the Bioresearch Division, let us concentrate on biology. We are not necessarily concerned with human standards in biology. We are interested in design criteria, to a certain extent. Techniques, we are interested in, and experiment definition is, obviously, our interest. Let us take an example of an experiment, defined and developed, at the time it might be selected by the appropriate committees and panels and recommended for flight. We would then turn it over to the Space Medicine Division for assignment to one of their programs. For example, as you will

see later, just two weeks ago I was finally able to justify an experiment for flight on Apollo 16. I have completed my responsibility, insofar as the management and administration are concerned. It now becomes the responsibility of Dr. Rufus Hessburg, who handles that division. The program functional flow is the routine type feed-back system. From Bioresearch, the experiment or solution goes to the cognizant Program Office. They, in turn, establish

VIEWGRAPH 2

certain criteria, certain problems that still remain unanswered. They also develop program requirements which are returned to the basic division. The next viewgraph (3) shows the Bioresearch Division objectives which I have been emphasizing. I want you to consider three things: (1) Biology required to support flight programs. What do we mean? Is there any problem in man, where the emphasis is right at the moment, that cannot be answered using man as the experimental animal? What analog, what surrogate or substitute do we use that we might be able to find the answer in an animal system? (2) Sponsor candidate flight experiments. I would say that the bulk of my program deals with this particular area. (3) Support space-oriented research. I believe, at this point in the research posture of this country, that not just space-oriented research but any supporting or meaningful information is necessary for our bank of knowledge for the future. We still use the advisory panel system. I would say that, for this conference, we are an advisory panel which —is reviewing each other's program and to give guidance for the general,

BIORESEARCH

OBJECTIVES

CONDUCT RESEARCH IN MEDICINE, BEHAVIOR, AND BIOLOGY REQUIRED TO SUPPORT FLIGHT PROGRAMS

DERIVE AND SPONSOR CANDIDATE FLIGHT EXPERIMENTS WHICH CAN SIGNIFICANTLY AMPLIFY KNOWLEDGE OF MEDICAL, BEHAVIORAL, AND BIOLOGICAL RESPONSES, AND HUMAN SUPPORTIVE TECHNIQUES

SUPPORT SPACE-ORIENTED RESEARCH DESIGNED TO PROVIDE MEANINGFUL INFORMATION ON LIVING SYSTEMS, WHICH IS NOT OTHERWISE OBTAINABLE

NASA HQ S-1-547H
8-71

VIEWGRAPH 3

overall program. There are two kinds of advisory systems that we use (viewgraph 4). One is our own in-house NASA people who ultimately decide from the advice of the outside committees as to what we are going to do. The other, the Life Science Committee, is a new one established in the Space

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Program Activities Council. There are five such committees of SPAC, and Life Sciences is one. You can see what their functions are. They provide us with guidance, they help us determine our policy, they try to assign priorities to our efforts, and wherever necessary, they try to provide us with technical assistance of some specialized nature. For instance, two weeks ago, I went through the first phase of a review of our entire radiation biology program with a panel

NASA OFFICE OF LIFE SCIENCES

USE OF SCIENTIFIC ADVISORY PANELS:

NON-NASA SCIENTIFIC REVIEW AND EVALUATION SYSTEM

• EVALUATION SCIENTIFIC VALUE AND QUALITY OF EFFORTS

• SPECIAL TECHNICAL PROBLEM ASSISTANCE

NASA SCIENCE, TECHNICAL AND MANAGEMENT LIFE SCIENCE ADVISORY COMMITTEE

• PROGRAMMATIC GUIDANCE
• POLICY DECISIONS, STRATEGY TO BE USED
• PRIORITIES OF NASA EFFORTS
• SPECIALIZED TECHNICAL PROBLEM ASSISTANCE

VIEWGRAPH 4

selected by the Space Science Board of the National Academy. This review is going to continue until we complete the study of the entire program. We use outside, non-NASA, panels to evaluate our research proposals. Primarily these are panels established by the AIBS under contract to NASA. I believe that, overall, we are receiving adequate guidance, direction, and scientific review from the scientific community represented by, in at least two instances, the AIBS and the National Academy. I mentioned that we are currently undergoing a review of the Radiation Biology Program with the National Academy. To give you some insight into what has gone on, I am reviewing the number of times we have had that program assessed (viewgraph 5). In 1963, we called on the National Academy to help us establish criteria for the best type of radiation experiments we could fly on Biosatellites I and II. They recommended that the following types of experiments should be flown: those that are involved with a known quality and quantity of radiation from an on board source to determine if the radiobiologic effects were modified by weightlessness or other factors of the space environment. This was 180 degrees opposite of what the Russians were claiming, at that time: that any genetic effect they observed was attributed to the ambient radiation of space. The Academy also recommended strong

	1963	WHSS 1965	LANGHAM GRAHM 1967	SCSS 1969	GLASS 1970	WARREN 1970
HZE PARTICLES		•	•	•	•	•
RETRO/PROSPECTIVE						•
DOSE-RATE DEPENDENCE			•	•	•	•
MODIFICATION	•	•	•			•
RADIATION BIOLOGY REVIEW			•			
RISK EVALUATION			•			•
MANNED DOSIMETRY			•	•	•	•
EARTH-BASED	•	•	•	•	•	•

VIEWGRAPH 5

MEETINGS: JANUARY (HAWAII)   JULY (JPL)   DECEMBER (COSMOS CLUB)

PROPOSALS:(61)   16   27   18

SCIENTIFIC RATING: 1.0 = OUTSTANDING to 5.0 = UNSATISFACTORY

	1.0-1.5	1.6-2.0	2.1-2.5	2.6-3.0	3.1-3.5	3.6-4.0	4.1-4.5	4.5-5.0	OTHER
NUMBER OF PROPOSALS:	5	8	6	6	9	13	7	2	5
FUNDED BY NASA IN FY71	1	1	0	0	0	0	0	0	-

$\leftarrow$ OUTSTANDING ——————— AVERAGE $\leftarrow$ MEDIOCRE ——————— POOR $\rightarrow$

25   31   5

VIEWGRAPH 6

support of Earth-based re-search. We asked the Academy again, in 1965, for another review. This time they looked at the high energy, high Z particles of galactic cosmic radiation as being something that we should be considering for the future. They, again, included the modification of radiation-induced damage by space flight variables as very worthwhile scientifi-cally. Of course, these reviews supported strongly the Earth-based research, and not necessarily leading to flight experimentation. In 1967, you can see the areas that were covered by the Langham-Grahn Committee resulting in a publi-cation entitled "Radiobiological Factors in Manned Space Flight" and avail-able through the Academy. In 1969, the Santa Cruz Summer Study was convened primarily to review the Biosatellite II results and the future of space biology, and, again, further review by the Glass Committee in 1970 and the Warren Committee, also in 1970. You can see that the review of just one area of the biology program has been constant. We feel that these reviews are not harming us in any way. They are helping us because we keep the National Academy aware of what we are doing from day to day and they, in turn, help us with our problems. The next viewgraph (6) shows what one of my problems is. Look at calendar year 1970 at which time I was in charge of the Environmental Biology Program in Bioscience Programs, OSSA. We had 16, then 43 (by July), and finally 61 new proposals, submitted for consideration. These were reviewed during meetings in January, July, and December. As you can see, 1.0 is outstanding as rated by the scientific panel, whereas 5.0 is com-pletely unsatisfactory. The breakdown, going from 1.0 to 3.0 contains almost half of the proposals. Notice that of these 25, we were able to fund only 2. It now seems to me that I would have to con-sider it futile to look at this graph, tell the in-vestigator what my prob-lems are, and then ask if he cared to resubmit at a later time. It is very,

SPACE FLIGHT

	TIME →
EARTH ORBITAL	SKYLAB, SPACE STATION, GEOSYN-CHRONOUS STATION
LUNAR	APOLLO 3 MEN, EXTENDED APOLLO, LUNAR ORBIT STATION 4 MEN, LUNAR SURFACE BASE 6 MEN
PLANETARY	MARINER ORBITER, VIKING, HIGH DATA RATE ORBITER, GRAND TOUR
TRANSPOR-TATION SYSTEMS	SATURN V, SPACE SHUTTLE, TUG, NUCLEAR SHUTTLE

VIEWGRAPH 7

5

very discouraging and frustrating to see that number of excellent to very good proposals and to be able to fund only 2 in a given fiscal year. This fiscal year, 1971, is even worse. Leaving the fate of proposals and what we were able to accomplish, viewgraph 7 is one of the prettier slides that NASA has created recently. Dr. Musacchia always says what he likes about NASA are its "pretty slides." I don't know how many more beautiful slides we are going to be able to create with the present budget! But, the time period on this viewgraph is 1970 to 1980 with 1980-1990 on the following viewgraph. This was the program that NASA recommended to Vice President Agnew when he headed up the Space Task Group. Two skylabs were recommended, a space station, and a geosynchronous station as a 10 year program for the Earth orbital manned system. The lunar program included landing on the moon in early 1970. Of course we did that before 1970, but we received no bonus for it! The extended Apollo was supposed to be about 1973, but we are ahead of that schedule with Rover. Still, no bonus for having done a good job! Then projected to 1980, we would have a 6-man lunar surface station. Of course, at the same time the program was indicated to be balanced between manned and unmanned systems. Viking is being projected for Mars, a lander and an orbiter about 1975. Then the grand tour that will go by several planets, circa 1980. The transportation

VIEWGRAPH 8

systems for this time interval include the Saturn V, the space shuttle (now a design and feasibility study), and by 1980, hopefully the nuclear shuttle. The next viewgraph (8) moving on from 1980-1990, suggests a huge space base with 100 men in low earth orbit by 1990. In the lunar area, 24 men and, finally, a factory, a school system and everything else up there on the lunar surface. A manned landing on Mars is projected after Viking makes the initial entries, and, ultimately, a Mars excursion module. That is the program that NASA believed it could and would
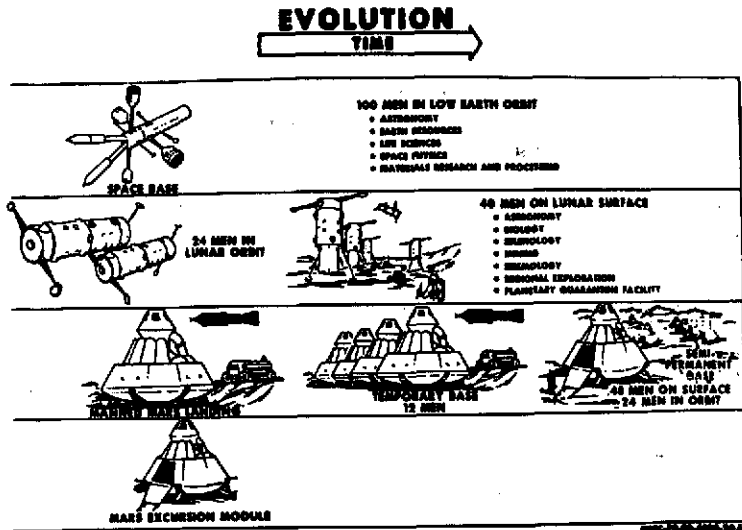
NASA OPTIONS-SPACE TASK GROUP
COMPARITIVE PROGRAM ACCOMPLISHMENTS
POST-APPOLLO

MILESTONES	MAXIMUM PACE	PROGRAM I	II, III	LOW LEVEL
MANNED SYSTEMS				
Space Station (Earth Orbit)	1975	1976	1977	-
50-Man Space Base (Earth Orbit)	1980	1980	1984	-
100-Man Space Base (Earth Orbit)	1985	1985	1989	-
Lunar Orbiting Station	1976	1978	1981	-
Lunar Surface Base	1978	1980	1983	-
Initial Mars Expedition	1981	1983	II-1986 III-OPEN	-
SPACE TRANSPORTATION SYSTEM				
Earth-to-Orbit	1975	1976	1977	-
Nuclear Orbit Transfer Stage	1976	1978	1981	-
Space Tug	1976	1978	1981	-
SCIENTIFIC				
Large Orbiting Observatory	1979	1979	1980	-
High Energy Astron, Capability	1973	1973	1981	1973
Out-of-Ecliptic Survey	1975	1975	1978	1975
Mars-High Resolution Mapping	1977	1977	1981	1977
Venus-Astmospheric Probes	1976	1976	Mid - 80's	1976
Multiple Outer Planet "Tours"	1977-79	1977-79	1977-79	1977-79
Asteroid Belt Survey	1975	1975	1981	1975

VIEWGRAPH 9

BIOMEDICINE IN

NASA CURRENT PROGRAM

<u>CONGRESSIONAL APPROVAL</u>

1972 .... SATURN-APOLLO MISSIONS 16 AND 17

1973 .... SKYLAB A (1 MOF)

<u>PLANNING OPTIONS</u>

1974 - 75 .... SKYLAB B OR CSM (POSSIBILITIES)

1974 - 77 .... BIORESEARCH MODULE

1978 .... SHUTTLE - BORNE BIORESEARCH MODULE

.... SHUTTLE (FMOF)

1979 .... SPACE STATION MODULE

1980 - 90 .... SPACE STATION

VIEWGRAPH 10

pursue for the future, given adequate funding. It was considered a well-balanced program. The Space Task Group reviewed and analyzed it. The STG reported to the President, recommending the program shown in the next viewgraph (9). They agreed to most of those efforts you saw in the previous slides (7 & 8), manned systems, transportation systems, and of course, the scientific programs which were primarily basic. By national pace, they meant that instead of going from 5.6 billion down to 3, they recommend increasing the budget from 5.6 up to 10 so that we could accomplish these milestones within the time period indicated. However, they were aware that funds would not become available that easily, therefore, if a gradual increase could be obtained over the current level from year to year, the goals could be completed at a somewhat slower pace. On the other hand, if an increase less than expected was forthcoming, the program would be delayed from 2-10 years, and the initial Mars expedition by man would be left open, perhaps in the 1990's or later. At a low level of funding, nothing would be completed in the manned or space transportation systems, and we would be concentrating on automated scientific flights. This recommendation was submitted to the White House, a recommendation by the Vice President and his task force. What happened is another story of which you are aware. The next viewgraph (10) shows our current NASA program.

By congressional approval, we currently have two programs. In 1972, we will complete missions 16 & 17 of Apollo. Skylab A will be first launched in 1973 by the Saturn 5 and the three subsequent visits will be by man in Earth-orbital Skylab missions. We do have planning options, however, which Congress accepts for the moment. We are permitted to "think" of 1974-75 with Skylab B or using the left over command service module for experiments. 1974-77 is "thought of" as the era of the Bioresearch Module

RADIATION BIOLOGIC RESULTS OF SPACE FLIGHT

MERCURY AND GEMINI

    •RADIATION DOSES WITHIN EXPECTED LIMITS

    •NO RADIATION SEQUELAE

APOLLO

    •RADIATION DOSES WITHIN PREDICTED EXPOSURE VALUES AND SUBSTANTIALLY BEL

    OPERATIONAL LIMITS

    •NO RADIATION SEQUELAE

    •LIGHT FLASH PHENOMENON

    •HELMET TRACKS IN VIII AND XII

    •INDICATION OF INCREASED INCIDENCE OF HZE FLUX IN XIV VERSUS VIII

BIOSATELLITE II

    •NULL, SYNERGISTIC AND DESENSITIZING EFFECTS IN LOWER LIFE FORMS

    •HZE PARTICLES .... $Z \geqq 20 = 10,1/cm^2/45$ HOURS

BIOSATELLITE III

    •NO RADIATION SEQUELAE

    •HZE PARTICLES .... $Z > 20 = 18,0/cm^2/8.5$ DAYS

VIEWGRAPH 11

(BRM), which we will ad-
dress ourselves to further
and which, hopefully, you
will think about. The 74-
77 BRM is an automated
system, non-recoverable and
launched from Wallops on
a Scout vehicle which Dr.
Holton will discuss later.
In 1978, we are "thinking"
of a shuttle-borne Bio-
research Module. It would
be launched with the shuttle,
kept in orbit for a prede-
termined time, recovered,
and returned in the shuttle.
The first manned orbital
shuttle flight is
"scheduled" for 1978. The
Space Station Modules would

RADIATION EXPOSURES
BIOSATELLITE II

● RADIATION SECTION
  1. RANGE: 200R TO 3600R
  2. FLIGHT vs GROUND CONTROL; DIFFERENCE IN DOSIMETRY
     READING BY PERCENTAGE: 48 POSITIONS – 0 TO 4%;
   4 POSITIONS – 4% TO 8%

● RADIATION CONTROL SECTION
  1. RANGE: .15R TO .77R

● NUCLEAR EMULSION MEASUREMENTS
  1. TRAPPED PROTONS: 40 millirad
  2. GALACTIC HEAVY NUCLEI:

ELEMENTS	Z	FLUX, NUCLEI /cm^2
Li, Be, B	3, 4, 5	30.3
C, N, O, F	6, 7, 8, 9	108.0
Ne → K	10 → 19	27.3
Ca → Ni	20 → 28	10.1, MEASURED

VIEWGRAPH 12

be shuttle-borne, launched, and placed in orbit in "1980-90." Many of these
modules would make up the large space station which you saw in a previous
diagram.

All that I am trying to convey to you is extremely important and will have
bearing on what we are going to do for the next 2 1/2 days. I continue to
refer to the Radiation Biology Program because of the careful scrutiny and
review it is getting. I know what I am trying to do with that Program and I
realize how effective it has been so far. My purpose here, then, is to see
if we can do something similar with the Regulatory Biology Program of which
Depressed Metabolism is a primary facet, in my way of looking at it. When
we begin our actual conference, there are some things I will have to say
about what we anticipate.

BIOSATELLITE II RADIATION EXPOSURE
G-5 EMULSION
EARTH CONTROL   BIOSATELLITE II
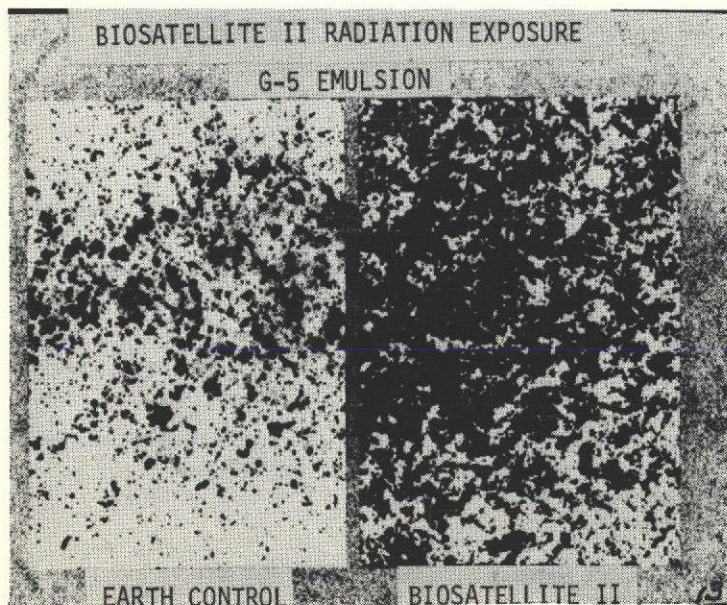
VIEWGRAPH 13

In the Mercury and Gemini
Programs we had no radiation
problems to speak of, at
least those that we looked
for (viewgraph 11). In
Apollo, we have had no seri-
ous radiation problems
either, except for the
light flash phenomenon, the
tracks that were observed in
the helmets of astronauts
during the Apollo 12 lunar
surface EVA, and an indica-
tion of the increased inci-
dence of the heavy particles
of galactic cosmic radiation
between Apollos 8 and 14
(none of these were seen in
the Apollo 7 or the Apollo

VIEWGRAPH 14

9 missions that were in Earth orbit). In Biosatellite II, we had positive results with an on-board source of γ-radiation delivered by ^{85}strontium. We found synergistic effects between radiation and presumably weightlessness. We found, too, that weightlessness desensitized certain biologic processes to radiation damage. We found that heavy particles, $Z \geq 20$, penetrated the spacecraft primarily as it passed through the South Atlantic Anomaly. In Biosatellite III, nothing happened to the primate insofar as radiation is concerned. However, we again observed evidence of these cosmic radiation particles and an increased flux over the 8.8 day period. The next viewgraph (12) shows some of the particles that we have recorded on Ilford emulsions. These are particles about which we are becoming concerned, particularly if they interact with and inactivate non-replicating tissues such as neurons, retinal structures of the eye, etc. These particles were actually measured. Based on these measurements, we now have calculations on those heavy particles which could be damaging. The next viewgraph (13) shows you one of the nuclear emulsions from Biosatellite II and the comparable Earth control. The next viewgraph (14) shows a heavy track on a nuclear emulsion of a particle about the Z number of calcium. We just do not know the biologic consequences should such a particle penetrate and traverse several layers of neurons in succession. Viewgraph 15 illustrates our radiation biology as it is today.

In terms of matrix we have worked with essentially all types of radiation. We have worked at the molecular and cellular level. The triangles, squares, diamonds, etc., indicate the functional entity such as mechanisms, linear energy transfer, genetic effects, ageing, acute effects, chronic effects, cell replication, or performance. The number inside of the geometric figure shows where the laboratory is and who is doing the work. We are looking at tissues and at systems such as


NASA RADIATION BIOLOGY PROGRAM
LEVEL OF BIOLOGIC ORGANIZATION
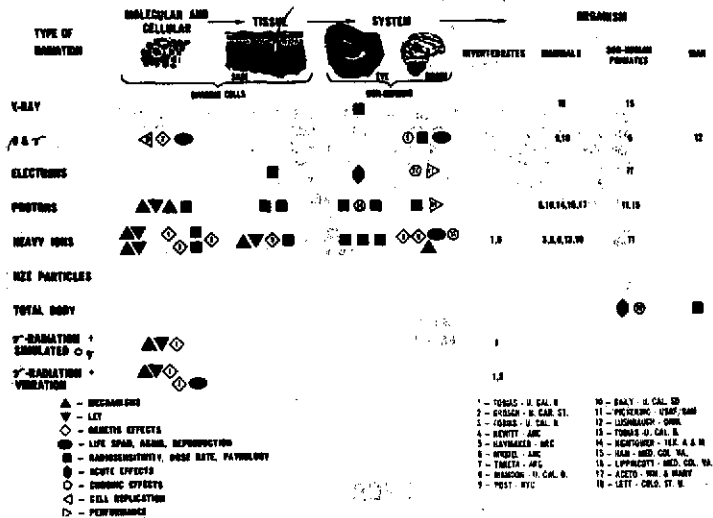
VIEWGRAPH 15

the eye and brain--the non-dividing types of tissues. We are working with invertebrates, mammals, sub-human primates, and man.

Earlier, I mentioned the Bioresearch Module. An example (viewgraph 16) of a BRM-type aboard a Scout, launched from Wallops, November 9, 1970, is this Orbiting Frog Otolith experiment flown by Dr. Gualtierotti from the University of Milan. Preflight, the bullfrog has this type of otolith response. During weight-

VIEWGRAPH 16

lessness at T + 48 hours, the response was still erratic, and it took about 54 hours, I believe, before it settled down. One of the conclusions, at least tentatively at this point in time, is that the vestibular system of the animal is the one that is the most sensitive to weightlessness. I mention this to you because the Bioresearch Module in an unmanned mode is the place for an experiment which I feel this group can design. This experiment could be launched, it could be productive, and it could give us information. That is the second point I would like you to think about. The first point is the matrix in so far as basic research is concerned - what are we doing, where should we be going, and is there anything we have missed. Secondly, the Bioresearch Module type of experiment.
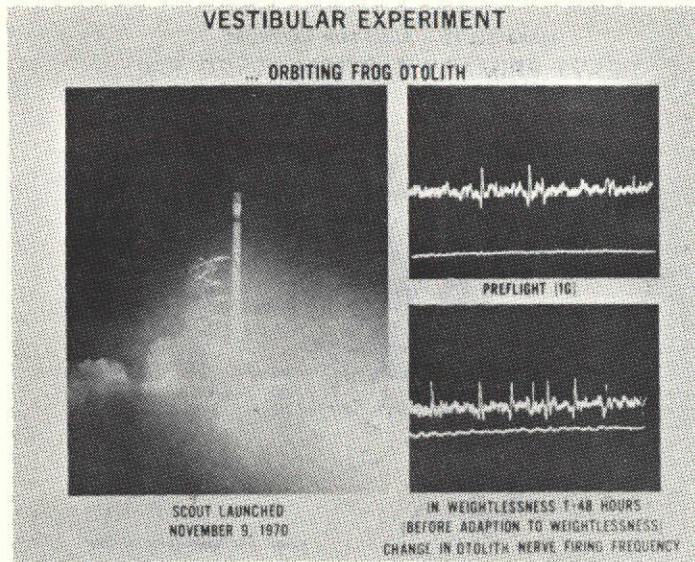
The next viewgraph (17) conveys the objectives of the Biology Program. This is the way I have set it up, initially at least. It is amenable to change, because I am sure that many changes will have to occur. The objectives include mechanisms which are probably 50% of the program, genetic disturbances based on the Biosatellite II and other experiments, and space radiation particularly the high energy particles of galactic cosmic radiation. The last point, I believe, is the one that you have to consider during this meeting and keep it in mind at all times. It is that this information is unobtainable on Earth.

VIEWGRAPH 17

10

BIOLOGY PROGRAM
BIORESEARCH DIVISION
OFFICE OF LIFE SCIENCES

PROGRAM MANAGER: JOSEPH F. SAUNDERS, PH.D.

OBJECTIVES:

ESTABLISH THE MECHANISMS OF FUNCTIONAL ADAPTATION AS INFLUENCED BY
SPACEFLIGHT VARIABLES AT DIFFERENT LEVELS OF BIOLOGIC ORGANIZATION

IDENTIFY POTENTIAL GENETIC DISTURBANCES IN LIVING SYSTEMS EXPOSED TO
SPACE FLIGHT

DETERMINE THE EFFECTS OF SPACE RADIATION ON LIVING SYSTEMS, ESPECIALLY
THEIR INTERACTION WITH HIGH ENERGY PARTICLES OF GALACTIC COSMIC RADIATION

UTILIZE THE SPACE ENVIRONMENT TO DEVELOP MEANINGFUL BIOLOGIC CONCEPTS
UNOBTAINABLE BY EARTH BASED RESEARCH

VIEWGRAPH 18

This is mandatory to over-
come the haranguing criti-
cisms, the questions and the
controversies, as to "why-
oh-why can't it be done in
an Earth-based Laboratory?"

This is the program (view-
graph 18) which is monitored
by Dr. Holton here at
Wallops Island. It is a
part of the Regulatory Biol-
ogy Program, involves you
people, and deals primarily
with problems of depressed
metabolism. It involves 5
university grants, 1 in-
house work unit, and 1 non-
profit organization. We
began with a Headquarters
guide of 250 thousand dollars for the program. The center response was for
280, not 250 as you see on the slide. The final allotment was 210 thousand
because of the budgetary constraints under which I had to operate.

Briefly, I would like to cite some of the effort in this program. The first
(viewgraph 19) is Dr. Popovic's research on cardiovascular adaptation to
weightlessness. He will tell you more about it later. This work was
summarized for the Congress last year in defense of the Biology Program.
Perhaps now you understand why we still ask for good pictures from which we
can make "pretty viewgraphs," ala Joe Musacchia. The next viewgraph (20)
is Dr. Musacchia's program which was submitted in our Congressional material
the year before. It depicts his hypothermia-helox technique vs. the normo-
thermic relative to protection against irradiation. Hypothermia-helox seems
to protect the animals.
Then, viewgraph (21), Dr.
Popovic's other aspect of
his program is differential
hypothermia and cancer
chemotherapy. I cannot
remember whose work is
illustrated by the next
slide, (viewgraph 22)
but it is somewhat re-
lated to Dr. Jordan's
work on the effects of
diluent gases. It does
not seem like the helium
depresses any of the
metabolic functions in
any significant way, un-
less you accept the 1°F
change in mean skin
temperature as significant.

VIEWGRAPH 19

11

DEPRESSED METABOLISM AND RADIATION PROTECTION

VIEWGRAPH 20

DIFFERENTIAL HYPOTHERMIA AND CANCER CHEMOTHERAPY

VIEWGRAPH 21

EFFECTS ON MAN OF 21% OXYGEN - 79% HELIUM
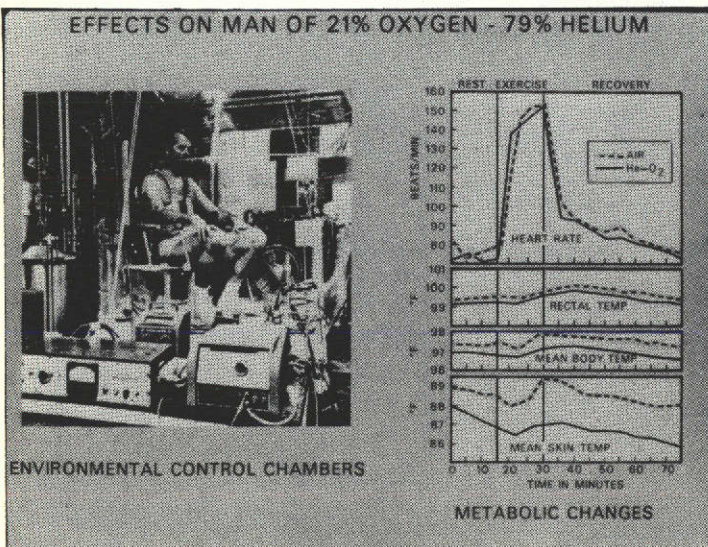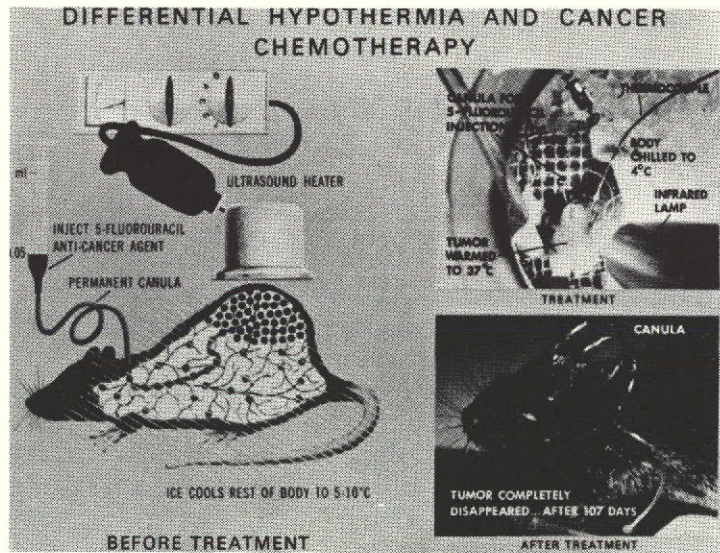
VIEWGRAPH 22

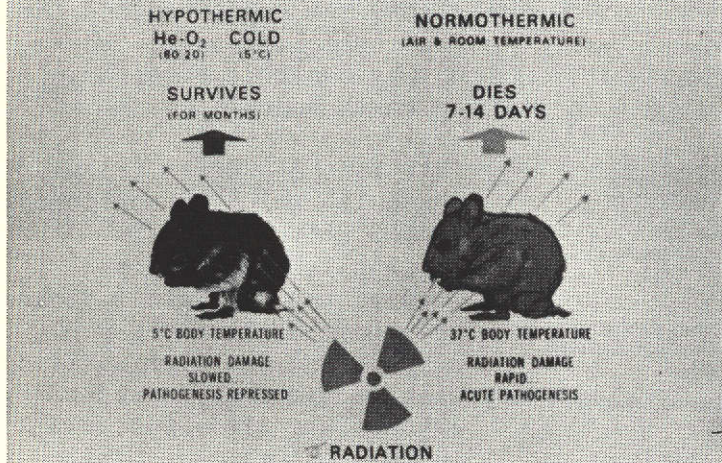The next viewgraph (23) is Dr. Smith's home furnace analogy where thermoregulation is compared to the thermostat of a furnace. He insists that you must treat each room individually. Another aspect of the diluent gases (viewgraph 24) is Dr. Sanford Siegel's in which he deals with embryonic systems and what happens to them in the presence of 100% oxygen vis-a-vis a normal atmosphere. I apologize to Dr. South for this older viewgraph (25). At this point, Frank was monitoring

VIEWGRAPH 23

nerve activity adaptation to hypothermia and the use of hypothermia as a means of radiation protection. The next viewgraph (26) is past history that has a strong bearing on this program. I mentioned successfully achieving acceptance of an experiment on Apollo 16. Two weeks ago today, I presented it before the Manned Space Flight Experiments Board. Prior to that the experiment was reviewed by an ad hoc panel which I convened. After the panel deliberated and recommended it, it went to a Life Sciences Committee. Finally, an evaluation was received from the Manned Spacecraft Center. After further review by Headquarters, we were asked to present it to the Manned Space Flight Experiments Board. The MSFEB following my presentation and scientific defense, approved the experiment. The Apollo Program Director then assigned it to Apollo 16. The experiment is from the University of Frankfurt and it is so simple and so beautiful that I cannot understand why one of us did not think of it. Viewgraph (27) shows the objectives of the experiment which are to study the interaction of heavy particles of galactic cosmic radiation at the molecular, cellular, and tissue level of biologic organization and to determine if, and why, and where these particles were doing any damage. Also, insight may be provided through this experiment so we might possibly duplicate them here in Earth-based laboratories. The biologic objects to be studied (viewgraph 28) are: (1) bacterial spores of B. subtilis; (2) plant seeds of
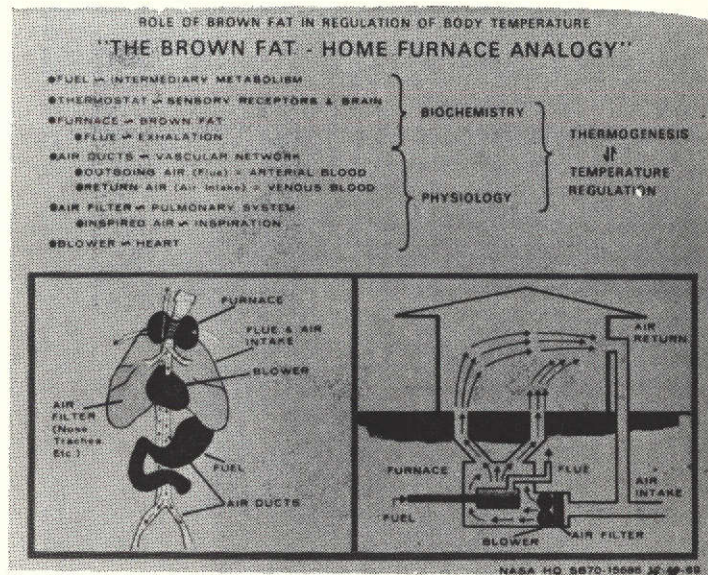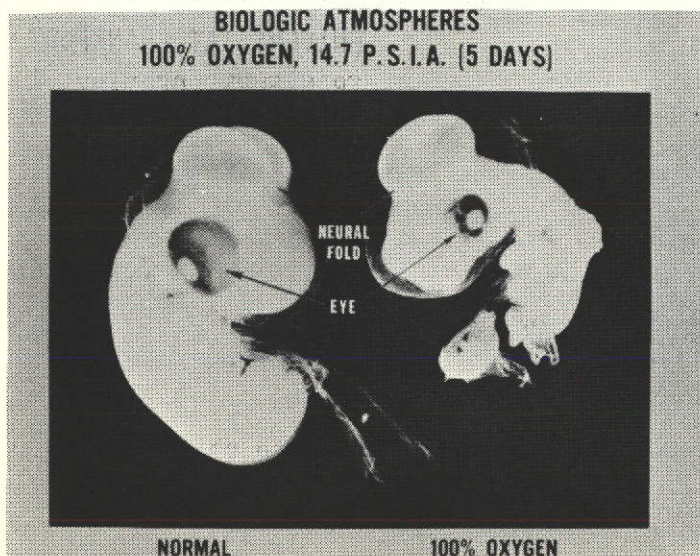
VIEWGRAPH 24

Radiation Protection During Hypothermia
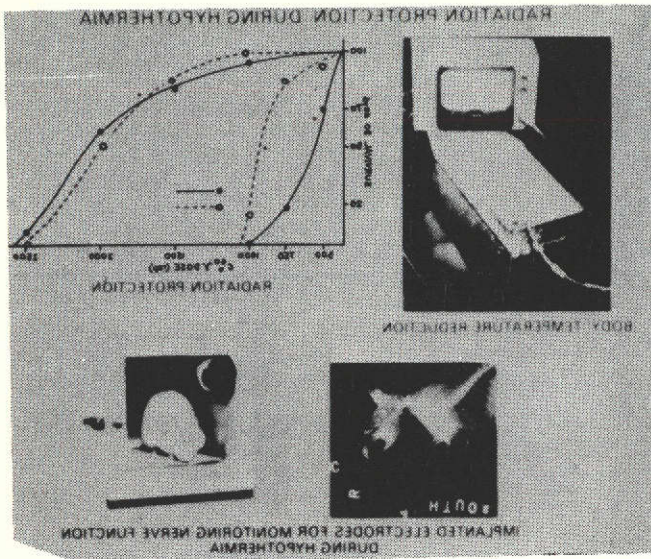
EXPERIMENT M 211

SHORT TITLE: - - - - - - - - - - - BIOSTACK

TITLE: THE COMBINED ACTION OF INDIVIDUAL HEAVY
NUCLEI OF COSMIC RADIATION AND SPACE
FLIGHT FACTORS ON BIOLOGIC SYSTEMS IN
THE RESTING STATE

PRINCIPAL INVESTIGATOR: DR. HORST BÜCKER
UNIVERSITÄT FRANKFURT
FRANKFURT, GERMANY

OBJECTIVES OF THE BIOSTACK

BIOLOGIC EFFECTS AT THE MOLECULAR, CELLULAR AND
TISSUE LEVELS OF INDIVIDUAL HEAVY NUCLEI OF
GALACTIC COSMIC RADIATION WHICH ARE NOT
OBTAINABLE FROM EARTH-BASED RADIATION SOURCES

SYSTEM	EFFECT TO BE OBSERVED	INVESTIGATOR	ORGANIZATION
BACTERIAL SPORES BAC SUBTILIS	INACTIVATION OF GROWTH AND CELL DIVISION	H. BÜCKER G. HORNECK	UNIV OF FRANKFURT
PLANT SEEDS ARABIDOPSIS THALIANA	INFLUENCE ON GERMINATION, GROWTH AND DEVELOPMENT-MUTATION INDUCTION IN F_1 AND F_2 GENERATIONS	E. REINHOLZ	MPI BIOPHYS FRANKFURT
RADICULAE OF VICIA FABA	NUCLEAR DAMAGE	H. SCHEUERMANN	TU OF HANNOVER
ANIMAL EGGS ASCARIS LUMB. ARTEMIA SALINA	INHIBITION OF DEVELOPMENT	W. RUTHER	UNIV OF MARBURG

VIEWGRAPH 28

Arabidopsis thaliana, the European watercress variety; (3) embryos of the bean, Vicia faba; and (4) encysted eggs of Artemia salina, the universal brine shrimp. A consortium of universities in Western Europe is collaborating in the effort. The BIOSTACK is depicted schematically in viewgraph 29. It measures about 10 cm by 10 cm by 10 cm. The organisms are sandwiched between layers of photographic emulsions and plastic detectors. If you look at the Arabidopsis thaliana section, you find layers of the plastic and nuclear emulsions, then the seeds, then again the layer of plastics and emulsions, then again the seeds, etc. Each experimental object will be treated in a similar manner so that one can track the particles and correlate their angle of penetration of the biologics. If the particles are very high energy and high velocity, they might go all the way through, or they might be stopped in the bean or the other systems, leaving a track or an ender, a lesion caused by a stopping particle. The container (viewgraph 30) is a hermetically sealed container made of aluminum. The BIOSTACK will be situated inside and sealed. The next viewgraph (31) gives you a different view of it, showing the outside dimensions of 125mm by 98mm. It will be mounted on the command module wall as clear as possible from any shielding so that the cosmic particles coming in can get to it. The next viewgraph (32) shows some of the package characteristics. (I am speaking to this experiment, deliberately, to show some of the processes of experiment selection.) I want you to think about dimensions, about requirements, about developments, and about the characteristics. The next viewgraph (33) will show you why BIOSTACK had little resistance to acceptance for flight on board the Apollo. It is completely passive. It is hermetically sealed. It requires nothing of the astronaut. It needs no command module power, no life support system, no mission-time measurements (that is mission-time while the spacecraft is on orbit), and, it requires no special spacecraft orientation. There are a few constraints,

SCHEME OF THE BIOSTACK

VIEWGRAPH 29
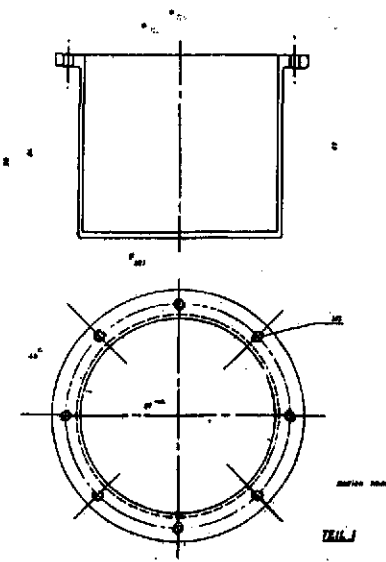
VIEWGRAPH 30

BIOSTACK HOUSING
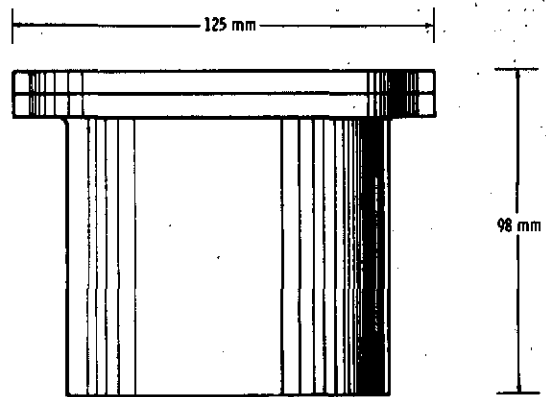
|←————— 125 mm ———————→|

98 mm

VIEWGRAPH 31

VIEWGRAPH 32

BIOSTACK EXPERIMENT COMPLETE

WEIGHT   =   2.4 ± 0.24 Kg

DIMENSIONS   =   125 MM (DIAMETER)
                          X 98 MM (LENGTH)

VOLUME   =   1000 CM^3

SHAPE   =   CYLINDER

16

however. It requires protection from extremely high temperatures, that is those greater than 100°F. It must be located where there is minimum shielding to the cosmic radiation, preferably on the bulkhead of the spacecraft and not down in a storage compartment. And, we do not want it exposed to other sources of radiation like some of the gammas or betas that are on board the spacecraft. This project (viewgraph 34) involves participation between two nations. The Germans have agreed that they will fabricate the flight hardware, they will prepare all the biologics, they will assemble the complete BIOSTACK in Germany (while we have our NASA people there during the assembly to insure that it meets all the reliability and quality assurance requirements), and then it will be transported to Florida to the Kennedy Space Center for flight. In the meantime, we will perform all testing, we will provide the documentation, we will integrate it with the spacecraft, and we will install it for launch. We will remove it at recovery and deliver it to the investigators. It will never be opened in this country. It will be sealed in Germany prior to flight and then opened in Germany following the mission. The next viewgraph (35) shows you the schedule for Apollo 16; schedules are critical. The German scientists were so determined that they were able to meet the schedule for Apollo 16. After the mission, the principal investigator has one year to complete his experiment and submit his final report to NASA. He must provide us with 30 day interim reports until his final report is published.

Another experiment we are defining (viewgraph 36) is that wherein the pocket mouse will be used to study cosmic radiation effects on the brain and eyes. Dr. Oyama is familiar with this experiment since it comes from his Center under the direction of Dr. Webb Haymaker. I am discussing this package because it opens up the possibility that some of the biologic

SPACE FLIGHT REQUIREMENTS AND CONSTRAINTS

REQUIREMENTS

- IS COMPLETELY PASSIVE
- IS HERMETICALLY SEALED
- REQUIRES NO ASTRONAUT TRAINING OR MISSION PARTICIPATION
- REQUIRES NO C/M POWER
- REQUIRES NO LSS
- REQUIRES NO MISSION-TIME MEASUREMENTS
- REQUIRES NO SPECIAL S/C ORIENTATION

CONSTRAINTS

- REQUIRES PROTECTION FROM EXTREME HIGH TEMPERATURE
- C/M LOCATION WITH MINIMUM SHIELDING TO COSMIC RADIATION
- NO EXPOSURE TO OTHER SOURCES OF RADIATION

VIEWGRAPH 33

BIOSTACK DEVELOPMENT

IN WEST GERMANY, THE PRINCIPAL INVESTIGATOR

FABRICATES   Flight Hardware
PREPARES   Biologics
ASSEMBLES   COMPLETE BIOSTACK, including leak tests under NASA R&QA supervision
TRANSPORTS   BIOSTACK (3 units) TO KSC

THE MANNED SPACECRAFT CENTER

Performs QUALIFICATION testing
Provides DOCUMENTATION
Installs BIOSTACK in C/M

VIEWGRAPH 34

4. SCHEDULE AND RESOURCE REQUIREMENTS – DEVELOPMENT SCHEDULE

Major Milestones	PLANNED DEVELOPMENT SCHEDULE											
SPACECRAFT MODULE SUMMARY	FY 71				FY 72				FY 73			
	QUARTERS				QUARTERS				QUARTERS			
	1	2	3	4	1	2	3	4	1	2	3	4
Experiment Approval (MSFEB)						X						
CCA Issued						X						
PI Contract Let						BA						
Hardware Contract Let						X						
Integration Specification Issued						X						
ICD Complete						X						
DEP Complete						X						
Mockup Delivered						X						
Prototype Delivered						X						
Crew Training Unit Delivered						X						
Quality Testing Completed						X						
Flight Units Fabrication & Delivery							X					
Acceptance Test Complete							X					
Ground Support Equipment Delivered						X						
Installation & Checkout							X					
Shipped to Launch Site							X					
Flight Analysis Complete									X			
Final Report											X	

VIEWGRAPH 35

CLOSED AND OPEN PACKAGE CONCEPTS FOR COSMIC RAY EFFECTS EXPERIMENT
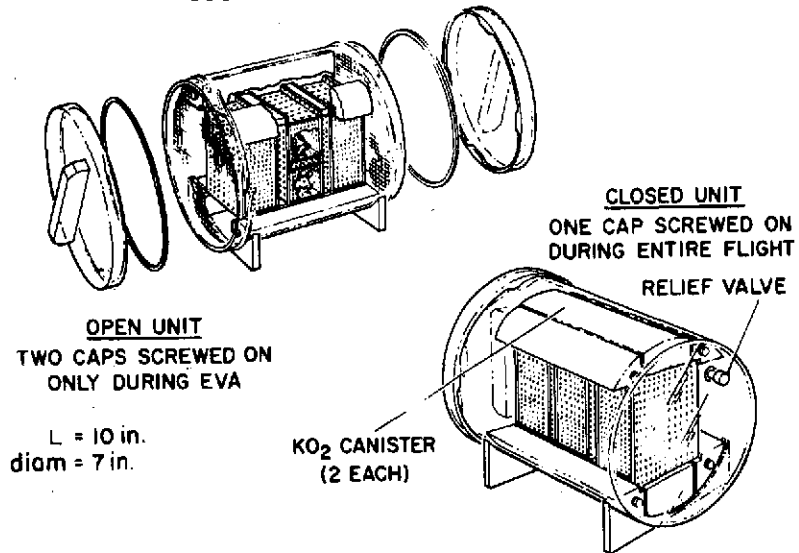
OPEN UNIT
TWO CAPS SCREWED ON ONLY DURING EVA

L = 10 in.
diam = 7 in.

CLOSED UNIT
ONE CAP SCREWED ON DURING ENTIRE FLIGHT

RELIEF VALVE

KO_2 CANISTER
(2 EACH)

VIEWGRAPH 36

18

INTAKE

FOOD:	1.0 gm/day Dried sunflower seeds	30.0 gm
OXYGEN:	2.12 gm/day	63.6 gm
WATER:	None required	0
	TOTAL	93.6 gm

OUTPUT

CO_2	2.4 gm/day	74.4 gm
FECES:	0.1 gm/day (dry and odorless)	3.0 gm
WATER:	0.49 gm/day (as vapor)	14.7 gm
URINE:	0.05 gm/day	1.5 gm
	TOTAL	93.6 gm

POTASSIUM SUPEROXIDE (KO_2):

356 gm to absorb

2.457 gm/day of CO_2
0.497 gm/day of H_2O*

* URINE (0.05) + WATER (0.49) - VENTED H_2O (0.043)

VIEWGRAPH 37

systems with which you work might be accomodated by it. This experiment uses the little pocket mouse, about a 10 gram animal, which is sometimes incorrectly called a kangaroo mouse because of its kangaroo-like characteristics. It requires hardly any life support system other than sunflower seeds mixed with several other types of seeds. This experiment was designed to fly on Apollo as a closed system, using potassium superoxide for life support. The seeds are there, he needs no water and his feces and urine excretion are minimal. I believe that the mice have been kept in this cage for about 6 weeks without any problems; however, the cages were not completely closed. Some of the characteristics of the little pocket mouse are shown in viewgraph 37. Notice the urine output per day. He conserves water metabolically for some reason and by mechanisms unknown to us.

Moving from these proposed experiments, we have several that will fly on Skylab A. Again, we can illustrate the benefit of microminiaturizing something complex and utilize it on board a spacecraft to answer a question assumed to be critical. The experiment which I selected as an illustration deals with the biochemistry and physiology of human kidney cells (viewgraph 38) and the effects of weightlessness on their metabolic function, cytoarchitecture, and replication. Dr. Montgomery of the University of Texas will have 24 biopacks inside the main housing. The inset depicts a single biopack. Each one will deal with a different type of experiment, using a different radioactive isotope for tracing and mapping discrete intermediary metabolic phenomena. What can we do with the ideas that we generate and the plans that we may have? The next viewgraph (39) shows the principal areas in my program. When I was reassigned from OSSA to the Office of Life Sciences to reorganize the program in biology, I

VIEWGRAPH 38

PROGRAM ELEMENT	CENTER $ - FY72				FY71 $		FY72	FY72 $ o/o of FY71
	ARC	MSC	WS	HQ	OTHER	975		
REGULATORY BIOLOGY	515		210		1250	190	725	58%
DEVELOPMENTAL BIOLOGY	245	100			850	20	345	41%
RADIATION BIOLOGY	305	485		165(a)	1500		955	64%
GENETIC BIOLOGY	55				600	145	55	9%
RESERVE				70			70	
	1120	585 2,150	210	235	4,200	355	2,150	

VIEWGRAPH 39

BIOLOGY PROGRAM

NATURE OF WORK DISCONTINUED IN FY 72

PLANT RESEARCH	215K	5 TASKS
THERMOBIOLOGY	110K	3 TASKS
THEORETICAL BIOLOGY	251K	3 TASKS
NUTRITION	40K	1 TASK
MSFC PAYLOAD INTEGRATION	200K	BLUE BOOK SUPPORT
EXPERIMENT DEFINITION (ARC)	180K	BLUE BOOK SUPPORT
APPLICATIONS	213K	3 TASKS
TOTAL	1209K	

VIEWGRAPH 40

20

inherited a total of 4
million, 200 thousand
dollars worth of effort.
I was given a budget of 2
million, 150 thousand
dollars. You can see
that I faced a dismal pic-
ture. There have been
projects that had to go by
the board (viewgraph 40).
One million, 209 thousand
dollars worth of work had
to be stopped immediately
or, at least, gradually
phased out in the next
fiscal year. Many tasks
are being continued. You
can see what impact this
has (viewgraph 41) had.
Four universities were

BIOLOGY PROGRAM
IMPACT
FY 71 (4.2M) vs FY 72 (2.15M)

	No. Institutions Affected	No. Tasks	$ (K)	Reductions $(K)	Total Decrease $(K)
UNIVERSITY	4	8	381	1134	1515
INDUSTRY	3	3	76	0	76
OTHER GOVERNMENT	-	-	-	75	75
IN-HOUSE	2	8	384	0	384
TOTALS	9	19	841	1209	2050

VIEWGRAPH 41

affected, 3 industrial organizations, and 2 in-house efforts. The number of
tasks was 19. The dollars--851 K. The total decrease over what I had to
work with in '71 was 2 million, 50 thousand dollars.

I hope that I did not bore you with the "nitty-gritty," but I planned this so
that you would have some cogent idea as to why we wanted to get together with
you, some idea as to what the options are, what we have in mind, what the
alternatives might be, what we can do to get the most out of our research,
how we can change the program if it needs to be changed, where can we put
the emphasis, where should we deemphasize, and particularly, how can we
collaborate in terms of a real, useful, bona fide, space flight experiment
using the science and the technology for which you have the expertise.

DISCUSSION BY PARTICIPANTS

MUSACCHIA: Before we get into thinking about space flight experiments, I thought it might be a nice idea to tell us again what types of space flight potentials would be open to us. In other words, you have a biomodule of some type, apparently they are going to bring experiments aboard Apollo missions, that's 2 -- there should be a list of available types of flights projected for us.

SAUNDERS: Let me put them on the blackboard and keep them there. Joe, that is a real good question, but unfortunately I can't answer it. All I can do is refer you to that slide that had planning options. The first option as I see it is Apollo 17 in December of '72. Skylab A I can't say anything about since it is full, but we do have 3 basic biology experiments aboard -- the pocket mouse circadian rhythm experiment, the vinegar gnat circadian rhythm experiment, and the tissue culture experiment which I described. Skylab B is a possibility, if there is such a thing, but it would not be flown until possibly '74 - '75, hopefully '74. We are told that if there is a Skylab B and if we want to fly those experiments that are already on board, there probably would be no problems, since the hardware is built, the integration has already been done and there will be minimal cost. If we want others, we have to start working on them now! For Skylab A, the 2 circadian rhythm experiments will not be recovered since they are in the service module rather than the command module where the tissue culture experiment is. If you fly experiments on Skylab B -- and there is no doubt in my mind that most people would like to have them recovered -- we face a problem relative to having them returned in that small command module where space is at a premium. The Bioresearch Module, if approved, will be launched on a schedule of one a year from '74 through '77. It will be launched here at Wallops as an automated, non-recoverable type. The shuttle-borne Bioresearch Module, I feel, is the one that most of you would be interested in, since it has recovery capabilities. And, of course, if it goes shuttle-borne, the payload weight and volume increase 3 to 1 over the non-recoverable type because much of the life support systems, power, and other requirements will be of a plug-in type for the shuttle. After that, it is the shuttle from '78 on. We believe that between '78 and '82 there will be 5 shuttle types dedicated to Life Sciences Payloads using what we call the RAM (research applications module) which is 15 feet in diameter and 58 feet long. I believe that it could handle a lot of life sciences, including man. After the shuttle, there is the space station in the late '80s and the 90s, perhaps. There are 2 life sciences laboratories in space station program planning. That means that the life sciences lab would be on orbit for 2 years with a projected life time of 5 years with revisit capabilities. I hope that gives you a better feel for what the possibilities are. Here we sit in 1971 planning for 1990 when we might not even be here to see it.

JORDAN: How about moon-based operations?

SAUNDERS: They are not in the current thinking as a real, concrete option, but there are still plans and ideas. Dr. von Braun is still quite enthusiastic. I have never seen previously such support or enthusiasm, as is displayed now by the people working under him, to get something done for Life Sciences.

Wallops and its Role in Depressed Metabolism

Dr. Emily M. Holton
NASA/Wallops Station
Wallops Island, Virginia 23337

We are very pleased to have all of you at Wallops for this meeting. I am not really intimately involved in research at the present time but I am in the process of setting up facilities so that hopefully in the near future we will able to do some experiments. So, rather than emphasizing this progress this morning, I thought it might be of interest to tell you a little about Wallops, where your grants will be monitored, and of some changes in the granting procedures.

Wallops was established in 1945 as a branch of Langley Research Center in Hampton, Virginia, under the auspices of the National Advisory Committee for Aeronautics. In 1958, when NASA was established by Congress, Wallops became a station unto itself and was no longer a branch of Langley Research Center. At this time, the Station consisted solely of the island. Wallops was looking around for land on which to place their main facilities when the Chincoteague Naval Air Station closed. NASA acquired the naval base; that property is presently the main station or main base where you are today.
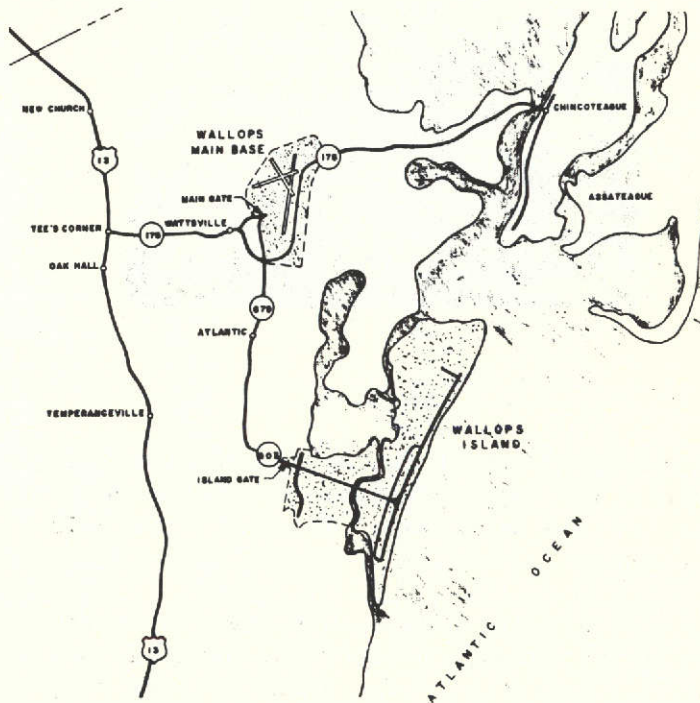
SLIDE 1

The first slide shows you the 3 areas of Wallops: the main base, which is the old Chincoteague Naval Air Station; the mainland, about 7 miles SE of the base, where the long range radars and the optical tracking facilities are located; and the

SLIDE 2

23

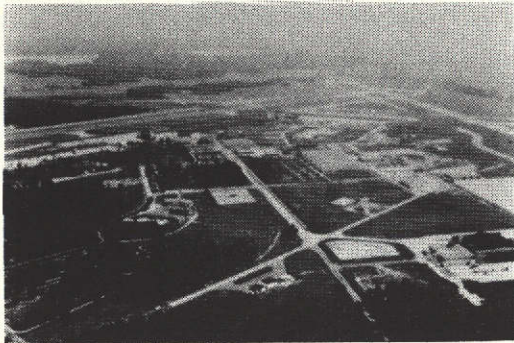island which is across the causeway from the mainland and is the site of
the launch facilities. The next slide (2) shows the island and the
various launch facilities, the dynamic balance facility, the blockhouses,
etc. If we have time, we might take a trip there tomorrow to show you
some of the assembly houses and the various stages of preparation for a
launch. The next slide (3) shows the main base. The airport facilities
stand out noticeably and were maintained from the naval station era.

Wallops is presently
establishing a research
program utilizing the
airport facilities and
involving not only air-
craft but also projects
requiring long paved
surfaces. For example,
some work on truck noise
is being done presently.
The big semitractor
trailor must have ample
room to accelerate to
70 mph and stop. Noise
abatement studies in
aircraft have been con-
ducted as well as some
testing phases on the
vertical short take off
and landing (V/STOL) air-
craft. The administration

SLIDE 3

buildings, most of the shops (machine, electrical, etc.), the biology
laboratory, chemistry laboratory, range control center, etc., are located
also on the main base. These will be pointed out during our tour
tomorrow.

Wallops primary mission has been to assemble, prepare, and launch experi-
mental payloads, to track the payload, and to acquire and process data
for the scientist. The scientist was responsible for any further re-
duction of data and interpretation of the results. Any bona fide
scientist can launch a rocket from Wallops; this means not only NASA and
government personnel, but scientists in the academic community, industry,
etc., with approved programs can get launch support. So far this year
(through August), we have launched 369 rockets. These were mainly test
rockets and small meteorological rockets, but it has been a very busy
year as we have already launched 3 Scout vehicles. The Scout is the
largest rocket launched from Wallops and we will discuss this vehicle
further tomorrow.

Wallops direction is changing somewhat from that of a strictly mission
oriented station. In July of this year, Headquarters approved an Applied
Sciences Directorate (slide 4). This new Directorate was triggered
primarily because the NASA center in Cambridge, Massachusetts, the
Electronics Research Center (ERC), was closed and 9 research oriented
scientists from ERC transferred to Wallops. You will notice that most of

the other directorates
are launch oriented while
the Applied Sciences
Directorate is research
oriented. This directo-
rate is the smallest
consisting only of 15
persons (somewhat less
than 500 people are
employed at Wallops).
No branches have been
assigned to this direc-
torate at the present
time. Most of the
people in the Applied
Sciences Directorate are
so to speak one-of-a-kind,
although the areas of
interest are compatable
and complimentary; for
example, I am the only
Life Scientist amidst

SLIDE 4

engineers, data processing people, physicist, astronomer, etc.
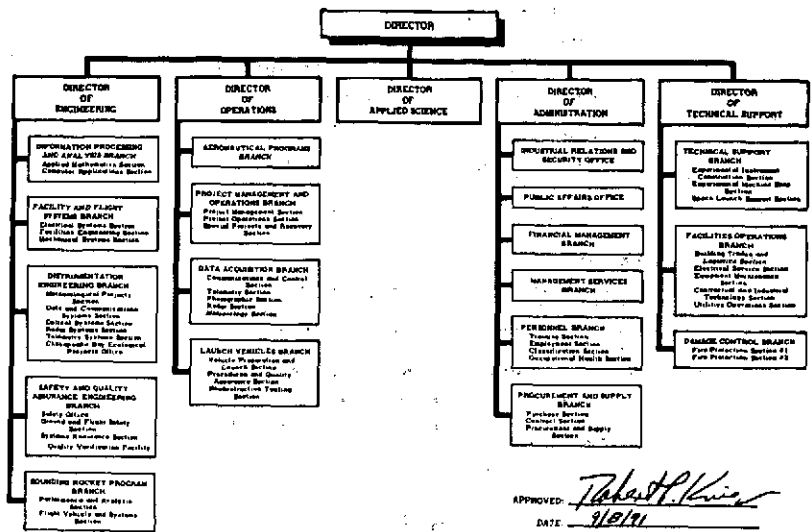
This is the first year that Wallops has administered grants. So, I
imagine that initially the road may be slightly rough but we'll iron out
the troubles as quickly and pleasantly as possible. At this time, I'd
like to introduce you to the gentleman at the back of the room, Mr. Elton
Scott, head of our contracts section. He will be the man with whom you
will be corresponding on your grants and renewals.

SCOTT: I probably will not be handling these grants myself, but for
any questions you might have contractually I would be glad to help work
your problems out. There are 5 or 6 grants that are being transferred
from NASA Headquarters and we hope to have them here by sometime this
week.

If you have any questions, I'm sure you will find Mr. Scott very amicable
and helpful. The next slide (5) shows what we are going to ask of you for
renewals and status reports. Your grants will be handled entirely at
Wallops; they will not go to Headquarters, they will come directly here.
For renewals, we would prefer that you send a letter containing these
3 items: (1) reference to the original grant or a later status report
depending upon which contains the protocol for the ensuring grant year,
(2) detail any major alternations, and (3) outline the budget. Seven
copies of the letter should be sent, 5 to Mr. Scott at the Contracts
Section at Wallops, 1 copy to me, and 1 copy to Dr. Saunders.

SAUNDERS: That letter doesn't have to be limited to a sentence or
small paragraph.

SLIDE 5

The renewal request, according to the Headquarters brochure, should be received at least 4 months prior to funding. From the date we receive this letter until the date you receive your funding will be on the order of at least 2 months. So, to avoid deadline delays, we are asking for your request ahead of time. Concerning the biannual status report, we are asking that you continue with your former protocol. We are not asking for any changes since letting a person report in the style with which he feels most comfortable is probably the proper approach. These reports are due every 6 months. We are again asking for 7 copies* but only 3 copies with reprints, preprints, etc. Send one copy with reprints to me, one to Dr. Saunders, and the remaining copies to Mr. Scott. If it is easier to send all copies with the reprints, etc., this is also perfectly acceptable; the only reason for suggesting just 3 such copies is to save you money and reprints.

Tomorrow we'll show you the existing chemistry and biology facilities. We are in the process of procuring equipment for the labs. When the equipment is obtained, we hope to set up some screening experiments concerning the effects of hypothermia and hibernation on mineral metabolism. Initially, this will be our primary area of endeavor at Wallops. Equipment procurement can be quite slow and quite frustrating. Presently I am doing a literature survey to familiarize myself with the area. A MEDLARS search for the years 1968-1971 on hibernation and hypothermia was conducted and we found 236 apparently pertinent references. The search was conducted in April and to date I have received 60 of these 236 references; since they must be obtained through the library, it's a bit slower. When our laboratory facilities are finished and ongoing, the facilities will be available to you for any studies you might wish to conduct here with equipment that is not available in your laboratory. We will be glad to help you with your research in any way that we can. We hope this will be a totally cooperative venture. I certainly hope that we have a long and very productive relationship.

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*This has been changed to 2 copies; one copy of the status report to Dr. Holton and one to Dr. Saunders. Mr. Scott should only receive the status report connected with the renewal.

Effect of Artificial Gravity
on Thermoregulation, Respiratory Metabolism
and Intermediary Metabolism of Animals

Dr. Jiro Oyama
Environmental Physiology Branch
NASA Ames Research Center
Moffett Field, California 94035

I would first like to make some brief comments about the chronic accelera-tion biology field which has developed into some prominence over the last 10 years or so. There are 3 major research facilities in the United States that are currently active in chronic acceleration research. They are at the University of Iowa (Dr. Charles Wunder), the University of California Davis (Dr. Arthur Smith), and our group at Ames Research Center. We've been involved with chronic acceleration work for about 8 years. The first three slides will give you an idea of our centrifuge facility at Ames. The first slide shows a centrifuge that we have been using in some of our earlier acute and chronic

studies. This is an 8-foot radius centrifuge. To expose animals to a different hypergravity environment, you can vary either the rpm or the radius arm. In this slide are shown cages that are mounted at 8-feet and 6-feet. There is also a 4-foot radius position which is not shown. This centrifuge was operational for about 6 years; it has now been decommissioned. The cages are on pivots and swing out with an increase in the rpm. The resultant G-vector

8-FT RADIUS ANIMAL CENTRIFUGE
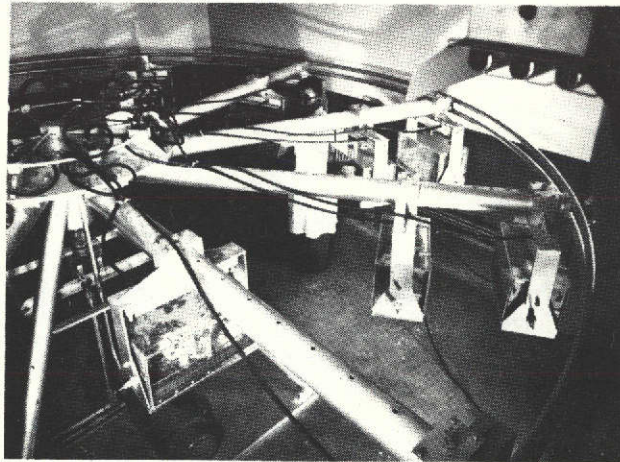
SLIDE 1

is normal to the cage floor. In chronic acceleration, what we are dealing with is the effects of a directional force field which is normal to the animal relative to the cage floor. This centrifuge had to be stopped period-ically, once or twice a week, for about 30 minutes. We have subsequently developed a major centrifuge facility for chronic work which is shown in the next Slide (2). This slide shows an overview of a 52-foot diameter centri-fuge. It enables us to centrifuge animals continuously without any stoppage whatsoever. The next Slide (3) shows the interior of the centri-fuge which gives you a visual feel for its size. The cages are along the periphery. The center platforms allows one to go on and off the
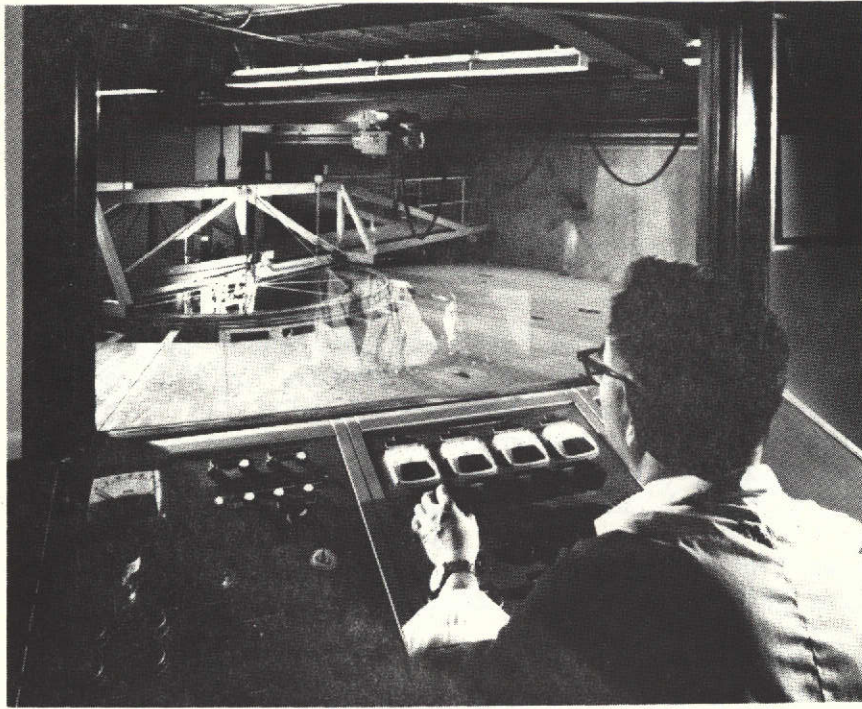
SLIDE 2
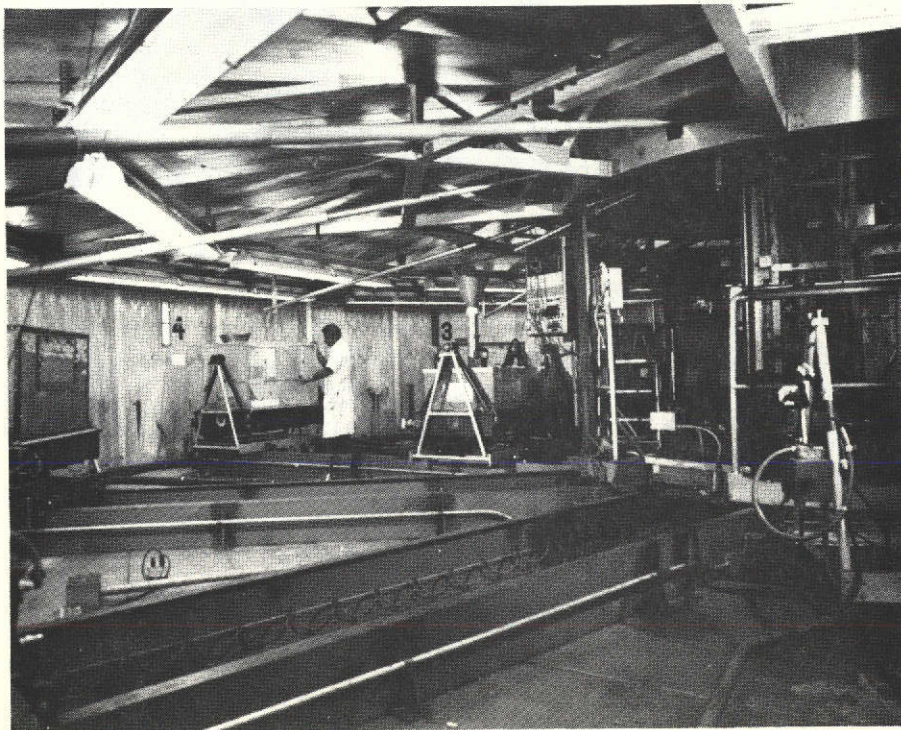
SLIDE 3

centrifuge while it is
rotating. These cages
are fairly large. We
have been working with
dogs primarily on this
centrifuge having gradu-
ated from rats and
mice.

The studies which I am
going to report on this
morning are largely un-
published. Some of the
data will be published
shortly, but most of the
material presented here
is unpublished. When
one exposes mammalian
organisms to radial

STRESS
IMPOSED

FUNCTION
RESPONSE

ADAPTATION

ACUTE
RESPONSE

RECOVERY

EXPOSURE TIME

VIEWGRAPH 1

acceleration (centrifugation) there are three distinct stages that the
animals undergo upon the imposition of the G load. Generally (viewgraph 1),
if the functional level here is taken as the precentrifugation value,
you will find an immediate stress response stage. In rats, mice, and dogs,
this can last for as long as 2 weeks, but generally the most dramatic changes
take place within several minutes to several hours. Then you have a
second stage, a recovery stage, which may again take as long as 14 days
or may be shorter depending upon the intensity of the G load. The third
stage we might appropriately call adaptation; this stage generally occurs
after 2 weeks. We've had animals subjected to life-time exposures and
you can consider them fully adapted. This is a general pattern of response;
it can be either positive or negative. Although this is an example of a
negative response, the increase in blood corticoids would be a response
in the positive direction.
These then are the general
stages in the response of
an animal to increased
gravity.
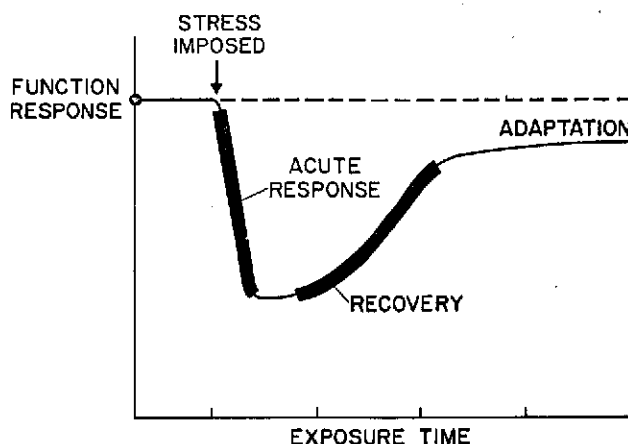
First, I'd like to present
some representative studies
that we've done on the
acute response stage and
then describe some of
the effects of prolonged
acceleration.

POPOVIC: What happens after
you interrupt acceleration
at the end of the adaptation
for a brief period? Do you
see similar changes?

RESPONSE TO HYPERGRAVITY

ACUTE	CHRONIC
↓ FOOD + WATER CONSUMPTION	↑ FOOD + WATER CONSUMPTION
↓ RESPIRATORY METABOLISM	↕ ↑ RESPIRATORY METABOLISM
↕ ↓ BODY TEMP	N/C BODY TEMP
↓ HEART RATE	N/C HEART RATE
↓ PHYSICAL ACTIVITY	? PHYSICAL ACTIVITY
↑ PITUITARY-ADRENAL FUNCTION	N/C PITUITARY-ADRENAL
↑ GLUCOSE MOBILIZATION	↕ ↓ GLUCOSE METABOLISM, TOLERANCE
↑ FAT MOBILIZATION	↓ FAT RESERVES
↓ URINE OUTPUT	↑ URINE OUTPUT
↓ ENERGY RESERVES	↓ ENERGY RESERVES
↓ BODY MASS	↓ BODY MASS
	↑ SELECTED ORGANS, (RELATIVE MASS)

VIEWGRAPH 2

OYAMA: There are differences that perhaps I'll report upon a little later.

Next viewgraph (2). We've covered a number of areas and this is a tabulation of some of the things that we have worked upon over the past 8 years or so. Here, I've arrowed some of the material that I'll cover in this morning's presentation: body temperature, some aspects of respiratory metabolism in chronically adapted animals, and some aspects of glucose metabolism again in the chronically adapted animal. Note here in the acute stress response stage that decreases occur in food and water consumption, respiratory metabolism, body temperature, heart rate, and physical activity; increases are seen in pituitary-adrenal function, glucose mobilization (a transient hyperglycemic stage), liver glycogen formation, and fat mobilization from changes in the free fatty acid levels in the plasma of these animals, i.e. increase in lipolysis. We have a transient anuria, a decrease in energy reserves and also body mass.

In the chronically exposed animal, what is rather interesting is that opposite to the acute effects, you do get an increase in respiratory metabolism which I'll cover later. There is essentially no change (N/C) in body temperature or heart rate. We do not know what the level of physical activity is, but we hope to record it shortly. We see no change in the pituitary-adrenal system. In terms of the glucose metabolism of these animals, they show an increase in oral glucose tolerance and an increased sensitivity to insulin. We also see a decrease in the fat reserves, an increase in urinary output, while the total energy reserve is lowered. A characteristic finding in chronic acceleration exposed animals of all types is a decrease in body mass. We also see selected increases in organ to body weight ratios.

The next viewgraph (3) shows a schematic diagram of the biotelemetry system that we have employed in our centrifuge temperature studies. A rat is usually implated about 2 weeks before we put the animal on the cen-

trifuge to get some baseline data. The shielded cage is placed on the centrifuge along with a receiver and demodulator. The slip ring assembly permits us to transfer the signal obtained from the animal off the centrifuge to the signal conditioner. The analog signal is converted to a DC output which is either stored on magnetic tape or punched on paper for analysis. The major effect of high G loads on a rat is shown in

BODY TEMPERATURE TELEMETRY SYSTEM FOR CENTRIFUGE
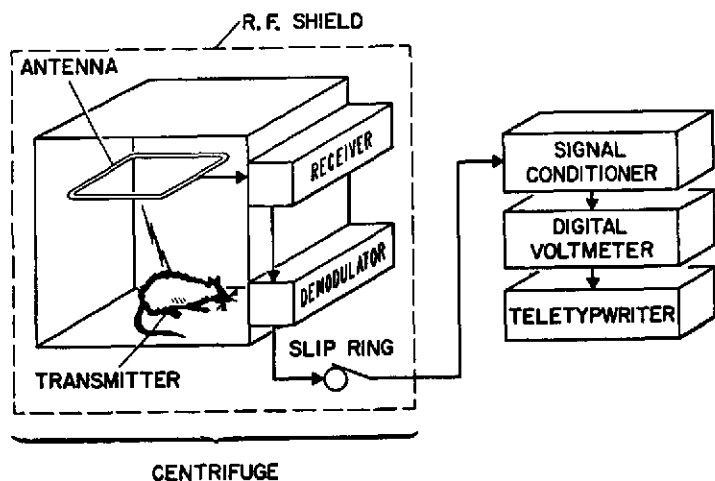
VIEWGRAPH 3

slide 4. On the vertical
axis, we have the deep
body temperature, (the
thermistor is placed in
the abdominal cavity);
on the horizontal axis,
we have a time period of
approximately 4 days.
The black horizontal
bars represent the 12
hours of night, the rest
represents the day--the
day-night cycle. The
black hatched curve,
which you will also see
in subsequent slides,
is the mean diurnal
temperature rhythm
taken for a period of
9 days before the ani-

TEMPERATURE OF FEMALE RAT
1st TIME PLACED ON CENTRIFUGE 2.5G
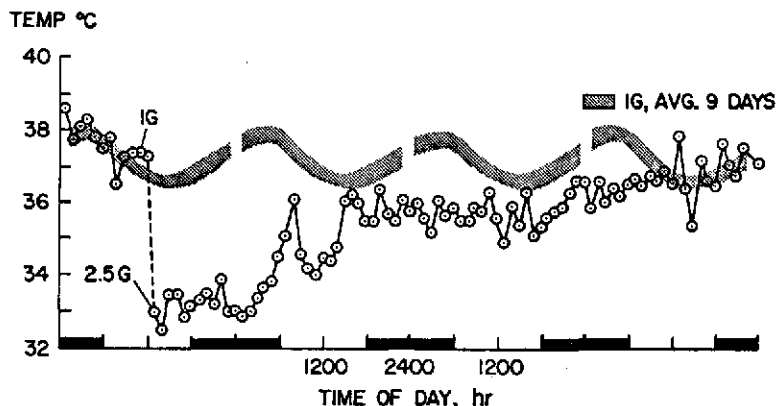
VIEWGRAPH 4*

mal was exposed to the G load. On the day of the centrifugation, the initial
values shown are hourly temperatures just before the animal was subjected
to the G load. You can see a very dramatic drop in the deep body temper-
ature of about 5 degrees over an hour's period. This is a linear drop and
you can see that the temperature does not return to normal until after 72
hours. This is really a severe stress. What happens to the animal if you
impose a lesser G load? This is shown on the next slide (5). Half the G
load seen on the previous slide, 1.25 G vs. 2.5 G, on a 4-foot radius centri-
fuge requires about 23 rpm rather than 40 rpm. At 1.25 G, you see a
similar precentrifugation pattern and drop in body temperature which is signi-
ficantily less than at the higher G level. At a much lower G level (slide 6),
1.12 G, we find no effect, i.e. no decrease in body temperature. This is
approximately 10 rpm on the
4-foot radius centrifuge. You
may even see a small increase
in body temperature. Note
that at this stage, that
apparently no rhythm is estab-
lished over the subsequent
3-4 day period; there is
no decrease in body temper-
ature, yet the temperature
pattern does not appear to be
normal when compared to
the prestressed period.
The next slide (7) summar-
izes these findings. The
first hour temperature drop
is plotted against the G load
imposed. There is almost
an exponential fall in the
body temperature as one

INITIAL EXPOSURE BODY TEMPERATURE
FEMALE RAT

VIEWGRAPH 5*

* Amer. J. Physiol. 221:1271, 1971

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1st HOUR TEMPERATURE DROP
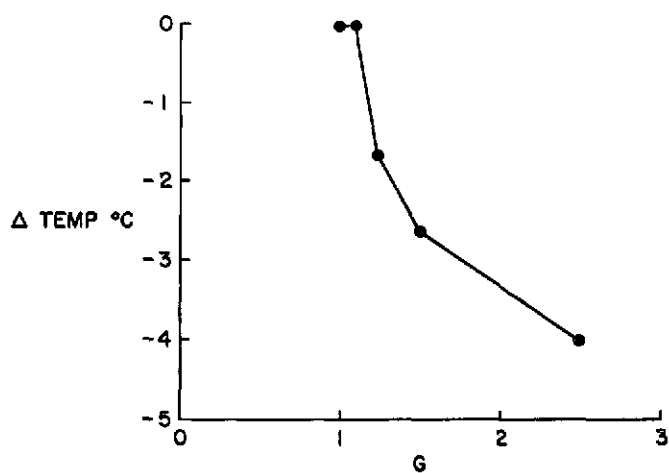
VIEWGRAPH 6

INITIAL EXPOSURE TO 1.12 G ON BODY TEMPERATURE
FEMALE RAT NO. 14
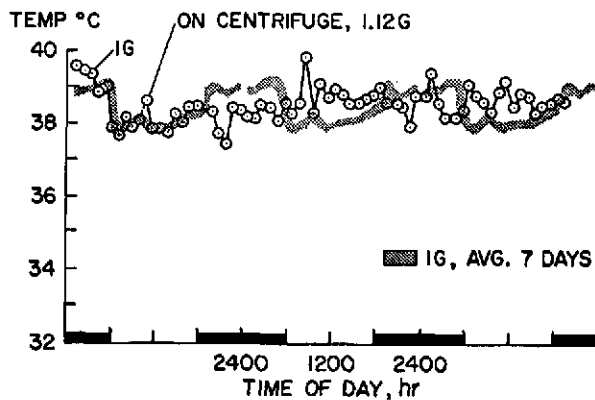
VIEWGRAPH 7

INCREASE TO 3.5 G AFTER 2 WEEKS AT 2.5 G

TEMP °C

VIEWGRAPH 8*

goes up the G level scale. We haven't gone beyond 2.5 G, but one might estimate the limit to which the first hour temperature will drop--possibly 5-6 degrees no matter how high the G level. What happens when you adapt an animal to 2.5 G for 2 weeks? This is shown on the next slide (8). We took a rat which was first subjected to 2.5 G. After the animal had established its rhythm as shown by the black curve, we imposed an additional 1 G going from 2.5 G to 3.5 G. You will note the significant drop in body temperature. Thus, when you preexpose an animal to a high G level, 2.5 G, and then impose an additional G load, there is still a temperature depression although perhaps less marked than the initial exposure. If you go the reverse way, (slide 9) and take one of the animals from the previous slide that had been exposed to 2.5 G for 2 weeks, and to 3.5 G for 2 weeks and then reduce the g level from 3.5 G to 2.5 G (shown by the blue curve), no change occurs in the body temperature. The pattern is very much akin to that at 3.5 G. When you decelerate the animal, there appears to be no temperature change. If we then take the animal off the centrifuge, from 2.5 G to 1 G (viewgraph 10), we find very little temperature change, maybe a very slight transient rise. We find this transient rise sometimes in some animals possibly because the centrifuge has stopped rotating and the noise level may change and the animal may move around a little bit more. In summary, whenever you impose a G load on the animal, there is a hypothermic response; when you decelerate, (reduce the G level), no change occurs--no significant effect on the body temperature.

What happens when you take an animal and you repeatedly expose the animal to an on-off type of treatment? The results are summarized in slide 11. We placed an animal on the centrifuge for 2 weeks, then removed it and left it at 1 G for 24 hours. The animal

DECREASE FROM 3.5G TO 2.5G

TEMP °C

VIEWGRAPH 9 *

1st TIME REMOVED FROM CENTRIFUGE
FROM 2.5 G TO 1.0 G
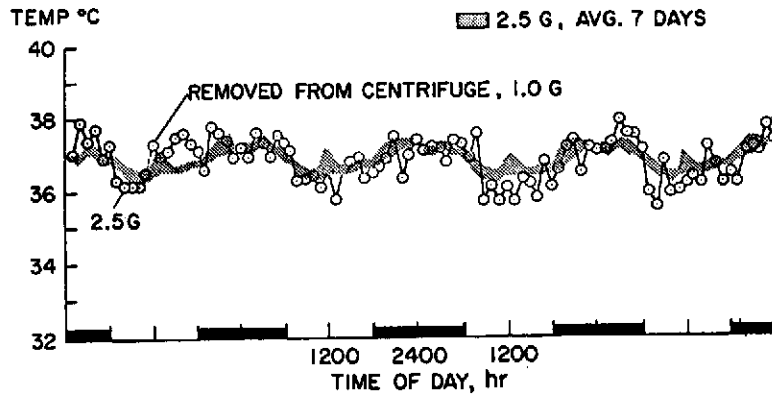
VIEWGRAPH 10*

REPEATED ON-OFF-ON TREATMENTS

VIEWGRAPH 11

34
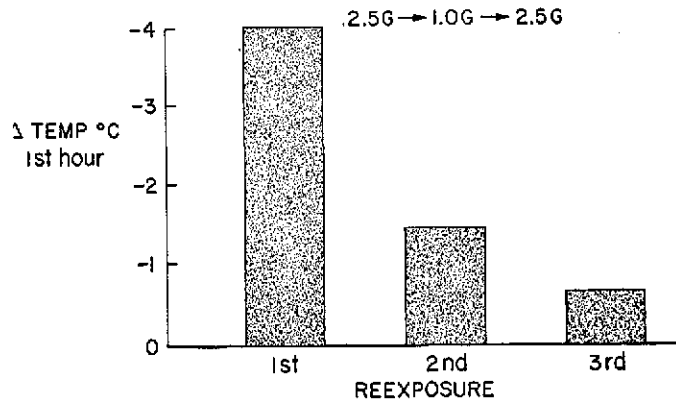
was reexposed to 2.5 G
and during this first re-
exposure, you get a
temperature drop during
the first hour of approx-
imately 4 degrees. We
continued the centrifu-
gation for 2 weeks, then
took the animal off for
24 hours, and again re-
exposed it to 2.5 G.
During the second re-
exposure, some habituation
occurs. With repeated
on-off exposures, the
response is significantly
diminished. The next
slide (12) shows a
chronically centrifuged
animal; this animal

FOOD CONSUMPTION

VIEWGRAPH 12

was centrifuged for over a 3-month period. We recorded the temperature con-
tinuously; these points refer to a mean 24 hour value (the temperatures
were averaged over 24 hours). The first exposure caused a very profound
drop in mean temperature. After two weeks an additional G load from 2.5
to 3.5 G, is imposed. The animal is returned to 2.5 G and finally the
animal is taken off. During this interval while the animal is off the
centrifuge, which is about 5 weeks, the animal's temperature is at a normal
value. If you then reimpose 2.5 G on the animal, you will see that com-
pared to the initial response this response (after 5 weeks off the centri-
fuge) is much smaller. This is a very consistent finding. Once you cen-
trifuge an animal, that animal is never the same animal; it apparently has
a very long retention time, in terms of recall. Whenever you recentrifuge
an animal, the response is always less in terms of the initial exposure.

NORMAL CONTROL RESPONSE —
ADRENALEOTOMIZED ONLY
ADRENALECTOMIZED
PREINJECTED — CORTICOSTERONE
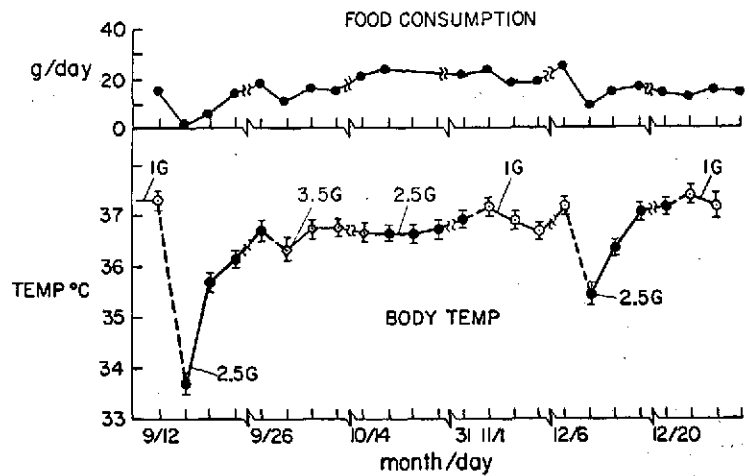
VIEWGRAPH 13

In this slide, we also
have measurements of
food consumption. You will
note that during the first
exposure to 2.5 G the food
consumption falls to zero
during the first day. The
animal does not eat, it is
very sick, and immobile.
When the animal is adapted
to 2.5 G and you impose an
additional G load the
response is a decrease in
food consumption which
corresponds to the decrease
in body temperature. You
can also see that the drop
in food consumption is also
correlated with the second
1 G to 2.5 G temperature

35
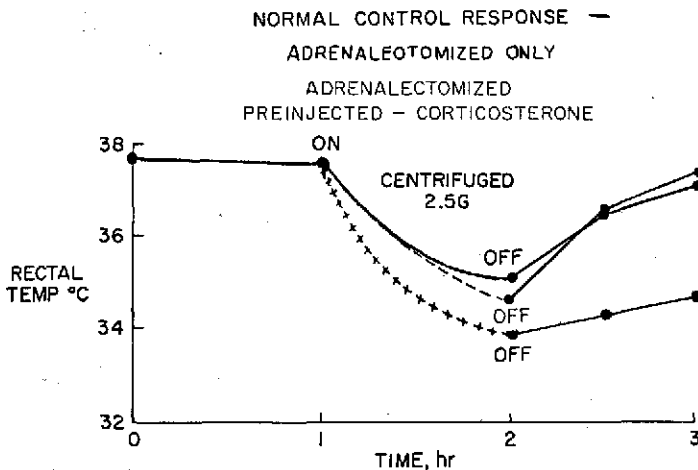
reexposure response. We have found that there is a very good correlation between decreased food consumption of these animals and the hypothermic response.

I have been showing you some results of our acute and chronic temperature studies using implant biotelemetry. It is very elegant test procedure that gives you data that is unobtainable by any other means. At the same time, the procedure is very tedious--it has taken a lot of time to get the telemetry system to work on the centrifuge. This work has not been done previously and I hope that next month it will be published in the American Journal of Physiology.* The procedure has its limitations because it is a rather tedious way of obtaining data--preparing the animal, etc. In this slide (13), we have studied the temperature responses of animals that were centrifuged for 1 hour at 2.5 G; the centrifuge was stopped and a rectal probe was used to determine time required for each animal to recover from the increased G load. The rectal temperature was measured about every 5 minutes before centrifugation. The points are the means of 6 animals. The precentrifugation temperatures are those usually obtained in normal control animals. The animals are subjected to one hour of centrifugation, during which time no temperatures are taken. The temperature is measured as soon as we are able to get at the animals, after the centrifuge stops. Within 2-3 minutes after the centrifuge stops, we usually have the first rectal temperature. The solid line represents the response of a normal group of animals. If animals are adrenalectomized 2 weeks before being tested (hatched line), the temperature response is significantly lower than the normal controls and the recovery pattern shows that even after one hour postcentrifugation, the temperature has not returned to its precentrifugation value. If you pretreat animals with the adrenocortical steroid, corticosterone, 24 hours, 4 hours, and 1 hour before centrifugation, the temperature response is intermediate between the adrenalectomized and normal animals. The recovery phase is practically that of a normal animal. This shows very clearly that the adrenocortical steroids play a very important role in the recovery of thermal regulation. Not only will surgical ablation cause a more profound hypothermic response, but it also can be produced by blocking the adrenocortical secretion with metropirone (slide 14). When the adrenals are blocked with metropirone, the temperature response is comparable to the adrenalectomized animals-- there is practically no recovery during the one hour postcentrifugation period. This indicates that the adrenals play a very significant role in thermal regulation of animals under stress. This demon-

ADRENAL BLOCKED
(METOPIRONE)

VIEWGRAPH 14
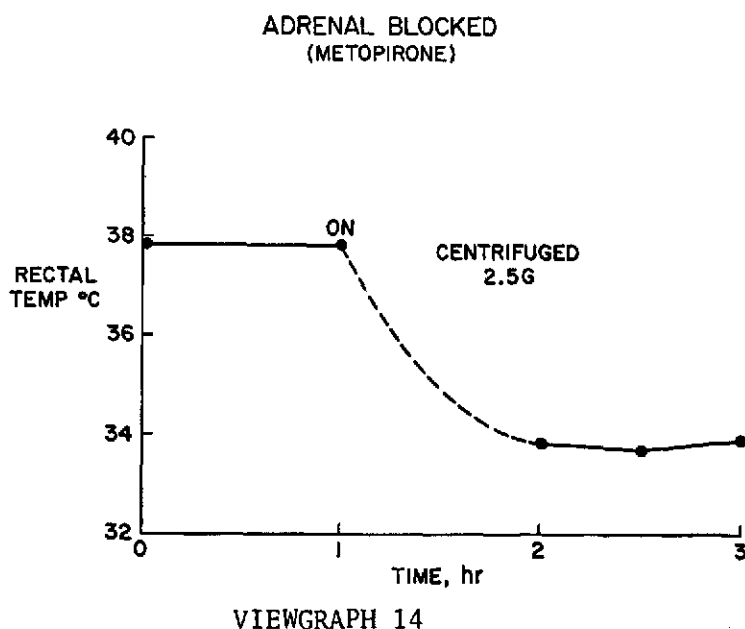
strates that the periph-
eral inputs have a pro-
found effect on tempera-
ture recovery following
acceleration stress.

Now I'd like to shift
from the temperature
studies to the area
of metabolism. Before I
touch upon some of our
experimental studies, I'd
like to review some basic
principles. Slide 15
shows the principle of
allometry. Equation 1 is
an equation that simply
states that many biolog-
ical functions and
structures are related

PRINCIPLE OF ALLOMETRY

1. $Y = bW^{\alpha}$ $\begin{cases} \alpha > 1 & \text{BONE} \\ \alpha \approx 1 & \text{LUNG VOLUME} \\ \alpha < 1 & \text{BMR} \end{cases}$

2. $Y = bW^{\alpha} + cT$

3. $Y = bM^{\alpha} + cG$

VIEWGRAPH 15

to the body weight of the animal in an exponential fashion. For example,
the skeletal weight of an animal, (Y), is related to the body weight by
an exponential function. If alpha is greater than one, it means that the
skeletal weight of an animal increases more with an increase in the total
weight of the animal--i.e. the relative skeletal weight increases. Or,
expressed another way the percent of skeletal weight in an elephant is
greater than that in the mouse. When alpha is less than one, as in the case
of basal metabolic rate, the basal metabolic rate expressed in terms of
calories output per unit of body weight decreases as the animal's weight is
increased. Interestingly, Kendeigh very recently has found that the basal
metabolic rate of birds can be expressed in terms of this elementary
equation with a basal metabolic rate that is equal to the weight of the
bird to some constant plus the ambient temperature to which the bird is
subjected times C which a constant. The last equation is a hypothetical
one which I have patterned after the equation of Kendeigh which expresses
the basal metabolic rate of an animal as a function of its body mass (M)
and its weight (G). This concept is a bit more clearly expressed in the
next slide (16). One of the chronic acceleration program's major objectives
is to delineate the mass vs. the weight contribution. What we are trying
to do is to establish for any given biologic function how much depends on
the gravitational factor vs. the mass of the animal. The importance and
value is whether we can extrapolate down to 0 G. This is still simply a
matter of conjecture. In many cases, the maximum body size that an animal
will achieve is rectilinearly related to the G level. I won't go into the
various studies on body mass changes with G load. These are reported in
the references that are included in the handout. Chronic centrifugation
studies help us to delineate those functions which are very responsive to
gravitational changes. We hope to establish some type of generalized
expression that includes both mass and the gravitational load in one equa-

EXTRAPOLATION TO "ZERO G"?
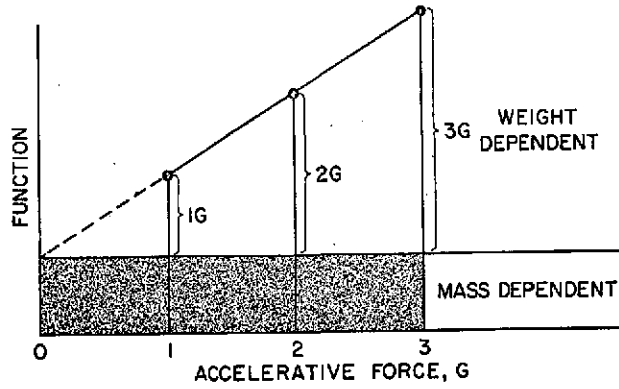
VIEWGRAPH 16

log RATE = 1.83 + 0.75 log w
(KLEIBER)
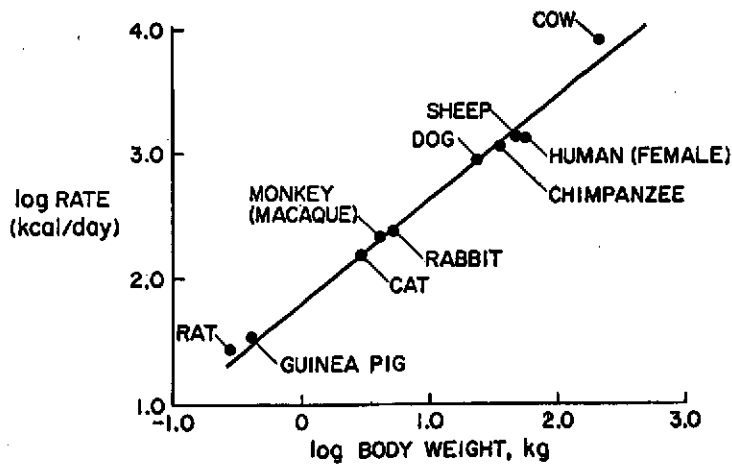
VIEWGRAPH 17*

*From Kleiber, Max. Fire of Life, An Introduction to Animal Energetics,
p. 201, John Wiley & Sons, Inc., N.Y.

tion. The next slide (17) is taken from Kleiber's text (Fire of Life) in which he plotted the allometric equation in log form. The log of the metabolic rate is equal to 1.83 (a constant) plus .75 times the log body weight. This is the so-called Kleiber's 3/4 power rule. If one plots a number of animal species on this log-log plot, one gets a linear regression ranging for species from the rat to the cow. These data have been compared for animals under
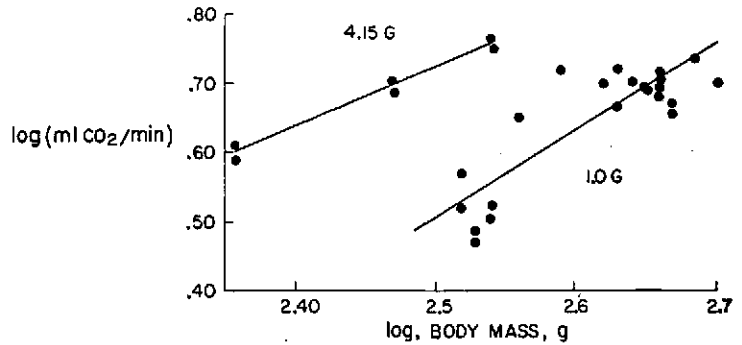
METABOLIC RATE vs G-LOAD

VIEWGRAPH 18

normal gravity conditions and the question posed is "What are the metabolic rates of animals that have been reared on the moon or under weightless conditions?" One might hope to get a linear regression curve for animals raised under high G that is above this line, for low G that falls below this line. From some food consumption studies and based on the fact that an animal exposed to high gravity should have to use more energy, the metabolic rate would be greater than under normal gravity. Some of our experimental results, which are preliminary, indicate that the CO_2 production rate of rats under normal gravity and under increased G are significantly different (slide 18). This slide shows the log of the CO_2 output per minute plotted against the log of the body mass of individual rats. You will notice that normal gravity rats show one kind of plot while the 6 rats that we have exposed to 4.15 G for as long as 3 months show a significantly higher rate of CO_2 output. This is predicted based on the food consumption patterns that we have already established. These results were very rewarding since our model predicted a higher energy utilization rate in high G adapted animals and we are now finding this to be the case. We are going to accumulate more data, but we are very impressed and encouraged since we have never had any of the high G adapted animals overlap the control animals. In addition to the in vivo studies, that is measuring whole animal metabolism,

GLUCOSE UPTAKE BY DIAPHRAGM TISSUE

VIEWGRAPH 19

39

METABOLISM OF GLUCOSE-^{14}C TO ^{14}CO$_2$

^{14}CO$_2$ PRODUCTION

CENTRIFUGED (4.1G)

CONTROL (1.0G)

EXPOSURE TIME, weeks

VIEWGRAPH 20

we have been interested in the metabolism of selected animal tissues (slide 19). We have been studying the glucose uptake capacity of diaphragm tissue from rats that were placed on the centrifuge. The precentrifugation glucose uptake is shown at 0 time. The glucose uptake is expressed in micromoles of glucose taken up by 100 milligrams of wet weight of diaphragm tissue in 90 minutes. The centrifuge exposure time is given in weeks. Note that the control animals show a very sharp drop from 0 to 2 weeks --this is a very normal pattern in young developing rats. The centrifuged animals show a small drop in glucose uptake rate between 4 weeks of age when they are put on the centrifuge and 6 weeks, but thereafter the glucose uptake is relatively constant. Groups of animals centrifuged from this period of time show a consistently higher rate of glucose uptake, from 30-50% higher than the controls. The next slide (20) correlates the CO$_2$ production by the diaphragm. We placed labeled glucose in the medium and measured the rate of labeled CO$_2$ production expressed in terms of micromoles of glucose equivalents per 100 milligrams of tissue per 90 minutes. This clearly demonstrates that the metabolic rate for glucose in the muscle tissue of animals adapted to high G loads-- in this case, 4.1 G--is consistently above that in the control animals, a magnitude of increase on the order of 90-100% in the CO$_2$ production rate. The CO$_2$ production rate does not account for the increase in glucose uptake as it represents only a small fraction of the total glucose uptake by the tissue. We are looking into this problem, that is, the distribution of the extra amount of glucose taken up by the centrifuged muscle tissue. We believe that there may be an increase in glycolysis or glycolytic rate-- we know that it doesn't go into muscle glycogen.

I mentioned before that the oral glucose tolerance of centrifuged animals is increased as well as their responsiveness

IN VITRO INSULIN EFFECT

INSULIN (0.1 mU)
CONTROL

μmoles GLUCOSE

+38%

+19%

NONCENTRIFUGE

CENTRIFUGE AT 4.1G. 3 months

VIEWGRAPH 21

to insulin. In vitro studies (slide 21) on diaphragm muscle confirm the
in vivo results. We have plotted the micromoles of glucose taken up by
diaphragm tissue (note that the scale starts at .80) and compared noncen-
trifuged animals with animals that were centrifuged for 3 months at 4 G.
The left column represents the control flask while the column on the right
represents the flasks that also contained insulin. You can see that in
the noncentrifuged diaphragm, the addition of 0.1 milliunits of insulin
produces a 19% increase in the glucose uptake. In contrast, the diaphragm
tissue from centrifuged animals shows a 38% increase in the insulin effect.
This clearly demonstrates that in vitro muscle tissue from centrifuged ani-
mals is much more sensitive to insulin.

Let me summarize (slide 22) what I have presented this morning. The tempera-
ture in the acutely stressed animal shows a very profound drop which is
correlated very well with the decrease in food consumption. Repeated ex-
posure of the acutely stressed animal causes a decrease in the hypo-
thermic response. With deceleration or reduction of the G load, there
is no significant change in temperature in these animals. The adrenal
corticosteroids play a very significant role in the recovery rate of these
animals. In the chronically centrifuged animals, no change occurs in the
mean body temperature pattern after they are fully adapted although there
is a suggestion that the
average temperature of
the adapted animals may
be a little less than
that of an animal under
normal gravity. No
change is seen in the
period or 24 hour temper-
ature rhythm. As regards
metabolism, the respira-
tory rate of the chroni-
cally adapted animals
increases very signifi-
cally in terms of the
CO_2 output; an increase
in glucose metabolism
or glucose uptake by
muscle tissue occurs;
and there is an increase
in insulin responsive-
ness or sensitivity.

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                              SUMMARY
                             TEMPERATURE

ACUTE                                       CHRONIC

  1. ↓ TEMPERATURE                            1. NO CHANGE OR
  2. ↓ TEMPERATURE ≈ ↓ FOOD CONSUMPTION          SMALL DECREASE IN
  3. ↓ TEMPERATURE RESPONSE - REPEATED           AVERAGE TEMPERATURE
     EXPOSURES
  4.   NO TEMPERATURE CHANGE WITH ↓ G         2. NO CHANGE IN
  5. ↑ IN RECOVERY RATE WITH ADRENAL             PERIOD LENGTH OF
     CORTICOSTEROID                              TEMPERATURE RHYTHM

                             METABOLISM

CHRONIC

  1. ↑ IN RESPIRATORY RATE
  2. ↑ IN GLUCOSE METABOLISM
  3. ↑ IN INSULIN EFFECT
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VIEWGRAPH 22

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DISCUSSION BY PARTICIPANTS

SOUTH: I was wondering if the temperature response might be at least in part artifactual because of the pressing of the telemeter against the central body wall and with increased muscle tone, of course, being kept away from the body wall somewhat more.

OYAMA: I'm sure that this is not the case. I have a feeling that the hypothermic response occurs because of a transient hypoxia. High altitude, I understand, will cause the same hypothermic response.

POPOVIC: You had some animals that were without your probe and they showed the same drop.

OYAMA: You can get the same drop with the rectal probe.

MUSACCHIA: But anoxia of high altitude is due to a decrease in ambient oxygen. You don't have this at sea level - no external anoxia, no ambient anoxia.

OYAMA: We have run animals with heart rate measurements also and it's surprising that one finds a decrease in heart rate which corresponds very well to the temperature drop. The amount of blood circulating in the vital areas might be reduced causing a generalized or specific tissue anoxia.

SOUTH: This might be analogous to the diving reflex so that inappropriately you have a tissue anoxia.

OYAMA: The animals are very inactive during this stress phase. They do feel cold - this is why we initially got off onto this problem. We noticed that under acute stress the animals appeared to be much colder to the touch than the control animals.

POPOVIC: I wondered about your second and third exposure to acceleration. If you see a smaller decrease in the temperature drop, it might be nonspecific because if you exposure animals to the cold for the second time after adaptation to cold, they show less of a temperature decrease. So the response may be very nonspecific, a response only to hypothermia per se.

OYAMA: One of the values of biotelemetry implant is that it allows you to look at the adaptation time. The rats are extremely sensitive to rotation. We have been working with biotelemetry at a 4 foot radius vs. a 22 ft. radius and the hypothermic response is the same irrespective of the rpm. We feel it is definitely a G effect. Dogs, in contrast, show only a transient decrease, depending on the particular dog and G level, of about 1/2 to possibly 1 degree - nothing like the rat. We had expected to see a very profound hypothermia in dogs because of the larger size. So, in a bioresearch module, if you want to look at the G effect, the rat is an ideal animal since it is very sensitive to changes in the gravitational field - 1.25 G causes a significant fall in body temperature.

POPOVIC: I don't understand why you expected to see a greater reaction in the dog since larger mammals including man regulate their body temperatures much better than the rat.

OYAMA: In terms of the greater hydrostatic pressure gradients. In general larger the animal the less tolerance it has to g loading. This is why we expected a greater change in the dog. It is true that the heat capacity of the dog is much greater than in the rat.

SMITH: Do you get any effect on the dogs?

OYAMA: Yes, we do but it is small. Again it is dependent on the g level. We haven't maximally challenged the dogs. We know that the dog can survive 2.2 G for 3 months. I haven't mentioned a lot of the other structural changes that we have observed in the dog - primarily in bone structure.

SMITH: Do you find a difference between little dogs and big dogs?

OYAMA: We have been working with male and female dogs ranging from 6 to about 15 kilograms. We thought that the female dog would be more resistant to acceleration since they are generally more resistant to a stressful environment but surprisingly we find the reverse. Male dogs appear to tolerate acceleration loads better than the female.

SMITH: Do you get a change in the testis? Is the density greater than the fluid - it might be a stress.

OYAMA: The only study I can refer to is one we did on rats concerning reproduction. Rats can reproduce on the centrifuge and we have several generations of such animals. Rats can mate and can produce viable pups at 3.5 G. They do not at 4.5 G. We thought it was simply a physical inability of these animals to copulate under the more severe condition, but recently MacFarland at University of California - Davis, has shown that at 4.5 G, male rats show testicular degeneration. Something is apparently happening to the testicles - they may mount, but they are infertile.

SAUNDERS: You showed that slide on the speculation going from 3 to 2 to 1 to 0, that you might be able to extrapolate from centrifugation-type experiments as to what might happen at 0 G. Yet, in the body temperature drop as you have been showing, you were going from a low G to a high G and getting the decrease. How can you say that something might be shown when going from 1 G to weightlessness and then return from weightlessness to 1 G again? How do you propose or can you speculate that there will be any significant change?

OYAMA: With respect to the body temperature response, I cannot explain why the animal doesn't show an opposite effect. We find that increased G levels produce a hypothermic response, a decrease in G level presumably if it goes in the opposite direction should show an increase in temperature. All I can say is that when an animal is subjected to an increased g level, I considered that a stressful condition - going to a decreased G environment is not stressful as it presumably does not disturb the thermoregulatory center. In predicting the effect of weightless environment on man or animal,

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I don't see any change in the thermoregulatory mechanism based on reduction in G level, but I do expect that when rats are fully adapted to the weightless environment one would see a significant drop in body temperature when they are returned to 1 G, their normal gravity environment. We are talking about animals adapted to low G and shifted to high G.

SAUNDERS: Can you speculate or predict how long the animal would have to be adapted to weightlessness before you would observe this effect. For example, the Russian Cosmos 110 with 2 dogs, and then the cosmonauts themselves.

OYAMA: We are talking now about the human vs. the rat. I would say that 2 weeks of weightlessness should provide enough adaptation time so that when the animal is returned to normal gravity you would expect a hypothermic response. With respect to other functions, I might add that when you talk about maximum body size that these animals (rats) attain under normal gravity and under hypergravity, you get growth curves like the following:
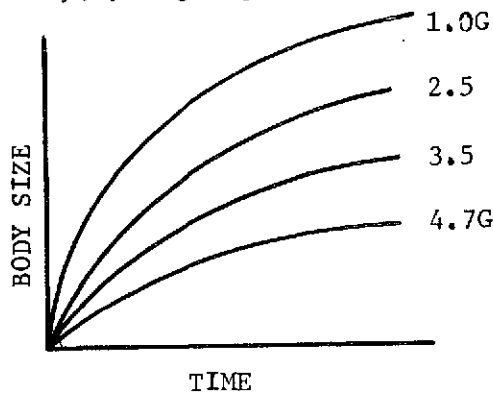
If you plot the maximum body size that these animals attain after 3 or 4 months of centrifugation against g level you'll find that you have a very linear response to the gravity field:
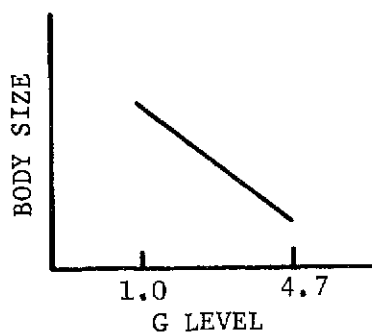
The value of this is that it enables us to predict, all things being equal, what the maximum size would be under 0 G. From our studies and this linearity, we would predict that rats under comparable conditions would be about 6% larger in body mass than under normal gravity conditions. With respect to basal metabolic rate, we would predict the same sort of thing except in the opposite direction - as you go up the G scale, the metabolic rate increases and under 0 G one might expect a decrease in metabolic rate. The 4 G adapted animal compared to the 1 G, is about 37%-40% higher. So, we might expect a

decrease under 0 G conditions. What we are talking about are function - G load relationships that could be linear or possibly bell-shaped:
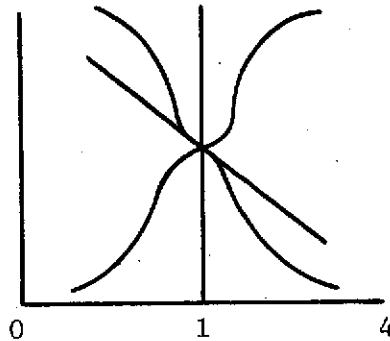
I can't predict what kind of function will behave in this manner, i.e. showing a change as an increase from normal gravity rather than displaying a linear regression. The value of this is that we can determine or delineate various functions in terms of the type of response to hyper G and then check them out under 0 G conditions. We have some estimated values based on linear extrapolation to zero G.

SAUNDERS: If we were to have such an experiment on the Bioresearch Module where you have the capability of altering the gravity gradient - going from a 1 G type load, through the acceleration phase (which you have already ruled out), until the animal and spacecraft have settled in orbit where you are in the weightless condition and then after a period of adaptation to weightlessness spin it up go say 1 G. Is your body temperature change a good indicator so that one could rely on a metabolic change based on the temperature and the resultant going from weightlessness to high G?

OYAMA: This is what we are trying to do now. We want to get a continuous CO_2 and oxygen consumption rate correlated with body temperature of animals that are being subjected to the whole G profile. We have just assembled the equipment to do this very thing - to correlate respiratory metabolish with the hypothermic response and the G level and food consumption. I would say right now that if you had to rely on any single physiologic index in space where it is difficult to measure food consumption the deep body temperature obtained by biotelemetry would be the best physiological indicator of the overall state of the animal. The unit is completely self-enclosed, no wires to worry about as in the EKG measurement. We've also shown that the heart rate and the body temperature fall in the same way; the heart rate can give you a little more insight into psychogenic stress and possibly physical activity whereas the temperature is less sensitive and less responsive but does probably give an indication as to whether the animal is asleep or awake. If I had to choose any single physiological parameter in rats, I would pick body temperature.

MUSACCHIA: I just want to get a few areas cleared up in my own mind. First, these telemeters are implanted in the animal - are they metallic?

OYAMA: No, they are enclosed in silastic and the tissue grows around them.

MUSACCHIA: What is the durability or duration - 1 month, 2 months?

OYAMA: The unit weighs about 10 grams and uses about 8-10 microamps. We use a battery that supplies about 160 milli-amp-hours and I think I calculated that you could get about 20,000 hours from the battery but body fluids eventually seep in and the unit usually degrades before this. The longest period of time that we have had these units in operation is around 6 months. I think they could operate longer under better conditions.

MUSACCHIA: Are the cages that the animals are in metallic?

OYAMA: The cages are copper shielded.

MUSACCHIA: What is the temperature of the entire room?

OYAMA: About 22-23 degrees.

MUSACCHIA: When the animal is put on and spun, does his body in any way come in contact with the cage?

OYAMA: No. We are very concerned about heat loss by conduction so we put the animals on a very thick layer of sawdust. The cages are practically sealed, but they have a few air ports on the rearward side of the cage. We tested this by blowing air over the animals under various conditions and no change occurs in their body temperature. So conductive heat loss is not a factor here.

MUSACCHIA: That's great. That answers a few of the things that were bugging me a little bit. The point about muscle glucose, the increase in metabolism, is keen. I'm wondering, since you had to sacrifice the animals at that time, if you possibly take the intestines from the animal and do some glucose uptake on the gut. You might help answer one of the questions concerning the nutritional story and that is if you see an increase in absorptive capacity of the gut or a decrease in absorptive capacity, you might be clueing in a little closer to the answer.

ENTENMAN: How does the amount of insulin that you used compare with the physiological dose?

OYAMA: We used about 33 microunits per ml in vitro. This is a very small dose as regards the bioassay of diaphragm tissue. If there is any error it is that we used too little. In fact, there may be a use for this particular group of animals (i.e., in insulin analysis) since these animals seem to be much more sensitive to insulin. You might be able to increase the response or sensitivity of the insulin bioassay by using diaphragms from chronically adapted animals. In general, when you have a group of animals exposed to centrifugation, if you take a look at the mean response and standard deviation, you find that the deviation is always smaller in the centrifuged animal. It seems that the scatter, the biologic variation, of animals adapted to high G is consistently less than normal controls. It seems to compress the animals so that their behavior pattern or response pattern is much more narrow. This seems to be a very consistent finding with

this typical animal - whether it is the amount of liver glycogen present
or the blood sugar level or any other parameter. So, they tend to be more
uniform.

SMITH: In reference to the ambient temperature in the centrifuge room,
could you give us a little more detail on how it was controlled?

OYAMA: We maintained the centrifuge in an air-conditioned facility.
When we put the animals in the metabolism cages, we measured the temperature
in the chamber before and after centrifugation. The temperature on some
occasions may rise a degree or two but generally the ambient temperature is
the same. No large changes occur in the environmental temperature within
the cages itself as measured by the biotelemetry system.

SMITH: What was the environmental temperature in the cage normally?

OYAMA: About 24 degrees.

SMITH: That's just about 4 degrees below the thermoneutral zone.

OYAMA: Yes, we're very much interested in this. We are now in the
process of devising an environmental chamber which will allow us to control
the temperature. We want to work at 28-32 degrees which is the thermoneutral
zone for the rat and get the basal metabolic rate under those conditions
coupled with implant biotelemetry of body temperature. Then we can take
measurements during the sleep cycle. In other words, work at the thermo-
neutral zone and take measurements during both the active phase at night
and when the animal is inactive during the day cycle. This is the type of
comparison of the data that we would like to have under the weightless en-
vironment because you want to divorce it from any physical activity that
might influence metabolic rate. It is very important that we get the temper-
ature coupled with the respiratory metabolism and activity. We are very
interested in activity. We have a TV monitor system that we hope to use to
monitor physical activity of animals under acceleration stress and during
adaptation. This is difficult since someone must score the movements of the
animals under these conditions over 24 hour periods. This is virtually
the only way that we are going to get a handle on metabolic rate of animals
under high g. We have that capability on our large centrifuge.

SMITH: As you are stressing the animal by increasing the G level, it
will be very interesting to see if the thermoneutral zone is shifted.

POPOVIC: Your animals will probably not become hypothermic but will
lose their diurinal rhythm during the first 4 days. What was the philosophy
behind your experiments in which you used cortical hormones? It is known
that the lack of cortical hormones will decrease the response of animals
exposed to the cold.

OYAMA: Yes, this is known. We are interested in the poststress
recovery phase primarily - how does the animal readapt to 1 G? We haven't
studies this any further, but I know that this isn't new. In adrenalecto-
mized animals, recovery response is different than in normal controls or in
corticosterone treated animals. The reason we are interested in adreno-

cortical steroids is that when you centrifuge an animal, you get a very large increase in the adrenosteroid blood level. We want to know what role the adrenal steroids have in acceleration stress, in general, and in the hypothermic response. Certainly, the central control for temperature regulation lies in the hypothalamus and we intend, ultimately, to look at some of the neurohormones and perhaps correlate them with changes in adrenal function.

POPOVIC: Do you measure the temperature continuously or do you sample at various time intervals?

OYAMA: In our biotelemetry system, the transmitter sends out signals continuously but we sample only at 10 minute intervals, otherwise we end up with too much data.

POPOVIC: How long do you sample at each 10 minute interval?

OYAMA: Only a matter of seconds - whatever we get on the recording device.

POPOVIC: I think that's fine because the temperature in rats does not change rapidly.

OYAMA: I would like to add that sometimes with the biotelemetry system you can get an animal that positions itself in a particular area of the cage. There are null spots within each cage. Sometimes the animal can creep into a null spot within the cage and you get spurious values with the temperature signal sometimes going way off scale. There are problems with the biotelemetry that people don't like to talk about; one is the integrity of the transmitters, sometimes you can get signal phase shifts, another more serious problem particularly with large animals is that due to the cage size you have difficulty shielding the cage properly thus producing interference or null spots within the cage. Usually with rats you only get 1% signal drop out but you can get up to as high as a 10% drop out.

SMITH: Where do you place your telemeter in the animal?

OYAMA: We put it in the umbilical region of the rat. Within a very short time we have tissue growing around it. When we open these animals up, the unit is completely encapsulated. Thus the unit is well placed and does not move around.

SMITH: Ralston has some very good data on goats which was in Science a year or so ago. They were taking temperatures in various places in the intestinal area, the abdominal area, and found interesting information. But, at least you are safe in one spot.

OYAMA: One spot is fixed, and as I said we establish the normal rhythm in rats at least a week before being centrifuged. The animal virtually serves as his own control. This is very nice since you don't have to compare

groups of animals; you are specifically comparing the individual animal to its precentrifugation rhythm.

HOLTON: If you take a rat that has been born on the centrifuge and spent most of its life on the centrifuge making it much more acclimatized to the increase in g force, and you then decrease the acceleration force do you still see no change in temperature?

OYAMA: We haven't done that with implant animals conceived and born on the centrifuge. I would predict that if you were to decrease the G load on these animals you would find no change in the body temperature. What I am trying to emphasize here is that any increase in G is a stressful phenomenon, a decrease in G is nonstressful as indicated by many, many specific responses such as corticoid levels, temperature, etc. The analogy here is that man can adapt to the weightless environment very well, at least from those measurements that we have made. I think the cost is going to be when he comes back to normal G. This is my feeling based on our animal work. Deceleration is nonstressful in most of the functions that we have measured. Any increase is stressful.

SAUNDERS: Can you really say that, Jiro?

OYAMA: The thing that I can see with respect to man may be in, for example, kidney stone formation from calcium mobilization, you might expect some deleterious effect. Aside from that if man or animals go into the weightless environment they won't have to use their muscles as much and their muscles may become atrophied, but for their existence in the weightless environment this is a natural adaptation. But when they return to normal gravity, they will have to walk, and that would be a stress at least for a while.

SAUNDERS: I'm not saying that it's a continuous stress but initially you have a stress in adapting to a different G level.

OYAMA: It really depends on how you define stress.

SAUNDERS: Yes, and what parameters you are looking at.

OYAMA: In terms of physiology, I don't think it (i.e., weightlessness) is stressful. The only thing that I consider that might be stressed would be the vestibular apparatus.

HORWITZ: When you return from high G to low G do you get a change in corticosteroids?

OYAMA: No, when we sacrifice animals that have been adapted to high G we find that there is no significant difference between the blood corticosteroid level in this animal as compared to normal.

HORWITZ: But I mean when you go the reverse way. If you take an animal that is adapted to high G and expose it to a lower G what happens?

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OYAMA: We have decelerated animals adapted to a high G and decapitated them as soon as the centrifuge stops. We find that there is no difference in the corticoid level of these animals compared to normal animals. We suspect that the resting level on the centrifuge is the same as under normal gravity and during deceleration you might expect some increase in cortico-steroids if it were stressful - we find no change. Quantitatively, I don't think that the pituitary-adrenal system is affected.

HORWITZ: I wasn't clear whether during this initial stage when you get a drop in temperature, you measure a drop in metabolism or have you done that?

OYAMA: We haven't done this. We have some very preliminary studies that indicate that there is a decrease in metabolism.

HORWITZ: There is a possibility that if you get a shift in vascular distribution it would be easy to explain a possible decrease in oxygen con-sumption as an artifact due to a decrease in visceral flow where your tele-meter happens to be, unless you really know that metabolism is decreasing.

OYAMA: We've run some very preliminary studies. We don't have a continuous CO_2 analyser; we have to use a collection and weighing sort of thing. We did have a device that we thought measured CO_2 continuously and we thought that we found a decrease in CO_2 production during the acute stress phase. I would think that it would correlate very well with the heart rate data and the body temperature.

HORWITZ: Is there any way that you can have these animals breathing a higher oxygen content than is in air?

OYAMA: Yes, we have a rotating slip joint.

HORWITZ: Can they breathe 100% oxygen under these conditions?

OYAMA: We haven't tried that. They just breathe room air. We hope that with our new chamber system we will be able to control the tempera-ture and gaseous environment.

SMITH: Did you find a CO_2 error in the chamber?

OYAMA: No, we measured CO_2 at one time but it only got as high as 2% in the outflow.

POPOVIC: 2% might be rather high.

OYAMA: It may be high, but if we can control the flow rate it should not be that high. I don't think 2% is that high.

POPOVIC: It isn't that high for normal situations but during stress you might increase the temperature drop due to the high CO_2.

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Metabolic Effects of Artificial Environments

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Outline of Talk

I. Chamber System
II. Marginal O_2 Toxicity
III. Protocol
   A. Survey
   B. Bracket
   C. Pinpoint
IV. Diluent Gas Effects

All of us take a great deal for granted -- our wives and families among other things. Perhaps one of the things we take most for granted is the air we breathe. Nationally there is considerable interest in environmental pollutants, trace contaminants in the air and so on, but I am talking about the major components of the air we breathe - the 80% nitrogen and the 20% oxygen. Then something happens that makes us ask questions that perhaps were not so obvious to us or did not seem too important before.

It reminds me of a friend of mine who has a hobby of making money. He makes it literally. He prints 20-dollar bills, that's his specialty. Doggone, he makes nice money. But his machine (he keeps it in the basement; he's rather quiet about his hobby) went on the fritz one day and started putting out 18-dollar bills. Now, what can a guy do with 18-dollar bills? He figures, "I'll have to destroy these." But he looked at them again and thought they do look pretty doggone good. So he thought well maybe if I move fast enough I can at least get some of these changed then I'll be on my way and we'll call it quits on these bills. He went to the druggist and he said, "Do you have change for an 18-dollar bill?" The druggist, quick as a wink, came right back to him and said, "Yeh, what would you like: 2 nines or 3 sixes?"

In our case, the question has to do with the selection of a gaseous environment for a prolonged manned space flight. Our initial problem in this area was one of engineering. So, we built a chamber system to hold animals in a test environment not for hours or days but literally for weeks and months on end. The chamber system (Figure 1) that we use is pretty simple in order to make it operable over a long period of time. Basically, we operate out of a large 8 man pressure chamber which constitutes nothing more or less than the elevator for us to take personnel to and from the test pressures, work with the animals, do injections, change food or drop trays or whatever has to be done. Inside we carry a series of smaller

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chambers in which the animals live (Figure 2). These are autoclaves about a yard long and 22 inches in diameter from which we have removed the distal end and replaced it with a one inch thick plexiglass window through which all the wires and other kind of material such as gaseous mixtures, etc. enter into the chamber.

Let's go to the motion picture and I'll narrate it for you (for schematic, see Figure 3). We'll start with a view of a consul for a single chamber. Since we've been in Colorado, we have nearly tripled the size of this thing. We've got almost 3 complete systems in it now. Each system contains a paramagnetic oxygen analyzer, a number of analytical and print out devices, and electronic feed back systems so that we can continually monitor temperature, pressure, oxygen consumption, and CO_2 production. Heat production is calculated from these data. We also monitor food and water consumption on a continuing basis. To hold pressure, we use one of the simplest systems one could imagine--a modified altimeter. We have the entire system hooked to an alarm which is activated in my house when there is a major malfunction, for example, if the animals are out of water. It takes less than 10 minutes for the duty officer to reach the chamber, even if he has been summoned from home. Through the plexiglass windows, you can see the animals moving around inside. We are monitoring for the contaminants within the chamber, not on a continuous basis but on a very frequent basis so that we can tell not only the major components but also the trace problems. The system is backed up with infrared spectrometry and other forms of analytical equipment. The animals are totally unrestrained. We usually house about 6, sometimes as many as 8, animals in one chamber. They are in as good or better shape than any animals we have in the animal unit. We always run a parallel chamber with air as the gaseous environment to serve as a control. We usually sacrifice the animals at the test pressure in the larger chamber and pass the remainder of carcass through an air lock to the people outside who continue with the autopsy.

After the tissues have been prepared for storage, we move as quickly as possible to the analytical steps. We also do a number of enzymatic assays as well. We don't do it on a general basis but only when we find specific enzymes that seem to us to have good reasons for analysis. From time to time, we will also harvest subcellular particles using a preparative ultracentrifuge; we have even gotten, in specific instances, down to a single protein prepared in fairly purified form. We then study its characteristics as influenced by the test environment in the analytical ultracentrifuge. The guts of the laboratory, though, involve the injection of radiolabeled substrates and the incorporation of these substrates into various metabolic pools plus the expiration of the label as $^{14}CO_2$. Consequently, a liquid scinillation counter is really the focal point of the laboratory activity. As you can imagine, not only from the analytical work but also from the environmental part of the program, we obtain increibly large amount of data which we then try to reduce to some manageable form using a small desk top computer to prepare it for the larger CDC 6400 computer.
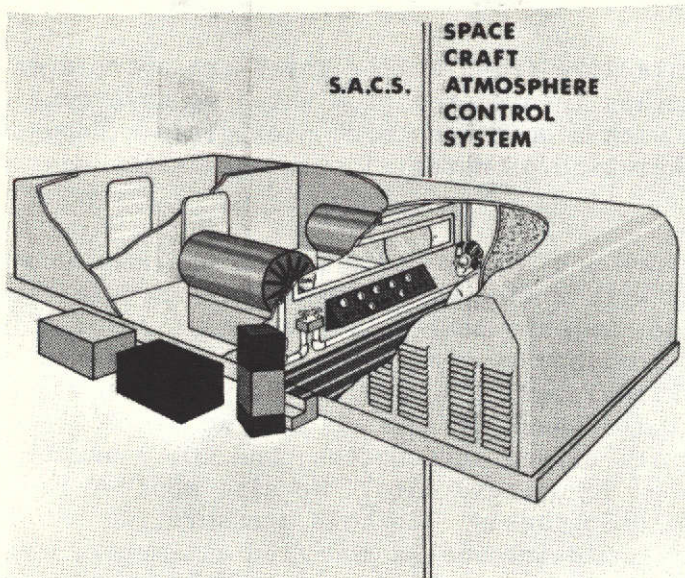
FIGURE 1

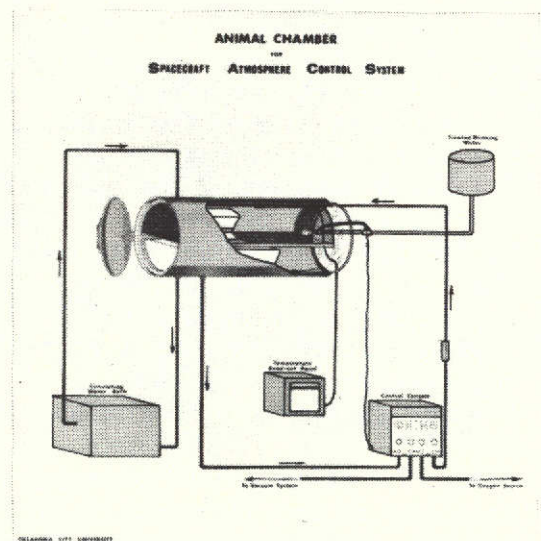S.A.C.S.

SPACE
CRAFT
ATMOSPHERE
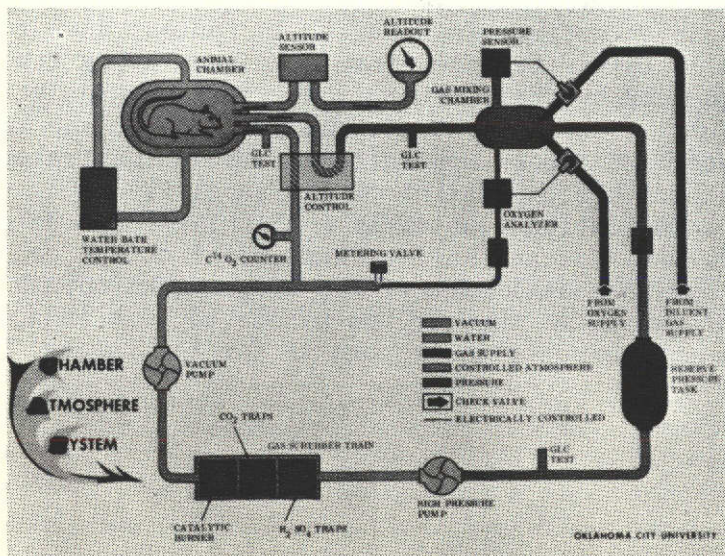CONTROL
SYSTEM

FIGURE 2

FIGURE 3

I would like now to tell you about a problem on which we have been working since the early part of the 1960's, marginal oxygen toxicity. Rats were placed in a 5 psia, 100% oxygen environment for periods of up to 12 weeks. We've run 2 very long experiments and many experiments of up to 4 weeks in duration. Among the things that we have seen in such animals, where the oxygen partial pressure is about 232 torr (by the time you calculate for moisture, CO_2, and a little trace of nitrogen in the system) are that they consume food at about the same rate as their normal counterparts in air at 1 atmosphere, consume the same amount of water, oxygen consumption remains fairly constant, CO_2 production is about the same, calculated heat production is about the same--in essence, the animals are very clearly in bioenergetic balance. In fact, their general state of health looks about the same. We do find some important changes though including the amount of coenzyme A in tissues. The aberration starts after perhaps a week of exposure to the test environment and shows a maximum reduction around 4 weeks after the animal under the 5 psia, 100% oxygen environment. It returns to about the normal level after 10-12 weeks in the test environment. We find this to be a general effect--a drop in coenzyme A in liver, kidney, spleen, and brain tissue. The most dramatic change is in brain tissue where over a 4 week period we see a decrease to about 50-55% of control values. This is startling to a metabolist because we normally consider this a rate-limiting compound and now we see that the animal in marginal oxygen toxicity can do without much of its CoA.

We went into this more deeply and found that the change was even greater when one measured specifically acetyl coenzyme A rather than total CoA. We also found some change in body composition; we found decreases in water and in lipids; these did not necessarily return to normal values. We found an increase in protein and carbohydrate over a 4 week period. Overall, however, most parameters returned to normal values--a rather effective demonstration of homeostasis.

There was, nevertheless, an apparent conflict because if we injected acetate -1-^{14}C and watched its expiration to ^{14}CO in the animal after 4 weeks in the 5 psia, 100% oxygen environment, the rate was about 78% of the rate of the comparable control animals or down about 22%. Yet, the animals were expiring the same amount of total CO_2. The animals were obviously in bioenergetic balance by any other type of measurement. So there must be an explanation for this. If we take a look at what can happen to intermediary compounds, perhaps we could get an idea of the answer to this apparent contradiction. Look first at the glycolytic sequence (Figure 4). The C-1 label is metabolized to the fructose-6-PO_4 state, then on the the fructose-1, 6-diphosphate stage at which time it is broken into two 3-carbon intermediates with both phosphates still hanging on to a radioactive carbon. So in independent experiments, we would inject the animal either with the 1-labeled carbon or the 6-labeled carbon and watch what happens. Theoretically, if you have adequate isomerization at the triose phosphate isomerase level, the two labels should behave exact the same. Therefore, as one goes on with the 3-carbon intermediate to pyruvate and then to the tricarboxylic acid cycle you should expire ^{14}C from glucose-1 or glucose-6 labels at approximately the same rate. It is clear now that this assumption is not quite correct because you have to correct for the lack a complete isomerization by determining the incorpora-

tion of label into the
L-alpha glycerol phos-
phate moiety and including
this correction factor.

If we take a look at the
pentose-phosphate path-
way as an alternative
(Figure 5), as the 1-
labeled glucose enters
that pathway it is
quickly lost as CO_2 and
6-labeled glucose is
maintained throughout
the various intermediates
as the ultimate carbon
in that chain. Any way
you put them back to-
gether again, there is no
way you can lose that
6-labeled glucose as CO_2;
the only possibility is
if you have a certain
amount of equilibration
with the trioses of the
glycolytic pathway, i.e.
glyceraldehyde-3-phosphate,
and then recycle it back
to glucose-6-phosphate.
The point of it is that
$^{14}CO_2$ expired from the
catabolism of glucose-6-
^{14}C represents only that
part of the glucose which
reaches the glycolytic
pathway and subsequently
the tricarboxylic acid
cycle while glucose-1-^{14}C
will be catabolized to

FIGURE 4

FIGURE 5

$^{14}CO_2$ by both the pentose phosphate pathway and the glycolytic pathway
plus the TCA cycle. If you'll now correct for the lack of isomerization
at the triose phosphate isomerase level, you can come out with a relative
importance of the pentose-phosphate pathway and the glycolytic pathway.
(Figure 6) shows the type of calculations involved.

Figure 7 shows results from animals weighing between 100 and 200 grams.
The significant point here is that these animals under the 5 psia, 100%
oxygen environment send a great deal more, roughly twice as much, of their
glucose through the pentose-phosphate pathway as opposed to the control
animals. It is almost equivalent, although not quite, with the glycolytic
pathway. We now have a mechanism which explains why we can come up with
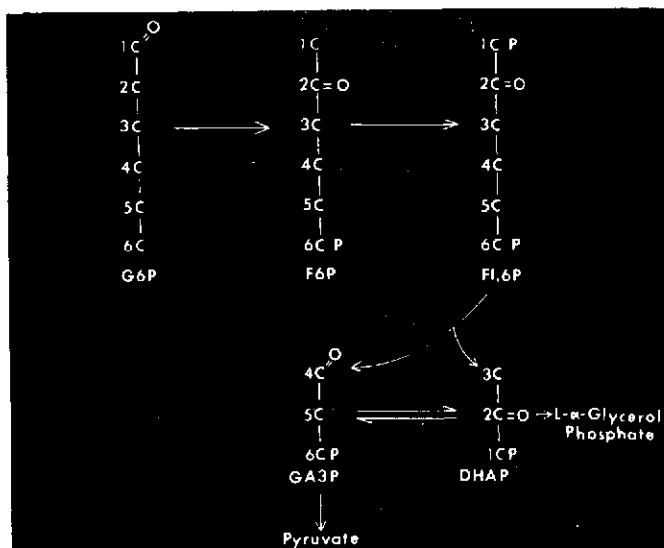the same total amount of CO_2 expiration with an obvious decrease in the
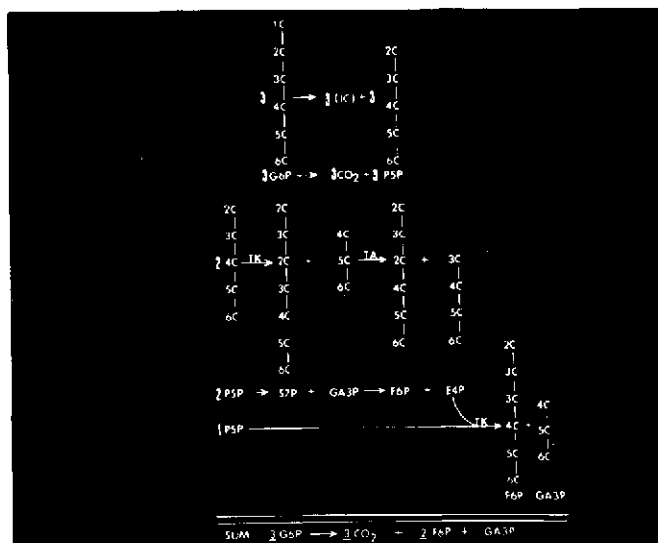
$$\frac{C^{14}O_{2(G1)} - \frac{1}{\delta} C^{14}O_{2(G6)}}{C^{14}O_{2(G1)}} \times 100 = \%PPP$$

where $\delta = \dfrac{C^{14} \text{ in glycerol from glucose-1-}C^{14}}{C^{14} \text{ in glycerol from glucose-6-}C^{14}}$

FIGURE 6

^{14}C-GLUCOSE SYNOPSIS

PERCENT *via*	EXP'L	CONT.	
GLYCOLYSIS & TCA	59.4	80.4	
PENTOSE P. P.	40.6	19.6	
			GLYCEROL dpm
	374	392	(1-^{14}C) GLUCOSE
	357	331	(6-^{14}C) GLUCOSE
			LAB

FIGURE 7

rate at which the tricarboxylic acid cycle is operating. We tested this theory by running not only glucose-1 and glucose-6 labeled compounds but also acetate-1 labeled and pyruvate-2 labeled ^{14}C. Under these conditions, we can demonstrate consistently that there is a decrease in the relative rate at which the tricarboxylic acid cycle is operating and a relative increase in the importance of the pentose-phosphate pathway.

There are also some interesting asides which I won't take time today to talk about, but incorporation of these compounds into fatty acids and steroids is another story.

The significance is obvious. We see how the animal utilized a pertinent pathway in order to adapt to the hyperoxic environment. We have run a number of other experiments (Figure 8) including glucose-6-PO$_4$ dehydrogenase, not only because it is an interesting sulfhydryl enzmye but also because it is the key enzyme at the entrance of the pentose-phosphate pathway. Its concentration in liver increases immediately and we can see it in a matter of hours after the animals have been put into a 5 psia, 100% oxygen environment. This is in the tissues; if you look in the red blood cells you may see a decrease, in fact, because it is a fairly labile compound. If on the other hand, we injected actinomycin D or cycloheximide to stop the synthesis of new protein, we did not find any increase in glucose-6-PO$_4$ dehydrogenase. So, we could demonstrate clearly from this that there was de novo synthesis of that specific enzyme in response to the 100% oxygen environment.

We also did some interesting injection studies of intermediates associated with the tricarboxylic acid cycle (Figure 9). The real crux of the issue is in the last two intermediates. If we injected α - ketoglutarate

57

|	SUBSTRATE OR ANTIBIOTIC INJECTED	ACCLIMATIZATION PERIOD	5 PSIA 100% O_2 EXPOSURE	INJECTIONS	NECROPSIES
BASELINE DATA	NONE	14 Day	14 Day	NONE	1,3,7, 14 Day
ANTIBIOTICS INJECTED	Actinomycin D	14 Day	2 Day	0,1,2 Day	2 Day
	Cycloheximide	14 Day	2 Day	0,1,2 Day	2 Day
RADIOLABELED SUBSTRATES	[1-^{14}C] glucose	14 Day	2 Day	2 Day	2 Day
	[6-^{14}C] glucose	14 Day	2 Day	2 Day	2 Day
SUBSTRATES INJECTED	Pyruvate	14 Day	2 Day	0,1,2 Day	2 Day
	2-oxoglutarate	14 Day	2 Day	0,1,2 Day	2 Day
	Succinate	14 Day	2 Day	0,1,2 Day	2 Day
TCA QUANTITATION	NONE	14 Day	2 Day	NONE	2 Day

FIGURE 8

FIGURE 9
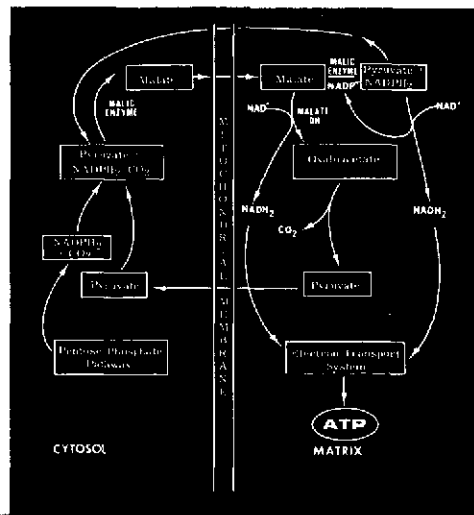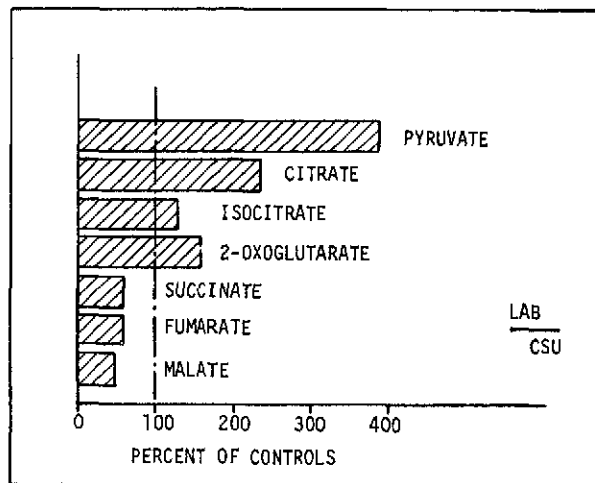
FIGURE 10

(2-oxoglutarate) into the animal, the situation was intensified; they synthesized almost twice as much glucose-6-PO_4 dehydrogenase as ever before. If, however, we injected succinate, the amount of glucose-6-PO_4 dehydrogenase synthesized decreased markedly. It's obvious that we are beginning to think in terms of α-ketoglutarate dehydrogenase as one of the key enzymes. The same argument can be shown for pyruvate dehydrogenase and it's not too surprising since the mechanism of action of these two enzymes, in so far as we know now, is identical, requiring the same cofactors, and, in fact, the complex of enzymes looks very much alike. When we took a look at the concentration of TCA intermediates in the liver of animals exposed to the 5 psia, 100% oxygen environment, you can see that everything before α - ketogluterate was increased. Pyruvate was increased about 4 times its normal level. As you get to the next point in the block, the concentration seems to jump a bit again. After you get past 2-oxoglutarate dehydrogenase, all intermediates are present at reduced levels. It is fairly clear that one of the blocks is at α - ketoglutarate dehydrogenase and probably a second block involves pyruvate dehydrogenase. To conclude this story (Figure 10) the significance of the study lies in the fact that to maintain bioenergetic balance, these animals utilize the pyridine nucleotide that has been reduced via the pentose-phosphate pathway for energy conservation reactions. Usually you look at TPN.H produced in the pentose-phosphate pathway as being associated with biosynthetic pathways and DPN.H as being associated with oxidative pathways that yield ATP. We've got to come up with an alternative for the utilization of TPN.H in an ATP yielding process. A high probably for this alternative, as shown by our experiments, is the existance of an isocitrate malate shuttle.

One of the interesting compounds we have looked at in addition to the sulfhydryl enzyme, glucose-6-PO_4 dehydrogenase, is the classic one, glyceraldehyde-3-PO_4 dehydrogenase. This substance is very sensitive to oxygen yet we see no change in this particular enzyme. Nor do we see a change in isocitrate dehydrogenase which might account for the fact that the concentration of the TCA intermediates associated with it is essentially normal under these conditions and perhaps, very important in terms of the shuttle of reduced pyridine nucleotides. This gives you a feel for the type of thing we are doing.

The importance of the story for this conference is simply to summarize our protocol--what we can do with it. We have here a situation where the effects of a particular environment were just not known to us; we could not predict them very well. When we began this work we had no feeling at all for where it might lead us. By entering into a survey phase with labeled acetate, for example, and certain key physiological parameters especially those associated with bioenergetics (oxygen consumption, CO_2 production, heat production, etc.), we were able to narrow the number of possibilities and even see a metabolic change in an organism which is remaining in very good bioenergetic balance. The sensitivity of the system, is demonstrated best, perhaps, in the oxygen toxicity story. The next step is to use certain key intermediates like glucose-1 labled and glucose-6 labeled pyruvate and so on, depending on the kind of problem one is dealing with, to bracket the areas in which you might be looking for change. Certain key enzymatic studies would perhaps be warranted based on such studies. Finally, we come to a point where we know approximately where the change

in the metabolic pathway and we can move to pinpointing the enzyme or
yme system involved. In the case of the oxygen toxicity story, it leaves
with only 3 alternatives. The first is that coenzyme A itself is
tially involved, but as I've shown earlier this doesn't demonstrate it-
.f until later in the first week and reaches maximum about the fourth
k in the 5 psia, 100% oxygen environment. So how do we explain a change
glucose-6-PO_4 dehydrogenase levels in a matter of 4-6 hours after
josure to the test environment? There are two other key compounds in
:h enzyme complexes; one of them, in each case, happens to be the sulfhy-
rl group on the enzyme itself, the other is lipoic acid which is a co-
:tor required in the system. We have not specifically identified which of
se two is involved in the initial step following marginal oxygen toxicity,
t if one took a look at the E'_0 of these kinds of compounds, one would
edict, fairly confidently, that it was the lipoic acid that was most
sily oxidized. Lipoic acid is the first to go and protect the CoA for a
ile. Later, perhaps the CoA takes over this kind of role.

have used the same protocol to study diluent gas effects on intermediary
tabolism. This all started back about the middle 1960's before the fire at
e Cape. Joe Saunders said that he wanted to call our attention to some
rk by Peter Bennett at the Royal Naval Research Laboratory in England in
ich he stuied neon as the diluent gas for diving purposes instead of
trogen. He took individuals, volunteer divers, and alternately put them
an air environment at 3 atmospheres or a neon-oxygen environment at
atmospheres, moving them back and forth so that would not know which
vironment they were in at any given time, t. Then they were asked to
complish certain psychomotor tests which included doing simple arithmetic
oblems--how many could you do in a minute and how many could you get
rrect, moving metal balls with forceps from one container to another and
w many could you move in a minute's time and how many could you get into the
xt container. Under these conditions, his volunteers performed 16-22%
tter in the neon-oxygen environment than they did in the air environment.

st of us that have been studying diluent gas effects are fully aware that
high pressures, gases in the rare gas family produce narcosis. The
rly, and some of the finest, work was done by Frank South and his
lleague, Cook, at the University of California, Berkeley. We began
oking at some of these gases: neon, argon, helium, and nitrogen in
s mixtures as well as 100% oxygen. We ran our first experiment (Figure 11)
th neon at 1 atmosphere for several reasons. If we were going to see
mething with neon, we thought that we should have enough neon in the
stem to get an effect before we looked at lower pressures and smaller
ounts of diluent gases. Thus in the initial experiment there was
sentially the same amount of oxygen in both environments; we simply
placed the nitrogen with neon. One of the mechanical problems that occurs
 that nitrogen becomes a contaminant, so we built perhaps the first
trogen scrubber in the world. Figure 12 summarizes the results. We
und an increase in the food consumption but the body weight did not
crease until about 2-3 weeks later; oxygen consumption was up, CO_2
oduction was up as was the heat production. When acetate-1-^{14}C was
jected, its rate of expiration as $^{14}CO_2$ was increased significantly.
ery parameter that we measured showed that animals exposed to the Ne-O_2

environment were metabo-
lizing at a significantly
higher rate than the con-
trol animals.   At the same
time we ran an alternate
experiment in which we
held the animals at 160
torr oxygen; neon was
added as the diluent gas
so that the total pressure
was 259 torr. We now had
a relatively small amount
of neon. In this case,
the increase in metabolic
rate was still there but
it was only about 10%
rather than 30%. The
significance of this is
that the easy explanation
for the increased meta-
bolic rate in the pre-
sence of neon is the
thermal conductivity of
neon as compared to
nitrogen; that is, it
carries heat away from
the body surface at a
faster rate. The trouble
with that explanation is
that if you take this
last experiment I de-
scribed to you, the
thermal conductivity of
that environment is only
about 43% of that associ-
ated with air at 1 atmos-
phere and yet metabolic
rate was increased.
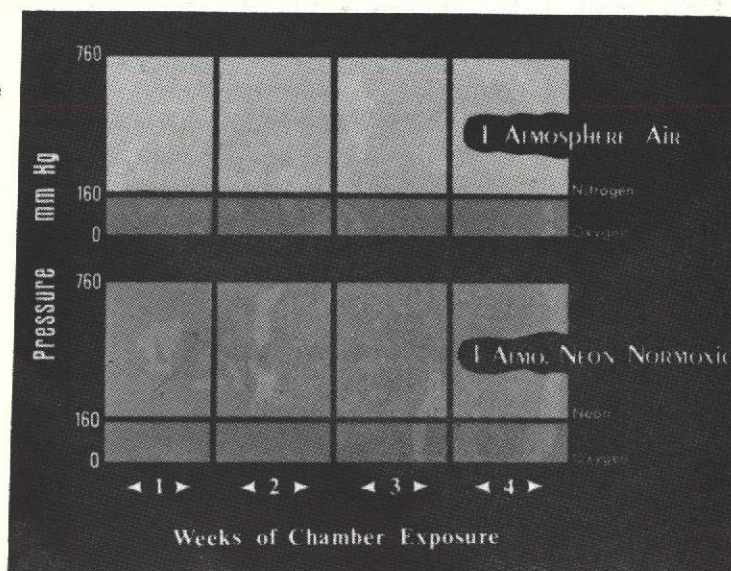
FIGURE 11

FIGURE 12

This was a tip-off and together with the work of Frank South (principally
his Warburg experiments with rare gases) we began to ask ourselves if it
was possible that there might be both a direct and an indirect metabolic
effect--the indirect effect being that due to thermal conductivity and the
direct metabolic effect in mechanism might parallel the narcotizing effects
seen at higher pressures.

In order to study this hypothesis, we began a series of experiments. One
set of the experiments involved a study of the thermoneutral zone for
animals breating nitrogen-oxygen, argon-oxygen, and helium-oxygen at one
atmosphere. Using a very small chamber (Figure 13) we measured oxygen
consumption, tail vein temperature, and body core temperature. We found
that for the air environment, the thermoneutral temperature in fed rats

FIGURE 13

lies between 27° and 29°C but for the helium-oxygen mixture the thermoneutral range is 31°-33°C (Figure 14). In the helium animals, you find a very sharp point at 31°. The animals died at temperatures above 33°C. For the air animals, it's not quite so sharp. If you look at argon, you'd have to say that the thermoneutral range is around 27°C but it's not sharp at all. I think this is significant because it demonstrates that with helium the thermal conductivity factor is a great deal more important than it is for argon and, consequently, the direct metabolic effect is significantly less in helium. Conversely, thermal conductivity is a minor factor in the argon environment but the direct metabolic effect with its concurrent reduction in metabolic rate is very significant.

Now I'll show you some data which corroborate these findings. The second experiment was designed to expose animals to thermally iso-

FIGURE 14

conductive environments containing the various test diluent gases; that is, where the thermal conductivity to each environment was identical with air at 760 torr and the pO_2 of the environment (Figure 15). Each group of animals was acclimatized in the chamber for a period of 2 weeks and then put in the test environment. The total pressure in a neon-oxygen mixture was about 515 torr; for a helium-oxygen environment, 259 torr; and for the argon-oxygen mixture, the total pressure was about 1000 torr in order to have a thermally isoconductive environment. We have also learned that the diluent gas does influence the transport of oxygen across the lung; this is especially significant in the case of helium. The expiration of $^{14}CO_2$ (Figure 16) from injected acetate-1-^{14}C was measured and compared to the air control animals; animals in the neon-oxygen environment show a curve shifted to the left indicating a higher rate of catabolism of acetate to CO_2. The last

time I was with you, I showed you one alternative. What I'd like to show you now is a far, far better alternative. In order to more clearly express these results, one simply remembers a basic physical chemistry rule--that you can express reaction energetics by simply considering the beginning and final state of the compound, disregarding the devious paths that compound has followed. You simply focus on how much of the labeled acetate has not been converted to $^{14}CO_2$. You can now plot the natural logarithm of the amount of acetate that has not been converted to $^{14}CO_2$ (the natural log of $(C_0^2 - P)$ where C_0 is the amount of acetate that you originally injected into the animal and P is the amount converted to $^{14}CO_2$) versus the time post-injection of the acetate (Figure 17). The plot (Figure 18) is very interesting because it follows first order kinetics until well after an hour after the substrate has been injected. This means that you can get many good sequencial measurements from this system until the reaction enters into multiple order kinetics--it obeys first order kinetics in air for about 70 minutes, and in argon (with the metabolic rate decreased) it follows first order kinetics for 120 minutes. You get a very nice plot and the slope of that curve is proportional to the rate of catabolism of that substrate. For glucose-1-^{14}C, it should also be a pretty close approximation of total metabolic rate. I would like to emphasize that these animals are not in a basal metabolic state, they are not restrained or wired, in fact, they are running loose in the cage. Using this procedure (Figure 19), the turn-over rate or half-life for the metabolic pool, which is independent of the number of acetate pools, may be calculated.
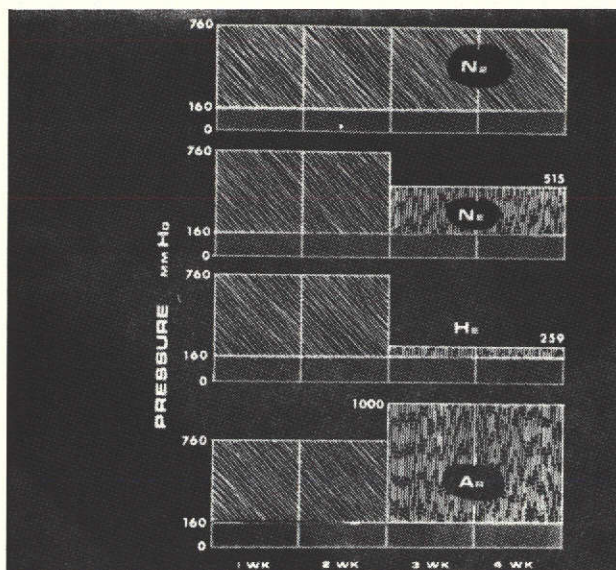
FIGURE 15
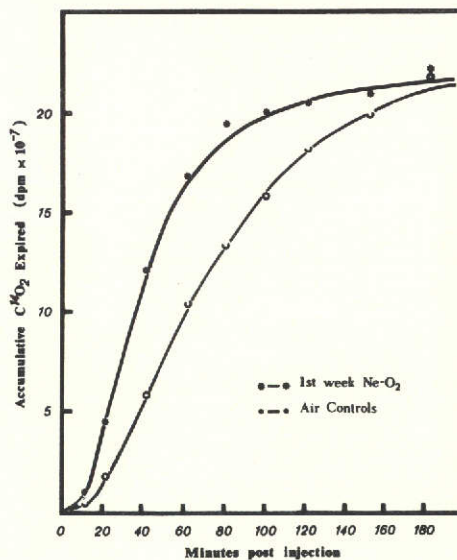
FIGURE 16*

* In Depressed Metabolism, eds. Musacchia, X. J. and J. F. Saunders, Elsevier, Publ., N. Y.: 87, 1969.

ln

¹⁴C-Acetate
Pool

FIGURE 17

POST INJECTION TIME

FIGURE 18

ln

¹⁴C-Acetate
Pool

k ∝ Catabolic Rate

POST INJECTION TIME

ln

¹⁴C-Acetate
Pool

FIGURE 19

$T_{1/2}$

POST INJECTION TIME
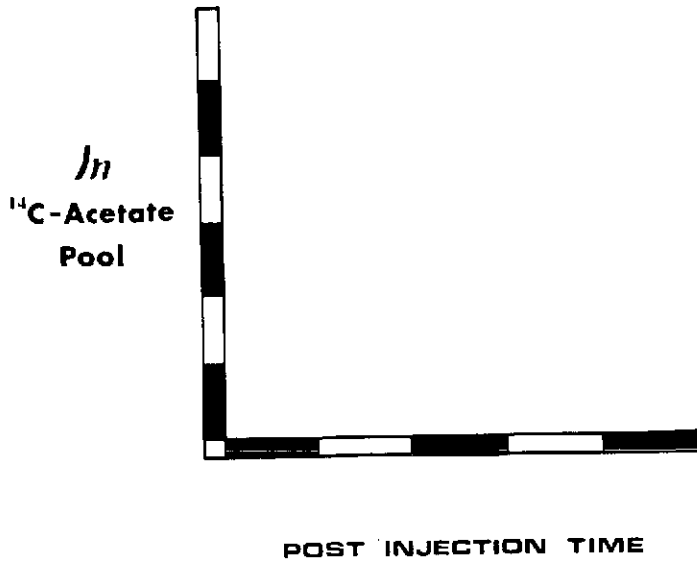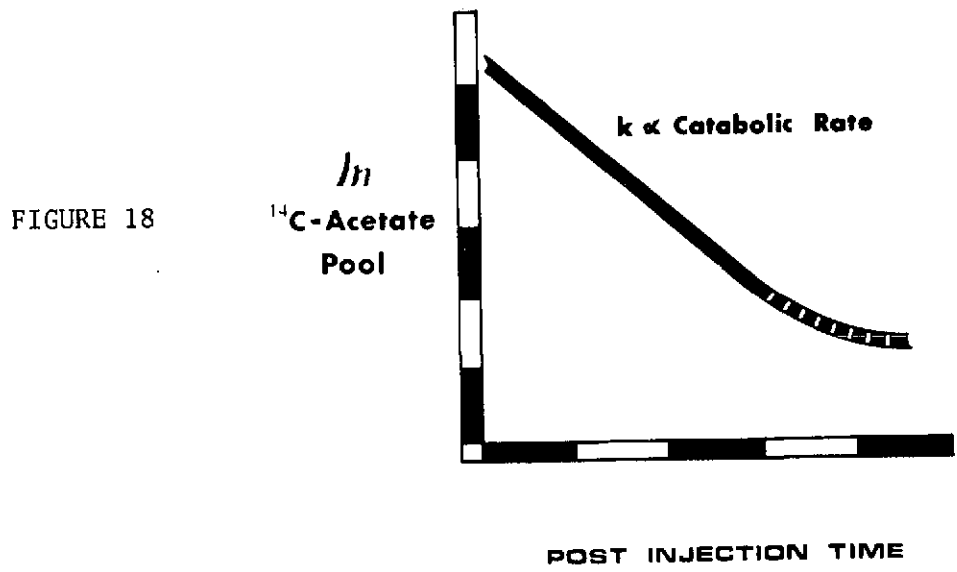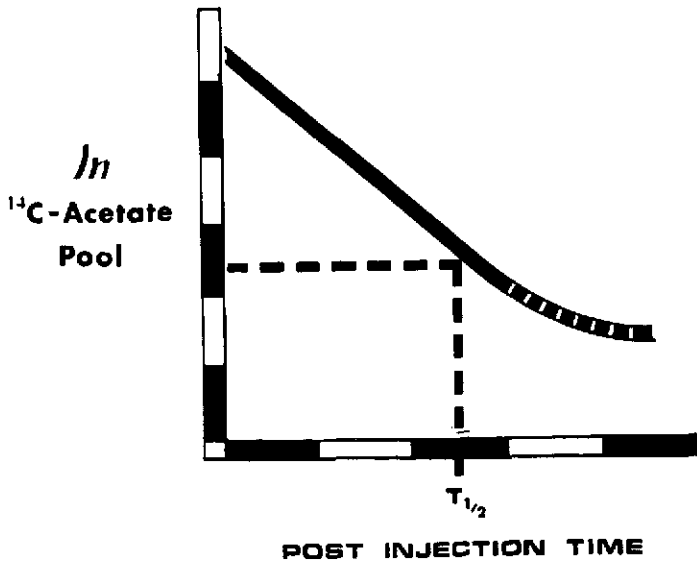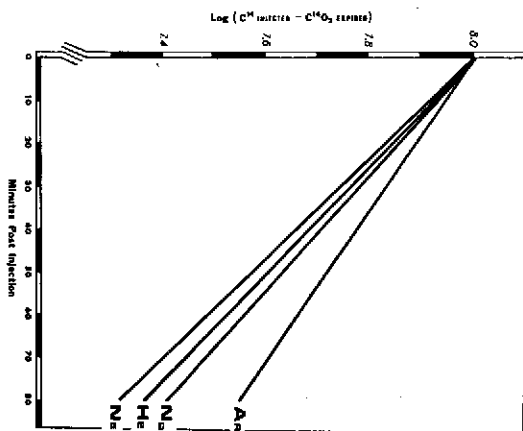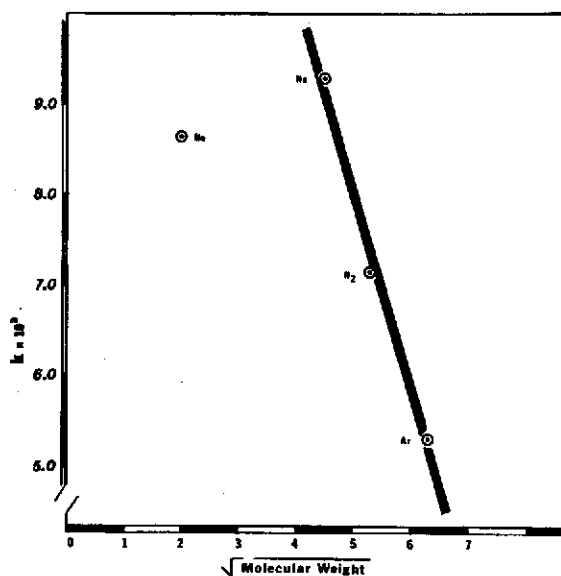
FIGURE 20

FIGURE 21

When you measure the k value for neon, helium, nitrogen, and argon as diluents gases, the result is a family of curves (Figure 20). If you now plot the k values for those curves vs certain of the physical parameters of those compounds which have been found to have a high degree of relationship with narcosis when the gas is present in high concentrations (Figure 21), they show a linear relationship, except for helium. We are fairly convinced from these curves that helium has a tremendous thermal conductivity effect and a rather modest (if any at all) direct metabolic effect. Figure 22 shows a measure of the molar refraction of these compounds vs the k value. Molar refraction was used by Linus Pauling to approximate the clathrate forming ability of these gases, i.e. the ability to form what are sometimes referred to as microice crystal which are associations of 5 or 6 water molecules with the rare gases. Figure 23 shows the k plot vs Hildebrand's "solubility parameter" which approximates solubilizing lipoprotein complex.

We assumed that all of the test environments should contain O_2 at 160 torr. As it turns out, the diluent gas does influence transport of oxygen across the lung and thus the original assumption must be modified. Using the dog (Figure 24), we determined what the environmental PO_2 would have to be to insure that the blood PO_2 was the same as it was in that dog in air at 1 atmosphere. When helium was the diluent gas, the partial pressure of oxygen in the environment must be 184-187 torr to have the same partial pressure of oxygen as you have at 160 torr in a nitrogen environment at one atmosphere. With argon, you only need 140 torr to have the same partial

65
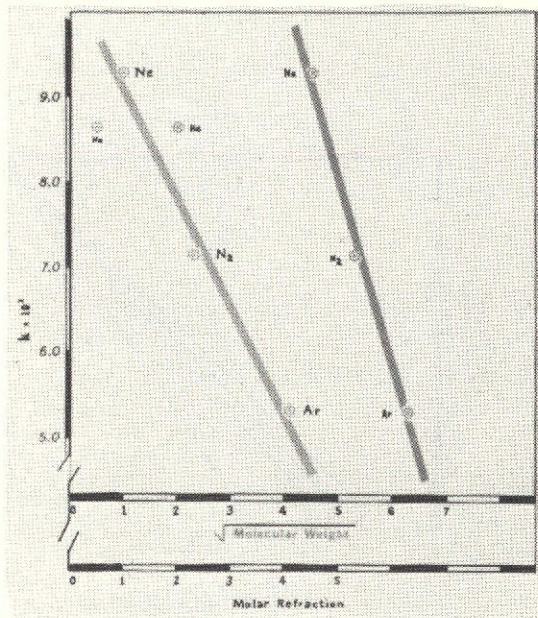
FIGURE 22

FIGURE 23

FIGURE 24

FIGURE 25

pressure of oxygen in the blood. We are now repeating the thermally isoconductive experiments with this correction factor in mind in an attempt to quantify the degree to which there is a direct and an indirect metabolic effect of the gases.

If you measure other characteristics such as CO_2 production or oxygen consumption against these same parameters (Figure 25), you find similarly linear curves. As you notice, helium again falls somewhat off the curve; that might be partly becuase the transport of oxygen in a helium-oxygen environment is different due to the physical nature of helium.

There are several points of significance in this work. It should be useful for prolonged space flight although it is not popular today to talk about such things. It may be necessary to have an astronaut in a depressed metabolic state to preserve his sanity, among other things, perhaps in an argon-oxygen or a xenon-nitrogen-oxygen mixture. But during periods requiring critical spacecraft maneuvers, we would like to sweep out the depressant gases and replace them with a neon-oxygen mixture so that the astronaut can perform maximally.

There are a number of alternative uses of these data, for example the approach to treating metabolic diseases. Hypertension may be related to a decreased sensitivity in some people to nitrogen narcosis. In fact, it is possible that a hypertensive patient could be treated by periodically placing him in a tent containing an increase in nitrogen partial pressure or perhaps a heavier gas could be used as the diluent, e.g., argon. As I have suggested before, we, living at one atmosphere in a nitrogen-oxygen environment, are possibly in a state of insensible narcosis, which is probably good for our longevity.

This presentation is dedicated to the memory of W. O. Fenn, distinguished Professor of Physiology at Rochester University who for many years served as consultant to my laboratory.

DISCUSSION BY PARTICIPANTS

SOUTH: I think that helium actually affects the mean free path of oxygen.

JORDAN: It sure does. It's probably a size phenomena too -- it sits in the alveolae and so on.

MUSACCHIA: Do you know anything about the Navy studies, the autopsies, etc. that were done on animals and, I believe, a few divers that did succumb while diving in a helium environment? As I understand it, the lungs apparently never show any particularly bad pathology from having breathed helium.

JORDAN: Those experiments are supportive of our work; they were done at an environmental temperature of 103°F in order to keep the individual comfortable. I'd like to emphasize again that the thermal conductivity factor for helium is extremely large and the direct metabolic effect is probably small. As a matter of fact, Ralph Brauer has studied helium in terms of narcosis and he's never been able to get his primates to show depression; instead he has seen an indiscriminant firing of electrical impulses which results in convulsions rather than narcosis. This occurs at about 55 atmospheres.

MUSACCHIA: Of course, in our experiments, we counted on the thermal conductivity to give us an appropriate depth of hypothermia. So far as actual pathology, longevity, or superficial behavior are concerned we have found no deletorius effect from helium exposure. I'm just trying to get a better fix on any potential effects that helium might have. As far as I can see, what you are calling an effect is the respiratory effect -- that there might be a problem with oxygen across the alveoli in the presence of helium.

JORDAN: It does two things. It does increase metabolic rate and at the same time, there is a physical effect of helium in terms of the transport of oxygen across the lungs. So if you want to see a total, overall metabolic effect, you've got to correct for this.

MUSACCHIA: Well, he certainly increases his respiration rate and is attempting to pull more oxygen in.

JORDAN: Anthropomorphically, maybe the cell is calling for more oxygen.

WUTOH: What is the chemical nature of the body weight gain?

JORDAN: I don't know the exact nature of the body weight gain in the neon-oxygen animals since we haven't done full carcass analysis on these animals, i.e. total water, carbohydrate, protein, and fat composition.

WUTOH: Actually, I was wondering if there was any difference.between the various gaseous mixtures?

JORDAN: I don't know. Our experience with 100% oxygen at 5 psia was that there was a modest change, but it took quite a while to demonstrate particular change in composition. In helium-oxygen, it would change more rapidly because metabolic rate has increased. We are doing carcass analysis on animals now but I don't have the data for you.

MUSACCHIA: You've brought up some real interesting aspects of the potential use of neon, helium, etc., etc., what about the thermal conductivity of neon?

JORDAN: It's not very much greater than that of nitrogen. In fact, quantitatively, the direct metabolic effect at 1 atmosphere is about twice as much as the indirect or thermal conductivity effect in neon. For argon, there is almost no thermal conductivity effect, while with helium most of the effect is thermal conductivity.

MUSACCHIA: Have you looked at any particular cellular constituents or structures such as mitochondria, organelles, etc?

JORDAN: Not yet, but we expect to in the next series of experiments. We're following this same protocol -- we're in the bracket phase, and we're running labeled substrate experiments in the hope of identifying the site(s) of effect.

MUSACCHIA: Are you going to do electron microscopy?

JORDAN: No.

OYAMA: Would you expect any change in an in vitro system?

JORDAN: That is a good question. We've got several Warburg-type measurements and experiments on the board for this winter. If we found no effect, we wouldn't know what to make of it because the data would be obtained out of context with the animal. If we see something, it still might be slightly difficult to interpret. We were very hesitant to do it until we can relate it to some whole animal effects.

SOUTH: One thing about Warburg experiments, I think you'd agree, is that sometimes there is a difficulty with the gas. The substances you are using tends to layer very readily because of sink in the gas flow.

JORDAN: One thing that is also sure about those gases is that they are not "perfect" gases in the physical chemistry sense.

SOUTH: Yes, so the best way of filling a Warburg or any experimental vessel is by evacuation rather than flow through because flow through takes forever.

JORDAN: I'd really like to emphasize that with our protocol one can start from almost no knowledge and move to a place where you can pinpoint the particular enzyme systems in specific tissues being affected.

SAUNDERS: Pat, you said that the original work was dealing with marginal oxygen toxicity. What specific endpoints would you say are indicators of the marginal toxicity that you have shown?

JORDAN: Do you mean what can you measure that shows that you have marginal oxygen toxicity?

SAUNDERS: For example, you said brain coenzyme A is decreased, cholesterol levels were increased....

JORDAN: That's correct -- I didn't say that here, but that's right. I think the best indicators that we've been able to monitor are 2 kinds. If one can inject ^{14}C-acetate, that's the fastest indicator; you see that right away. You can also do this with glucose-6-^{14}C and it will show you the same effect very quickly. The next best in coenzyme A -- we use to think it was the best but it's just too slow to tell you the very early stages.

SAUNDERS: Another question that is mission oriented. Remember when in the Gemini program the astronauts were breathing pure oxygen at 5 psia and we had the decrease in erythrocyte mass something like 14-20% in, I believe it was, the 14 day mission. All the sudden the order came to include a few percent of nitrogen to the breathing system during the Apollo program and we never experienced any decrease in erythrocyte volume. The conclusion, empirically I guess, is that the absence of nitrogen on the previous missions was the responsible agent for the erythrocyte loss. What do you have to say about that?

JORDAN: I think it is probably correct.

SAUNDERS: But why would nitrogen have such an effect with only a few percent added?

JORDAN: Let me start in another way and give you our own data which fully support that. When we were trying to identify whether the effects we saw and were labeling as an oxygen toxicity problem were, in fact, due to the oxygen, we had a series of experiments including running nitrogen-oxygen mixtures at 259 torr with oxygen partial pressure at 160 torr. We found that in 100% oxygen at 259 torr, we saw all these effects including the hematocrit and hemoglobin drops and the whole gammit of things that we have described. I won't burden you with all those data here. We also saw at 259, but 160 torr for oxygen, no effect when nitrogen was the diluent gas, no effect when neon was the diluent gas, in terms of decreased CoA or radioactive $^{14}CO_2$ from acetate-1-^{14}C and a whole myriad of measurements. All we saw was an increase in total metabolic rate in the neon-oxygen experiment. Then we thought that we would run the "critical" experiment -- run the oxygen partial pressure up to 233 torr (an equivalent amount to what we actually had in the 5 psia, 100% oxygen), then add nitrogen to 760 torr and we thought that we would prove that the effect was due to oxygen and that pressure had little to do with it. It turns out that we didn't see anything in that experiment. We are convinced that as nitrogen influences the burning of a candle, it also effects the "fire of life". In fact, you really don't need that much nitro-gen -- we are able to demonstrate the same effects with lesser amounts of nitrogen that we do with large amounts. We haven't run the proper experiment; that is, 233 torr oxygen and maybe another 100 torr of nitrogen. What we use to call an inert gas has been proven to be active not only by the chemist but also now by the biologist. Our real interest is the nature of inert

gas narcosis, but we're only about half-way down our protocol in moving towards that end. What I tried to show you today was the step-wise sequence and how we went all the way with it.

SMITH: Do you have any evidence for the noble gas effects on CoA, the pentose shunt, and key cofactors like DPN and TPN? It seems to me there is a hiatus between the addition of noble gases and jumping from that into biochemistry. I don't see the connection between those areas of interest - noble gases, what do they do to any reaction? We know absorption has a proportionality to the concentration and to partial pressure.

JORDAN: I think that is really the question we are trying to answer. We know some of the relationships that show up but we don't know the nature of the effect, for example, at the nerve synapse or at a membrane surface. I think that the mechanism should be closely related to those characteristics that allow those gases to be narcosizing at much higher pressures. I think there may be a continuum of effects; you have to get a certain amount of that gas before you see the gross physiological effects that you're hunting for in narcosis. Perhaps biochemical manifestations are seen at much lower concentrations. I think you are correct; and it is that hiatus area that we are headed for in our studies. I showed you the oxygen story to demonstrate how this protocol allows you to come to a specific definition, a specific answer, by bracketing and then beginning to identify specific mechanisms.

OYAMA: When you have these catabolic processes occurring, you have the evolution of heat, an exothermic reaction, and therefore an increase in conductive heat loss. In essence, you are drawing heat away at the pump and you can catalyze the kinetics in this direction. So, on a molecular basis, you might possibly consider the rate of heat dissipation as accelerating the kinetics.

JORDAN: Except that the argument is weakened when we run our experiments in a thermally isoconductive environment. The problem there so that pressure may have had an effect, and that is why we have done the other temperature experiments and intend, this fall, to run experiments parallel to the rare gas experiment described here within the range of thermoneutrality of these various gases and measure the effects again. In the third experimental approach we are going to implant a tracheal cannula with the hope of getting a different gas mixture inside the organism vs that which is outside on the surface of the skin; obviously we will only approximate that since there is some heat loss from the trachea as well.

MUSACCHIA: Will the animal be free running?

JORDAN: No, that animal will be restrained.

MUSACCHIA: You shouldn't have too much trouble then; we've been putting tracheal cannula into hamsters now. I'd like to return a minute to thermoneutral zone and ask if you know anything about body temperature in these animals under the various gas phases.

JORDAN: Yes, as a matter of fact, when we ran the thermoneutral range experiments we not only measured oxygen consumption but also tail temperature and core temperature.

MUSACCHIA: Do the animals maintain at about 37° - 38°?

JORDAN: If the environmental temperature is below the thermoneutral range, core temperature drops.

SMITH: Your temperature chamber isn't 37° though?

JORDAN: No, but we are running some animals now in a nitrogen-oxygen mixture at 28°, in a helium-oxygen mixture at 31°, and in an argon-oxygen mixture we run that animals at about 27° - 28° since temperature is not as critical in argon.

MUSACCHIA: You've already made the adjustment by going up to 31° with helium, but have you run any animals at room temperature?

JORDAN: In helium, we have measured only gross physiological data such as consumption and CO_2 production, not labeled compound studies.

MUSACCHIA: The total oxygen consumption in, say, an 80:20 mixture at room temperature does what?

JORDAN: It is minimal in the range at 31° - 33° for a helium-oxygen mixture. At room temperature, the oxygen consumption is probably a good twice and maybe closer to 3 times that measured in the thermoneutral zone (see Figure 14).

MUSACCHIA: The respiration rate will be just about twice as much.

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72-A

Hibernation, Sleep, and Thermoregulation

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Initially, this project was aimed, rather ambitiously, at trying to determine the differences in CNS activity during hibernation and hypothermia, and also to introduce our current theories on adaptation to hypothermia by nonhibernators. During the course of this, we were tripped up by a number of fairly difficult problems. These included not only procedural but also philosophical ones. These will become evident as we proceed.

Our general procedure, as typified by the marmot, may vary in detail from individual to individual. Animals are implanted with a series of cortical and subcortical electrodes. For the thermoregulatory experiments, the thermode was in the preoptic area, the thermocouple reentry tubes led to the preoptic and other thermocouple tubes frequently led to the amygdaloid-lentiform nuclei (Slide 1). When we wanted to record respiration, all we did was to put a very fine, sensitive thermocouple into the nasal bone and extend it to the nasal mucosa so that a variation in temperature was recorded with every breath even during hibernation. Also, we record nuchal EMG and, for thermoregulatory experiments, we also record temperature from the dorsal surface of the neck as well as subcutaneously using some safety-pin type of electrodes. After recovery from surgery, we have a typical, pleasant, happy marmot with a pedestal on his head which he wears with no problem.
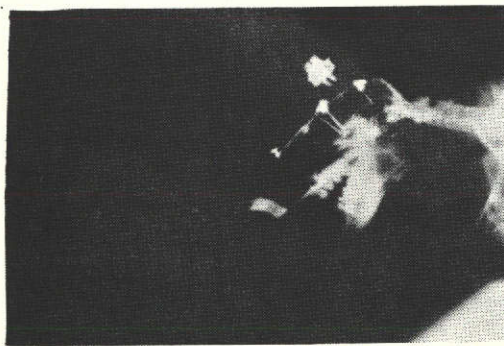
SLIDE 1

He bites well, sleeps well, jumps fine, and displays the usual forms of agressive behavoir. We usually attach our cables onto the pedestal (which you can barely see there) and screw them in place so they can't come off after hibernation is entered (Slide 2). You can see the cable going up to the slip ring assembly. When we attach him during hibernation, he will arouse but will go back with no difficulty.
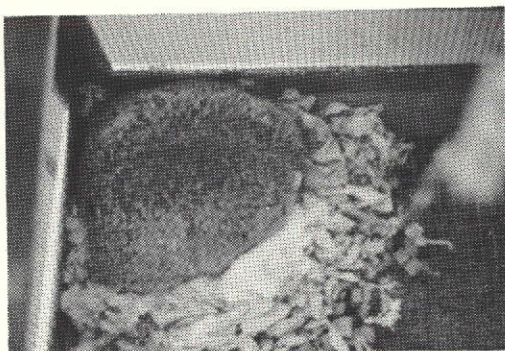
SLIDE 2

Hypothermia, Hibernation and Sleep

The overall differences in cortical and subcortical activities, between marmots entering hibernation, during hibernation, or arousing from hibernation as opposed to simply hypothermia are shown on Slide 3. You see that the cortical spindles start in around $28^{\circ}C$; a bit before the anterior hypothalamic preoptic area displays strong synchronous generator potentials, which occur at about $20\text{-}15^{\circ}C$. As entry continues, this activity drops out and the strongest thing left is the cortical spindle-- this is invariant in all the hibernating animals we have looked at.

SMITH: Frank, is that the hippocampus there?

SOUTH: This is not hippocampus but medial preoptic activity. I don't have a hippocampal trace on this particular one. I'll show a hippo trace shortly.

When he starts to arouse, you see activation of the EMG, EKG, etc. In hypothermic animals, you see similar waves (Slide 4), but if you look closely the power is much reduced and they are not as well organized. (The animal was made hypothermic by a modification of the closed jar technique of Andjus; the "jar" is very large and is made of double-walled steel.) The same type of wave pattern occurs at somewhat similar temperatures. As he goes into hypothermia, the power increases in the usual manner. The differences in this can also be shown by using power spectral analysis. Slide 5 shows autopower spectra of hibernation entry, from 38° to $7^{\circ}C$ at frequencies from DC up to twenty hertz (Hz). You'll notice here again, circa 20°, a huge amount of power coming from the medial preoptic at the lower frequencies, about five- to six-Hz. During arousal, just the reverse occurs. However, during hypothermic entry, it is much reduced, the temperature is slightly different, and if you look closely, you'll find

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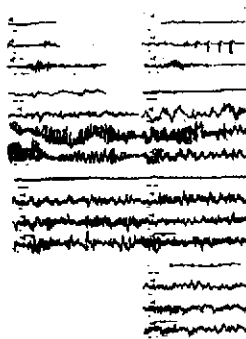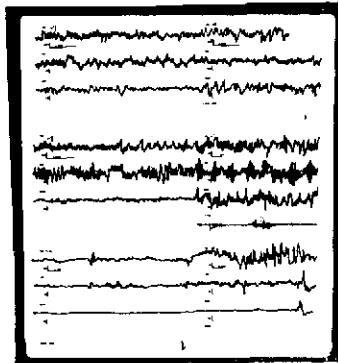
SLIDE 3

SLIDE 4

SLIDE 5

SLIDE 6

SLIDE 7

that the mean power is about 1 to 2 <u>orders</u> of magnitude less in hypothermia than it is in hibernation. Also, you'll notice some sleep recording. The hypothesis was generated that maybe hibernation is a form of sleep--at least this gives us a handle, or a hypothesis to work on in order to understand the entire thing. The next slide (6) shows a typically a-typical marmot slow sleep (SS) record -- frontal cortex, occipital cortex, hippocampus (which is asynchronous), ventro-lateral thalamus, pontine reticular formation. There is something that's interesting here which points out why marmot sleep is a little bit atypical. The EMG and EKG is taken in the nuchal region in order to obtain an EKG artifact as well as an EMG recording--but there isn't any EMG activity in this record. The marmot may or may not have EMG activity during slow sleep at normal room temperature or during cold exposure. The next slide (7) is of para-doxical sleep (PS) typically showing slightly irregular heart rate, cortex activated, hippo-campus, ventrolateral thalamus, and so on. This is a typical, good, PS recording. As should be the case, there is no EMG activity. The next slide (8) is a recording from an animal going into hibernation, the brain temperature is 20°C and dropping. It almost looks like a type of PS, (reading it in its own terms) an activated cortex, very good synchrony in the hippocampus.

POPOVIC: Frank, is that blip behind the complex the T wave?

SOUTH: Yes, the S-T segment in hibernating animals is typically much shorter than in nonhibernators. These are major differences; that is a very important distinction.

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In the next slide (9), we
see an animal in hiberna-
tion; he's gone through
the 20° loop. Once in a
while, we'll pick up this
type of activity from the
hippocampus. Note the
frontal cortex spindles.
EKG artifacts are the
rule since they are
irreducible at low
temperatures because
the electrodes are sep-
arated and because of
the simple overwhelming
power of the EKG. Fre-
quently we will pick up
spike complexes out of
the occipital cortex.
Slide 10 illustrates
our thinking about the
organization of fast
waves of the frontal
complexes, but it
doesn't always look
that well organized.
In hibernation, it does
appear that these things
originate from the
frontal cortex --it
appears that this part
of the limbic system is
through. We frequently
see (Slide 11) this
type of relationship,
but now we are picking
up spikes from the
lateral geniculate.
When we do pick up the
lateral geniculate spikes

SLIDE 8

SLIDE 9

(we don't always get them), we also get spikes in the occipital cortex.
So, the sleep hypothesis looks just great, in that we've got a lot of
characteristics of sleep.
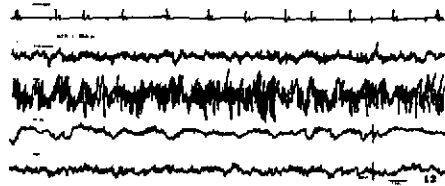
Behavioral Thermoregulation

Periodically we have gone a bit mad because the polygraph seemed to lose
its mind while animals were going into hibernation (Slide 12). So, we'd
tiptoe into the cold room and look at the animal and we'd find nothing--
absolutely nothing. We'd tiptoe out, close the door, and the polygraph
would go wild again. Then we got closed-circuit TV--we had to find out
what was happening and, indeed, a lot was going on. We've termed what we
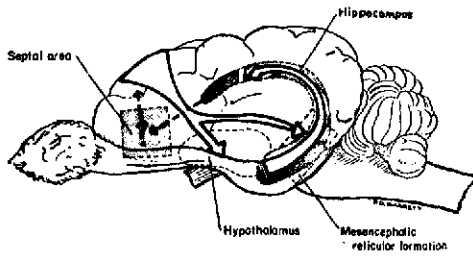
SLIDE 10

SLIDE 11

SLIDE 12

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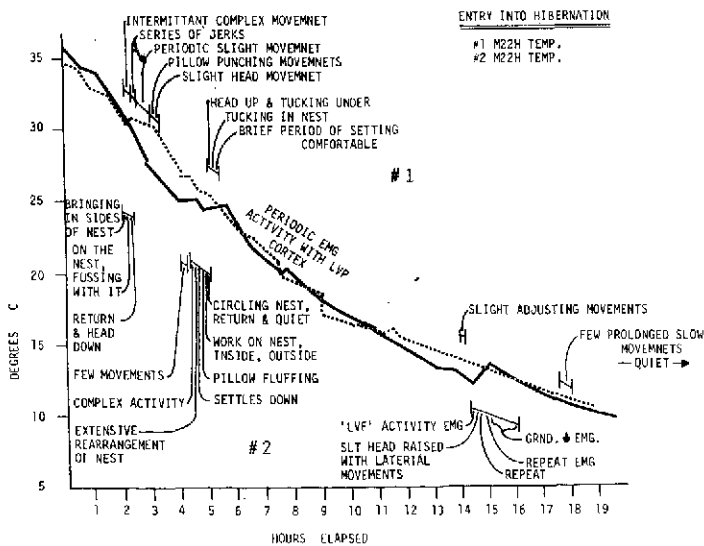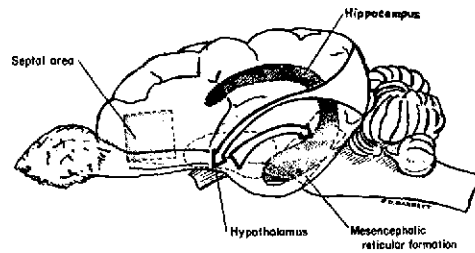
observed as "paradoxical behavioral thermoregulation." The animal goes to sleep and enters hibernation from a stage that looks like SS. Intermittant complex movements of the dashed line occur during this animal's first entry into hibernation (not the very first time but time #1 here). As he enters and body temperature falls, one may note the following: intermittant complex movements, jerks, periodic slight movements, pillow punching, slight head movements, head raised, tucked under, tucking in his nest (this is a brain temperature at 27°), he gets up and pulls on the nest, punches it, and curls up with his head down. He settles in and get comfortable. The temperature keeps going down (note the periodic EMG), low voltage fast activity in the cortex following it, quiet adjusting movements, than a few, slow, long movements--head raising and lowering. The second time around, he should be practiced right? He goes into sleep, and apparently wakes up at 25°C. He tucks in the sides of the nest, arranges it; he may get out of the nest and move it in from the sides at $20-25^{o}$C. He circles the nest, pushes it in, arranges it, then "pillow fluffing", raising the head, lowering it, and so on. This was watched time after time; we have record after record of these beasts performing this sort of nonsense. It is sort of nonsensical when you think about it. Here's an animal that "wants to" lose body heat and yet he's doing everything he can to prevent it by increasing his nest arrangement, making it tighter and more comfortable. At this point and rather superficially it appears a bit paradoxical.

POPOVIC: Could he possibly be preparing for arousal? If so, what would be paradoxical about that?

SOUTH: The other part of the "non-paradox" is that what he might be doing is to increase his heat capacitance. In other words, and I think it might emerge later here, that it is desirable for an animal in hibernation to have as much heat capacitance as possible to give him enough time to be "awake" to thermoregulate. There are two problem here. If we look back at our record again, depress the time scale, and amplify the voltage, we note that even during these periods of quiescence that there are alternating periods of what could be interpreted as PS and as slow sleep. Is it a form of sleep that he enters into?--Yes. Does he remain asleep? The point also is that there are a couple of other idiosyncrasies about these creatures. One is that normally when an animal sleeps, he will usually show considerable EMG activity during slow sleep and none during PS; yet you put these animals in the cold room or at room temperature and they may show EMG activity at either time. Another characteristic of sleep in most mammals is that when they go to sleep, their brain temperature will decrease during SS and increase slightly during PS; yet these animals show nothing but a decrease during normal sleep, they show no increase in brain temperature during PS. It would appear that this is even more pronounced at other times. One asks then if they thermoregulate like a normal, everyday, stick-in-the-mud, critter.

There are two ways to approach this question. We chose the more difficult one by putting the thermode in the brain and heating and cooling while looking at various responses (Slide 13). The solid line is the preoptic temperature as controlled by running warm or cold water through it. This shows a record obtained from a typical normothermic marmot at an ambient

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SLIDE 13

PREOPTIC COOLING & WARMING DURING NORMOTHERMIA

AMBIENT TEMPERATURE = 21° ± 1°

MARMOT T14

PREOPTIC TEMPERATURE

△△△△ PONTINE RETICULAR FORMATION TEMPERATURE

□□□□ DORSAL SUBCUTANEOUS SKIN TEMPERATURE

●●●● DORSAL SURFACE SKIN TEMPERATURE

of $21° \pm 1°C$. We cool the PO off, then the pontine reticular formation temperature, which is a good indicator of the direction of the body temperature, increases as it ought to. The thermal clamp is released and it decreases. Heating the thermode, the body temperature drops as it ought to; releasing the clamp, returns it to normal. During heating, the animal may start panting and frequently it will start drinking water. A rather interesting point here, another pecularity of the thermal makeup of this critter, is shown by taking the subcutaneous temperature of the dorsum of the neck and the surface temperature of the dorsum of the neck. Drop the temperature and the temperature goes up, it reflects body temperature, and vice versa, always maintaining a set difference. In other words, skin circulation does not change relative to these central thermal stresses. It logically follows that the marmot and possibly all hibernators, depends very strongly on chemical thermoregulation and relatively little on physical.

Hibernation and Temperature Regulation

We've looked at the animal now during normothermia, but does the animal thermoregulate during hibernation? In a sense, this is an anachronistic question. Slide 14 shows a simple, straightforward experiment. We We measured brain temperature, skin temperature, EKG, EMG (we didn't have an integrator working at the time so we did it by brute force), and established a baseline for these parameters in an animal hibernating for a number of days at $5°C$ ambient temperature. We dropped the room temperature down to about $1.5°C$ and observed any changes that occurred in addition to the EEG changes. Interestingly, the brain temperature went up. We lowered the temperature further and the brain temperature went up again. Heart rate seemed to be strongly correlated with all the thermoregulatory activity and, indeed, would be exactly what you might expect if the animals were thermoregulating purely by a sympathetic nervous system response along with an EMG controller. So, we looked at these things specifically (Slide 15) and got a cross-correlation function on them from the computer. We find very highly significant results but we need a lag time of about 22 minutes between external thermal response and maximal response. It would appear that the animal does thermoregulate and resist changes in temperature-- a positive response to a lowering of the ambient temperature.

SLIDE 14

SLIDE 15

We also plotted time
(Slide 16), it went along
here with the previous
plot, as shown by the
phase-plane relationship
as a function of time <u>vs</u>
heart rate and brain
temperature. If the
responses were unregulated,
the plot would simply be
a scattergram. If it
were regulated but re-
mained in one place, it
would either be a point
or a circle. If the set
for this response be-
came more precise (if
regulation became more
precise), it would be a
concentric spiral. How-
ever, should you get
improvement in perfor-
mance along with a change
in functional set, it
would do exactly what it
did, moved down in this
direction. Each time it
improves its performance
and changes in relation-
ship with other temper-
atures as an analog of
capacitance in the
system, a leaky capaci-
tance I should say.
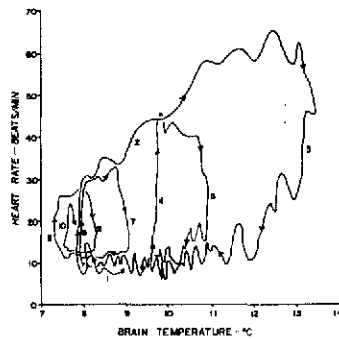
SLIDE 16
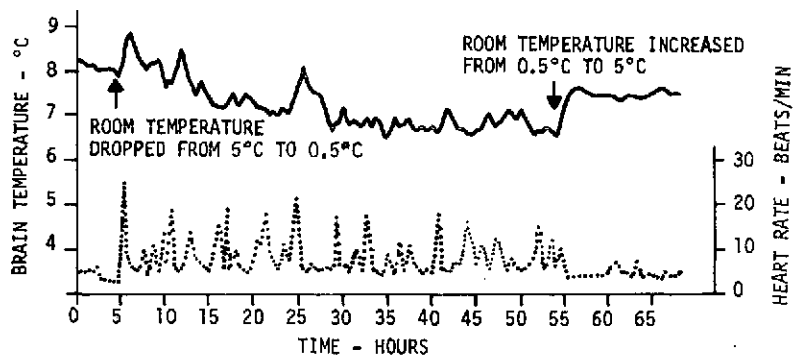
SLIDE 17

Slide 17 is a somewhat
similar experiment. We
lowered the room tem-
perature from 8 to 7
and from 5 to 0.5°C and got these coordinates. The next one (Slide 18)
shows a similar diagram except we didn't change room temperature so it is
more-or-less concentric and flat. There is one other little trick here.
We did another experiment in which we changed the room temperature in
square waves. I won't show you this because it gets too complex, I'll
just diagram it. What we did was to change the room temperature like this:

SLIDE 18

PREOPTIC COOLING DURING HIBERNATION

SLIDE 19
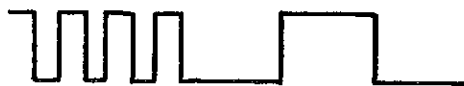
We went from 5° to 0.5°C and so on, first for one hour and then for longer periods of about 3 hours. This is where it really gets spooky. With the response and learning at room temperature, heart rate went up each time. Let's make it simpler and say that we did it like so:
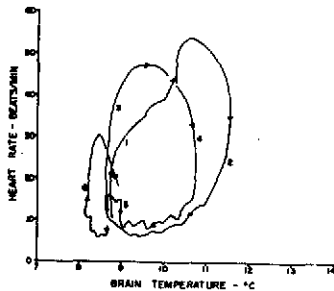
and heart rate:
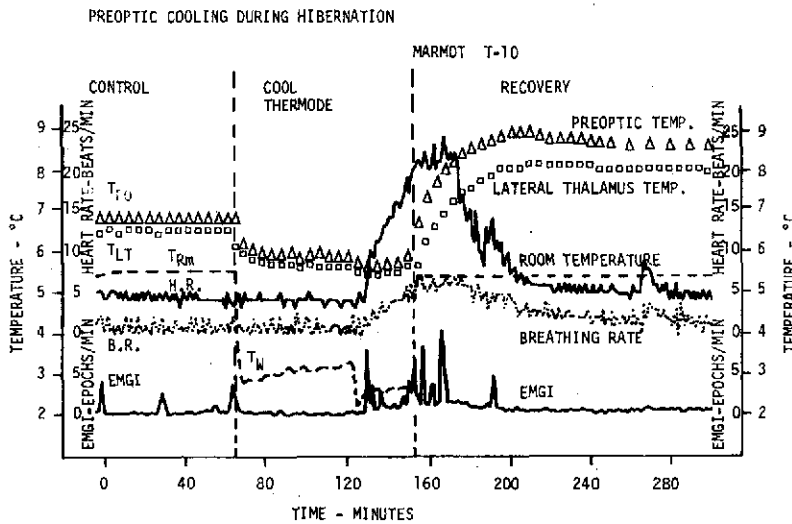
We gave him a Zeitgeber; in hibernation, he showed autonomic learning! That applies to heart rate response, brain temperature response, EMG activity, etc.

Going back to the more complicated experiment again (Slide 19), we cooled the thermode in an animal during deep hibernation. If he should not be regulating, it should make no difference to the animal. Were he regulating only in response to an "alarm temperature," he should come into full arousal. You can't have it both ways. In this experiment we lowered the temperature in 2 stages, from 8 to 7.5° to 6.8° or so. What is interesting here is that the first lowering was subthreshold and we got no significant change in heart rate or EMG activity. The temperature returned and there was no overshoot. If it had actually generated heat during this period, the brain temperature should have shown an overshoot. We lower the temperature slightly more and bang, the EKG shows an increase but the skin temperatures really don't change that much. Upon releasing the clamp, the EKG returns to baseline. If the animal were generating heat, the brain temperature would rise as warm blood comes in from the periphery and an overshoot becomes apparent.

We did a little different
experiment here (Slide
20) in which we lowered
the brain temperature
in 2 stages. In the
previous slide, there
was no EMG change; in
this slide, we've some-
how come upon the
threshold for EKG and
EMG response. When
you release the clamp,
you see the overshoot
from the lateral thal-
amus and preoptics as
the warm blood from
the periphery enters.
These are examples of
a number of experiments.
It is interesting,
especially at room
temperature, that if
you lower the room
temperature from say
5° to 3°C, the only
response you usually
get from the hiber-
nating animals is EKG;
if you lower it from
5° 0.5°C, you get a
direct response in
volving both EKG and
shivering.
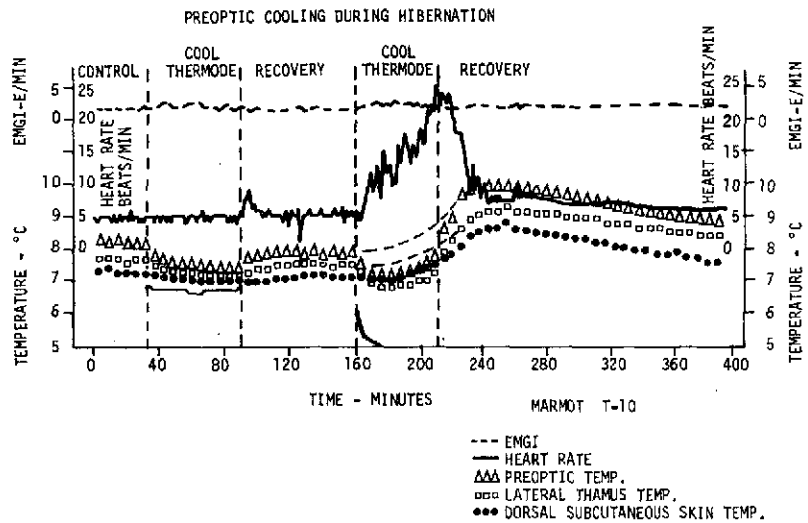
SLIDE 20

SLIDE 21

What about the other
side of the coin? The
hibernating animal defends
himself against central
cooling but what about
heating? This question
really makes no sense
because the animal's body temperature is so low $(7-8^{\circ})$ that when you heat
it up with a 16° or 20° thermode, you add significantly to the heat content
of the body. So one doesn't know whether the effects
obtained were due to a heating of the preoptic area or to generalized heat-
ing of the brain opening up circuits which were not functioning. We
tried this experiment a few times (Slide 21). This is the preoptic temper-
ature going from 10° to 13°. As may be seen from the temperature of the
dorsal skin surface and the other areas, we've added significantly to the
heat content of the body. It is interesting that before we did this,
the heart rate was quite variable - a little bit above what we call a
minimal level. We socked the heat to him and bang--down it went to a
minimum. This is rather typical; the heart rate will go down to a minimal
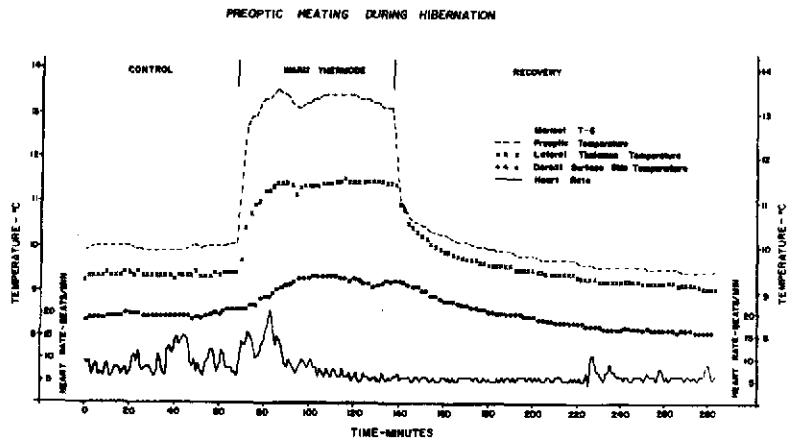
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PREOPTIC WARMING DURING HIBERNATION

MARMOT T-10

SLIDE 22

HIBERNATION ENTRY FUNCTION
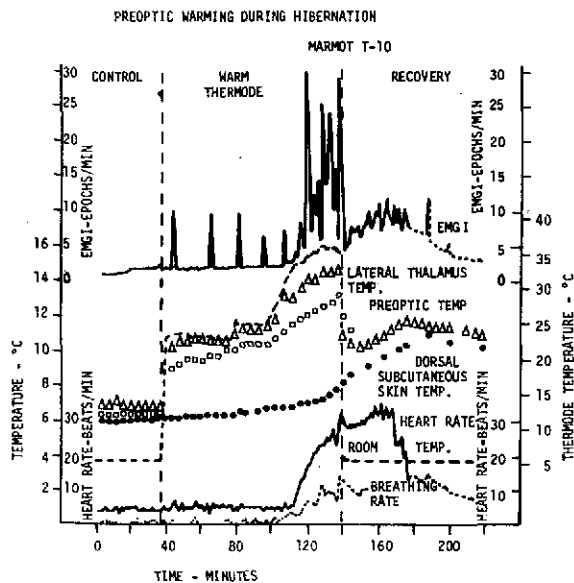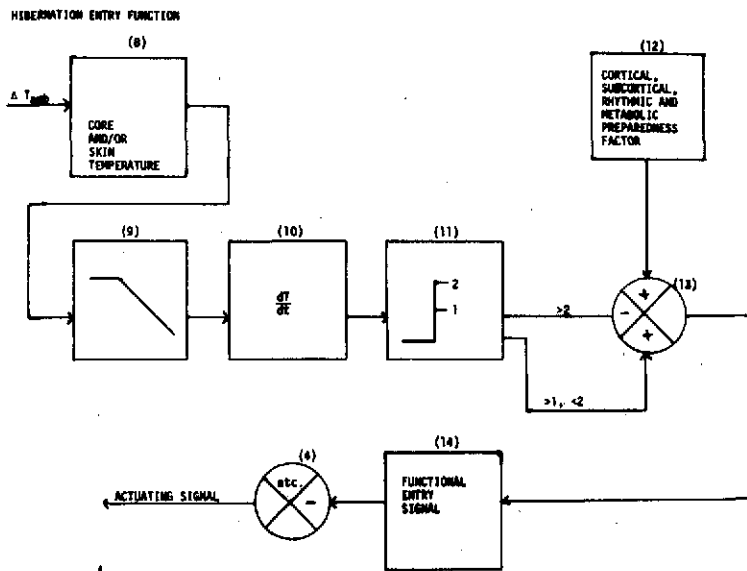
SLIDE 23

level as a consequence of a thermal load of this sort or should a cold stress be relieved, it will fall to a minimal level. This is exactly what you would expect if thermal regulation is a sympathetic response.

In another experiment (Slide 22), we again heated the thermode; no response was obtained so the temperature was increased further with interesting consequences, something that happened about 3 other times.

As the heating take place (the brain temperature was initially 6-7°) the animals slowly, but very powerfully, arose partially and crawled out of the nest and lay prone on the gridwork underneath - loosing heat to the conducting surfaces. Also they spread out for maximum radiant heat exchange. After the thermal change is relieved, they return to hibernation. This particular one was a very long experiment. We got tired of watching him lying there, so we left and went home and came back about 8 hours later. He was back in his nest and hibernating. We did keep the recorder running and it showed no sign of arousal on the part of this animal or others subjected to such stimuli.

We've tried to put some of this together (Slide 23). We assumed that the normal animal and the hibernating animal are similar in their control system. That is, we have a disturbance and a controller acting through a set point. Perhaps the distinguishing thing is the hibernation entry function (we don't know what else to call it) and an intrinsic reference input. While the diagram is self-explanatory it is obvious that a great deal of work remains to be done on how the pieces operate and, in some cases, on their identity,(Slide 24). Certainly, the primary outputs of the controlling

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system is the EMG and the sympathetic nervous system. Additional evidence
for this statement is that we've been injecting, both in the normothermic
and the hibernating animal, biogenic amines in to the third ventricle and
the preoptic areas. In response to epinephrine or norephinephrine in-
jections, the thermolytic response that might be expected, is obtained.
When we use a drug such as 5-hydroxytryptamine thermogenic responses
are obtained. During hibernation, we were successful in administering drugs
without waking the animal. Here also norepinephrine resulted in a minimi-
zation of EKG activity.

Some of the characteristics of these hibernators bother me considerably;
here they are, they generally lie or orient themselves in antigravity
posture in their nest and the CNS shows relatively little activity with
respect to this. However, they may hibernate in any position. Well,
this, has generated another idea: that is the notion of a physiological
"dither effect." A "dither" is a device, a mechanical tapper, used by
engineers to keep instruments from sticking. Let's suppose that gravity
has a function other than orienting things--that of a dither. How would
the dither work? It would work via the vestibular apparatus and pro-
priocepters using inputs to the CNS to simply keep the limbic functions
going, to keep the brain stem operating, keep cycles moving, to keep things
from "sticking." Thermoregulation, as an example, may well depend upon
a dither to keep going, to keep set in reference to something else. Sleep-
wake cycles, the quality of sleep, may depend on a dither. There is little
evidence to support such an idea other than the newspaper reports of the
Russian Cosmonauts complaining a great deal about feelings of malaise, dis-
comfort, disturbance of sleep cycles, and so on. Also, the Russians are up
there for a long time and they don't do a great deal of work relative to
American Astronauts, it would appear. Perhaps the reasons for the number of
Russian complaints is that they are not busy enough. The American
Astronauts are always occupied and therefore getting a lot of proprio-
ceptive "dither" input; their brain is constantly having its "meter face"
tapped as compared to the Russians whose "meters" stick because of the
inactivity. How could you demonstrate this? In order to make a beginning
it will be necessary to put an animal outside gravity and in three-space
in such a manner that it would not have any input. Possibly the way to
do this is by putting a marmot-on-a-stick. Suppose that you have a
hibernating marmot on the ground. When he goes off on a space shot, he'll
wake up. In gravity, he'll be down like this (hibernating):
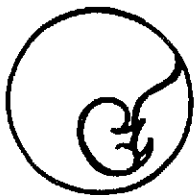
A hollow tube with flexible cable is attached to the skull. The cable
becomes rigid when we put tension on it. We send the animals into a gravity
free environment and tighten up on the cable which is attached to the skull
of the marmot. We end up with a marmot-on-a-stick:

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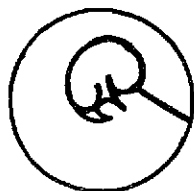
In free space there is no sensory input outside the system as long as he doesn't wake up. If he wakes up, he's going to be wiggily but he might go back to sleep as happens during bouts of hibernation. Another possibility is to do the same thing with smaller animals that won't whip around so much, such as a rat-on-a-stick. Once he's off on a space trip, he can't build up too much rotational inertia so as to rip the pedestal off. As long as that pedestal is strong, he's going to be floating with no sensory input--a perfect sensory deprivation experiment. What do we look for? The possibilities are legion, I would like to know what is going to happen during the arousal periods, sleep periods, and so on.

REFERENCE INPUT ELEMENTS

CORE

SKIN

(1)

CORTEX
RETICULAR SYSTEM
LIMBIC SYSTEM

(2)

HIBERNATION
ENTRY
FUNCTION

(3)

INTRINSIC
REFERENCE
INPUT

(4)

(5)

CONTROLING
SYSTEM:
αEMG, αPANTING
αSYMPATHETIC
ACTIVITY,
ENDOCRINE
ACTIVITY,
BEHAVIOR

CONTROLLER ACTION (R-Ro)

(6)

CONTROLED
SYSTEM
(THERMAL
CHARACTERISTICS)

DISTURBANCE

(7)

FEEDBACK
ELEMENTS
(HYPOTHALAMIC
RECEPTORS)

$T_{hypoth.}$

SLIDE 24

It should be noted that very significant contributions have been, and are being, made to this work by my colleagues, Drs. R. H. Luecke, Steven Mills, Dennis Wright, and William Hartner as well as students such as Kurt Jacobs and Virginia Miller.

DISCUSSION BY PARTICIPANTS

SAUNDERS: Frank, you said, "What are we going to look for? The possibilities are legion." Would you interpret this in laymen's terms?

SOUTH: I think that what we really have to look for are discrepancies or dyscrasias in limbic function in thermoregulatory activity, autonomic nervous system activity, states of arousal. These are the obvious, the easier things to look for such as sleep habits, thermoregulatory patterns, possibly evoked potentials of auditory and visual pathways but this means more electrodes and the more electrodes, the more difficult the experiment becomes.

SMITH: I was wondering about this, Frank. In the null gravity, having one of your critters up there might be very interesting, but to elaborate a little bit on the proprioceptive and the reticular activating system might be more important than the limbic. Only in space can we check out the proprioception and input in the medullary area.

SOUTH: You surely can't do it with bouyancy.

SMITH: I don't know about that. Have you tried it?

SOUTH: With bouyancy? No--there go my Sundays.

MUSACCHIA: Frank, one of the areas that is still nebulous arises from something you just said. That is the area where we generally accepted for a number of years that a hibernating animal would respond like a poikilotherm. As you drop the temperature, it would go along with the temperature to a certain point, a real low point, and then he would wake up. This is a myth of hibernation. I begin to see from your work and also from things Jack Twente has said that there are certain points of discrepancy. It is possible that smaller hibernators, the 200-300 gram guys, respond in a poikilothermic manner?

SOUTH: Yes, this is why the paradoxical behavioral thermal regulation may be a non-paradox. The little guys are resting on a knife edge; they have no thermal capacitance--they cool too easily, they don't have enough degrees of freedom. This is also probably why they tend to bury themselves; a hamster in a barrel of trash will burrow down to the middle, this probably gives him enough heat capacitance to thermoregulate. A larger animal has his own built-in capacitance so he's not resting on that knife edge and he can control his temperature somewhat--the control is there, but it isn't as sharp as during normothermia. As an example of this, we had to vary the brain temperature rather widely to get a good thermogenic response during hibernation but when we vary room temperature, we always get a thermoregulatory response before we can see any variation in brain temperature no matter what gain we use. We've turned the gains up so high that we can't record. We always get a thermogenic response before we see any appreciable change in brain temperature.

POPOVIC: Frank, I don't quite understand your message. What did you want to tell us?

SOUTH: We are looking at regulation in a particular series of animals; we're looking at thermoregulatory activity and wherever else it's going to take us. Part of this function is looking at state of arousal. We are making the assumption, and indeed our basic hypothesis is that, states of arousal, thermoregulatory activity are part of the same set of functions in an animal. That one exists in order to serve the other.

POPOVIC: Let me ask you another question. Was your message that hibernation might have something to do with ordinary sleep?

SOUTH: Yes, that's right.

POPOVIC: If so, did you try to electrically stimulate the brain in order to find the sleep center?

SOUTH: Yes, we've done that. As you undoubtedly know, a number of people, Harnandez-Peon and a number of others, have stimulated the brain both chemically and electrically and gotten changes in states of arousal. We have also. Sleep is a change in the state of arousal. One of the interesting experiments is very hard to repeat; we've only been able to do so twice in different animals, in the same animal it works time after time. We used low frequency stimulation of the midbrain reticular formation (MRF) when the animal came out of hibernation and was regulating (about 4 hours after arousal). The animal was given a 6 Hz stimulus to the MRF and it went into behavioral and EEG sleep. We didn't get much change in thermoregulatory set that we could see in this short of time. This was with a bipolar square wave. We left the stimulator set as it was, and since at that time we didn't have video, we sent a co-worker, Joan Baumber, in to observe the animal and waited until it was going into hibernation. The idea of this experiment was to look at thermoregulatory states vis a vis electrical stimulation and slow sleep. When the animal's brain temperature hit 15° we turned the stimulator on. We thought that maybe it would wake up, maybe it would go into hibernation faster, or something else. What it did do was something we weren't prepared for. It got out of the nest, went over to the corner, vocalized for a period with some nondescript "oofs", and (interestingly) just stayed in the corner. It took a full minute for the traverse. We turned off the stimulator. Several things are possible but 2 things immediately come to mind; if he were reversing physiologically, he would turn around and go back to the nest. On the other hand, if he were reversing behaviorally, he'd back up. Behavioral reversal is not the same as psysiological reversal because it takes a different set of muscles and coordination to back up. So what he did do, rather surprisingly, was to back up into his nest, curl up, and continue into hibernation. Another time we did this, we stimulated a different animal probably in a slightly different system and he got up out of the nest, circled the cage once, ended up back in the nest and continued on after turning off the stimulus. We haven't carried these experiments further due to a lack of time.

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POPOVIC: May I ask another question, please? You did show that a small change in brain temperature may cause the animal to move. Remember when you increased the temperature? Why do you think the EMG changes that you observe with small changes in the temperature of the brain represent shiver?

SOUTH: When I heated it up, that wasn't shiver, that was just a consequence of movement--he was moving out of the nest.

POPOVIC: I know this time, but the other time when you saw EMG changes, how did you know it was shivering?

SOUTH: Because we visualized the animal directly with video. There was no change in posture--it was shivering.

SMITH: This "ditherosis" you have is very interesting.

JORDAN: Other than the availability of marmots in Colorado, what prompted the use of marmots?

SOUTH: Two things: 1) They have a nice, big, heavy, thick skull; they hibernate easily in the laboratory and we can put trash in or on their skull and it will stay there; 2) the reason I really used it was that I wanted to do some cord physiology. I tried for about 4 months to do a successful laminectomy on ground squirrels and on rats. (I now know why rats and ground squirrels are not used for cord work; it is absolutely impossible--by the time you've done a decerebration and a laminectomy and h..ve gotten out your hunk of nerve, the animal is dead. He always dies just as you are putting on the electrodes.) I thought that a marmot would be just great. He's big and I could work with his cord. That's wonderful and it's true except that the marmot has a very peculiar brain stem; something like this:

That is, it takes a 90° turn directly at the corpora quadrigemina. And how one can do a decerebration on this critter, I don't know. I haven't figured it out yet except for going through the basalar artery or using a bucchal approach.

SMITH: They don't have a tentorium?

SOUTH: They don't have a tentorium at all. That's good in a way though, because it means you can get the electrodes down to the pontine reticular formation without the tentorium getting in the way. But that's the only thing good I can think about it.

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Radioprotection in Depressed Metabolic States:
The Physiology of Helium-Cold Hypothermia

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There are 4 topics which will be discussed in this presentation:

1) Radioresistance and depressed metabolism

2) Physiology and biochemistry of helium-cold hypothermia

3) Improvement of the helium-cold technique

4) Helium-cold technique in various species

Initially, I'll talk about radioresistance and the studies with hibernation and hypothermia. Then I'll discuss biochemical and physiological parameters in helium-cold hypothermia. Next, there will be a review of improvements of the helium-cold hypothermic technique. Lastly, I'll show you some results of studies with rats and mice. The use of the helium-cold hypothermia method is being broadened with experiments utilizing other mammals.

I'd like to point out at the onset, that there have been several colleagues associated with this research, Dr. Ron Barr, Dr. Wynn Volkert, Dr. Gary Anderson, also, several technicians and graduate students. These colleagues and co-workers have had a significant input in all the research to be discussed.

The first slide is to orient you to various types of depressed metabolic states. In particular, hypothermia and hibernation are classical states of depressed metabolism. Hibernation, as you know, is a natural, seasonal phenomenon, and the animal possesses the inherent ability of self-arousal. Hibernation is inducible in the laboratory. It is restricted to several species of mammals and some birds. Cold torpor, another form of depressed metabolism, can be overlooked insofar as this presentation is concerned; it pertains principally to cold-blooded animals, e.g., poikilotherms. Hypothermia is a laboratory experimental technique. There is no self-arousal and for all intents and purposes the investigator decides the stimulus for arousal in the hypothermic animal.

There are a variety of experiments that have been done with depressed metabolic states and radiation resistance (slide 2). Generally speaking, the work with goldfish, frogs, and lizards falls into a very difficult area for categorization. From the reports in the literature, we seldom know whether or not the animals were cold torpid. There are hibernation experiments with animals such as dormice, ground squirrels, and bats. A

SLIDE 1   SLIDE 2

generality which repeatedly emerges, particularly in textbooks, is the
suggestion that an animal is protected only as long as it is hibernating
and once it awakens from hibernation, it shows all the pathology that
a normothermic non-hibernating animal would show if it were similarly
irradiated. Lastly, with reference to hypothermia, there are again a
variety of studies in the literature dealing principally with mice and
rats. There is an excellent, but dated, review by Weiss (1961) and a
more recent discussion of the subject by Musacchia and Barr (1968). Again,
insofar as hypothermia is concerned, the primary consideration is that the
radio-protection seen in hypothermia is due to varying levels of tissue
hypoxia. The mechanism(s) of radioprotection in hibernating animals is
not known; it is not defined.

The instrument used (slide 3) in all of our studies is a ^{60}Co irradiator.
Slide 4 shows the instrument with a plastic container. Exposure takes
place inside this lead-shielded apparatus. The animal receives whole
body uniform exposures of irradiation. The animal is surrounded by a
grouping of pencil-like structures, which contain uniformly spaced cobalt
discs. Inside the cylindrical chamber where the exposure takes place,
the animal is totally irradiated, and "sees" a rather uniform level of
exposure. A key factor in radiation studies should be the uniformity
of the radiation exposure, particularly when comparisons are to be made;
a similarity of irradiation apparatus is essential. It can be readily
seen, in the literature, that a variety of instruments have been used and as a result, it's been very difficult to draw specific conclusions about radiation responses in hibernating animals. I want to show you dose responses (slide 5) which illustrate a relationship between the dose and the survival time in rats. The same general type of response is also applicable to hamsters and ground squirrels. With very
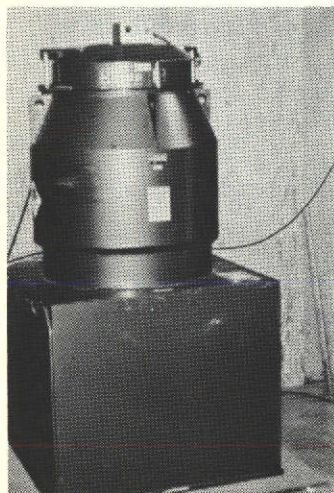
SLIDE 3
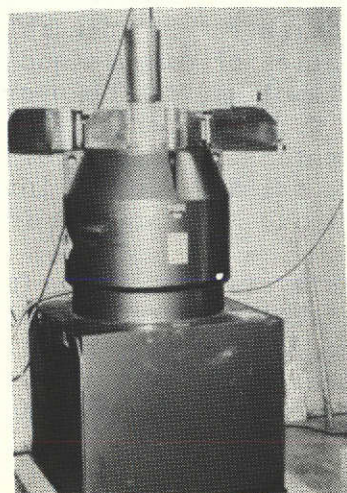
SLIDE 4

low levels of irradiation it is very difficult to define the particular pathology, however, there is a shortening of life expectancy, i.e., shortened life span. With exposures increased to 500-800R, one is dealing with a hematopoetic syndrome, a little higher and the intestinal syndrome becomes evident. Lastly, at exposures of 10,000 and 12,000R or more, one is dealing with CNS damage. As a physiologist would look at it (slide 6) with graded doses, first there is arrest of hematopoiesis, then the intestinal function is disrupted, and finally the central nervous system is damaged. The initial and most responsive tissues are those which are mitotically active. This type of dose-response curve is applicable to our results with the ground squirrels.

The next slide (7) shows a ground squirrel in the event some of you may never have seen one. Slide 8 shows a plastic container of the type we use for hibernation and radiation exposures. These clear plastic containers permit an excellent view of the animal at all times. The next slide (9) shows the first dose-response curve we obtained with hibernating and active ground squirrels. The hibernating animals show a total shift of the curve to the right; there is an increase in the mean survival times in a wide range of exposures. The hibernating animals show an increase in radiation resistance and the results are significantly different than those in active animals. We have looked at radiation resistance in hibernators using a host of experimental variables. In one experiment (slide 10), animals were forced into hibernating in the summer and compared with the "normal" winter hibernating animals. In our experiments, comparisons are often made between littermates in an active and hibernating series. We are able to use siblings in experimental comparisons since we purchase pregnant animals which are delivered to the laboratory so that the litters can be delivered and raised under laboratory controlled conditions. As a result, one

SLIDE 5

SLIDE 6

SLIDE 7

SLIDE 8

SLIDE 9 *

TABLE I

PERCENTAGE OF SURVIVAL AT 30 DAYS POSTIRRADIATION OF HIBERNATING AND ACTIVE GROUND SQUIRRELS

	1000 R	1100 R	1250 R	1500 R	1750 R
Summer					
Hibernating	--	--	57	29	0
Active	80	--	17	0	--
Winter					
Hibernating	--	100	100	83	40
Active	100	67	0	0	--

SLIDE 10 *

can do experiments comparing littermate with littermate. The $LD_{50(30)}$ for winter hibernating ground squirrel is between 1500 and 1750 rads and between 1100 and 1250 rads for active animals. Before I explain the data on this Slide (11), I would like to point out one thing which other investigators have done. In many previous studies of hibernating animals, the hibernation phenomenon has been determined visually. The animal is curled up and appears to be hibernating (it may well be), and it is assumed that it is hibernating. The animal is often taken from the hibernaculum and given a radiation exposure. One immediate way to know if an animal is hibernating at the time of irradiation is to take its temperature. In our dose-response curve experiments, the animal was permitted to hibernate in the usual manner but then body temperature (T_b) was taken either during hibernation or immediately (within 30 seconds) following irradiation. Thus, if one records a T_b of 7°C immediately after irradiation (within 30 seconds), one can be certain that the body temperature in that animal wasn't much different than T_b 7° at the time of irradiation. It may have been a couple of tenths of a degree higher, but such a slight increase is relatively unimportant. When these studies were being done, a number of questions were asked. One question, perhaps an obvious one, was concerned with the fact that animals hibernate at different body temperatures. Therefore, one might ask, is there a temperature relationship to increased radio-resistance? We thought about this and proceded to hibernate our animals at a variety of temperatures. One feature worthy of consideration was the probability that various levels of hypoxia may be in progress and may be related to increased radio-resistance to radiation damage. A variety of temperatures were used and in one experimental series squirrels were irradiated while at hibernating temperatures of 5°, 13°, or 37°C. Looking at a percent survival it turned out that the 13°C animals consistently showed better survival than the 5°C hibernating animals. We've done this experiment not only at 1250 rads but also at 1500 and 2000 rads. We usually do a variety of exposures and I'm showing you one which is typical of these various responses.

SAUNDERS: What is that bottom line, Joe? Days, time?

MUSACCHIA: Sorry, this is days.

SAUNDERS: That runs up to . . .?

MUSACCHIA: It runs up to 90 days. In fact, we have some survivors up to 180 days. I'll show you some pictures of survivors.

* Rad. Res. 33: 348, 1968.

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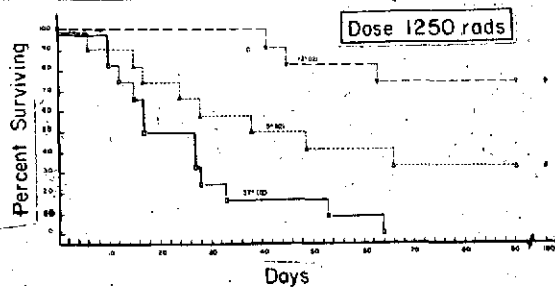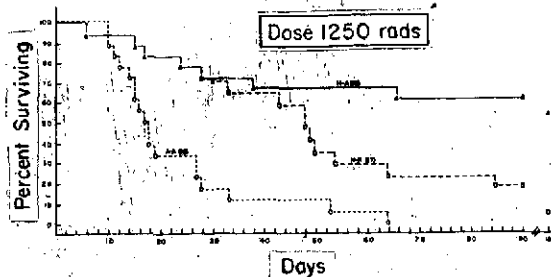
SLIDE 11 *   SLIDE 12 *

Another question which arose (slide 12) was concerned with hibernation
after irradiation. In other words, if one permits animals to hibernate
after irradiation, will they show delayed responses or improved mean
survival times? This question was tested experimentally. Animals from
the same litters were separated into three groups: designated A → A,
H → H and H → A. A → A animals were irradiated while active and then kept
active; H → H were animals irradiated while hibernating and maintained in
their hibernacula, and H → A were animals which were irradiated while
hibernating and then permitted to awaken. The results supported those
obtained earlier: the best survival time was found in the animals that
were irradiated while hibernating and then permitted to awaken as com-
pared to animals irradiated while in an active, normothermic state.
Particular consideration was given to the H → H animals, i.e., the animals
which were not awakened but rather maintained in hibernation. These
animals were not disturbed; they were not awakened; they were not trans-
ferred from one cage to another, and the ambient temperature was maintained
throughout the radiation procedures. The container and hibernating occupant
were irradiated and then returned to the cold room. The ^{60}Co irradiation
was equipped with chilling coils so that even during these exposures
animals were maintained in a cold ambient atmosphere. There was a minimal
amount of disturbance, if any, and as far as we're concerned, it's about
as tight an experiment as we could possibly design. You'll notice that the
H → H curve starts at day 30 which means essentially that animals were kept
undisturbed in their hibernaculum for 30 days. During those first 30 days
a number of deaths occurred. Interestingly, the number of deaths were
comparable to the number which occurred among those who were irradiated
while hibernating and permitted to awaken. At this point the rate of dying
was a little more rapid than that in H → A animals, but not as rapid as the
animals irradiated while in the active, normothermic state.

Long term survivors (slide 13), except for greying of the hair or a
slight discoloration, are not grossly affected. They do not show too much
obvious pathology. We've had a pathology group in the Veterinary School
do some necropsy studies on these animals and they've found some slight
increase in the incidence of various tumors but nothing unexpected. There
is little point in discussing accelerated aging because there simply is
inadequate knowledge of aging, even in normal squirrels. The next
slide (14) is another demonstration of survivors and normal animals.
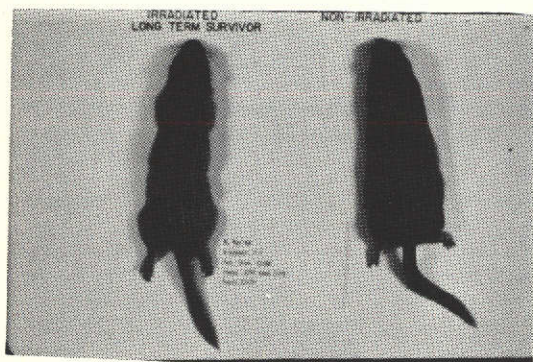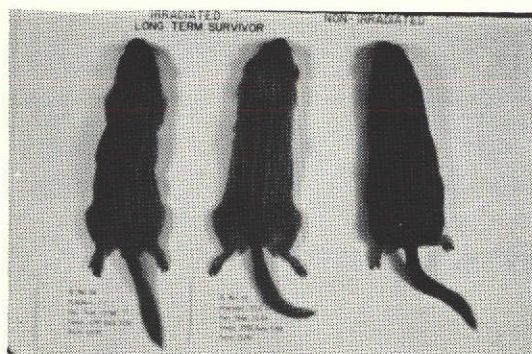
SLIDE 13

SLIDE 14

POPOVIC: I'd like to ask a question. During hibernation, even in non-irradiated animals, some animals die from nonspecific causes, did you subtract this number?

MUSACCHIA: No. I realize that some animals die in hibernation, however, that number is very small in wintertime. If one has 30 animals hibernating, there may be 1 or 2 deaths in a period of 30 days.

SAUNDERS: Joe, after irradiation have you done a recovery response? Do any parameters that you're looking at show and follow what is changing in their recovery kinetics following irradiation?

MUSACCHIA: At this point, we have chiefly used survival as a criterion. In retrospect, when we started this work, we were looking at the intestinal absorption following irradiation. We found that the gut, even after 1000 and 1500 R, did not show the same kind of damage syndrome that a rat intestine would show. Even after these high levels of radiation exposure, they do retain the absorptive capacity. We have extensive histologic studies on the intestinal mucosa and we know there is some histopathology of the intestinal mucosa following a large dose of irradiation. Long-term survivors as with ground squirrels in general, after an initial loss in body weight, resume weight gain. These animals are given an ad libitum diet of food (Wayne Lab Blox) and water. Food intake is apparently sufficient and permits a normal energy balance as indicated by maintenance of weight. Insofar as any specific parameter, the only thing we have looked at is the intestine and all we can say specifically is that it takes larger doses of irradiation to destroy, or damage the gut of the ground squirrel than it does in the rat, the mouse, etc.

SAUNDERS: What is the time period for recovery after irradiation?

MUSACCHIA: It depends on a number of things, for example, whether or not it is an active animal or a hibernating animal, and also, the level of irradiation. But, let's make a generality, we'll say about 2 weeks postirradiation. At this time, one knows fairly well about how many survivors there will be; you can begin to predict the survival response-- within limits we can predict it now, we couldn't several years ago. I think that after a couple of weeks one can essentially begin to determine that the animals are recovering from the irradiation damage.

SAUNDERS: One more question, have you irradiated them and then challenged them, again after they started their recovery?

MUSACCHIA: No, and I know the split dose story also.

SAUNDERS: No, I don't mean split dose. I mean, you give them the full blast and then they recover and then you give them another blast.

MUSACCHIA: No, we have not done that type of experiment.

Then we reached a point where we were satisfied that hibernation offered an animal a considerable level of increased radio-resistance. There continued to be one major problem with the hibernating animals, namely, you can work with them readily in wintertime, however, in working with them in summertime, one has a different kind of animal. From a practical point of view, NASA is not going to be interested in an animal which we can prepare in wintertime and which they might conceivably want to orbit in the summertime. So, we went over to another type of experimental approach, namely, hypothermia. Hypothermia offered a depressed metabolic state which we could control at any time of the year and could well provide a more efficient experimental system. Hamsters are used in these studies (Slide 15).

Let us now review the research concerned with radio-resistance in hypothermia. We induce hypothermia in hamsters (slide 16) by using a combination of helium and oxygen (helox), 80% helium, 20% oxygen, and a low ambient temperature (Ta). The hamster becomes hypothermic, a form of depressed metabolism, in about 6-8 hours. The animals become flacid, with hypothermic body temperatures of about 7°C. They remain depressed for relatively "long" periods of time; for example, 24 hours. The helium-oxygen mixture is commercially prepared and readily purchased (slide 17). Slide 18 is essentially a schematic diagram of the flow system. The gas is moved through the chambers at a rate of about 150 ml/min, water and CO_2 can be absorbed as required. Essentially what occurs is that a normal, active animal put into a helium-cold environment becomes hypothermic (slide 19). Then the animal is taken out of the helium-oxygen mixture and put into an ordinary refrigerator at low ambient temperatures. The animal is merely kept in the cold and it will remain hypothermic for various periods of time. If we take it from the cold air at any time and bring it out into ordinary room temperature, i.e., 20-22°, and place the flacid, hypothermic animal on an ordinary laboratory table, it will revive in about 2 hours. The animal will return to normothermia again. It needs
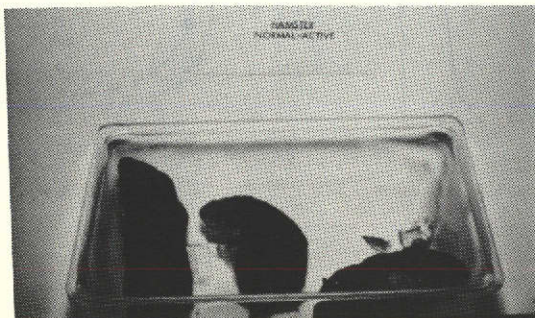
SLIDE 15
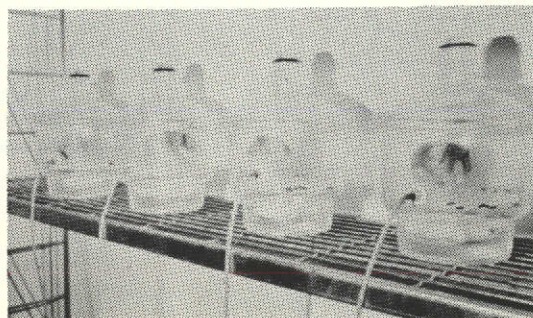
SLIDE 16
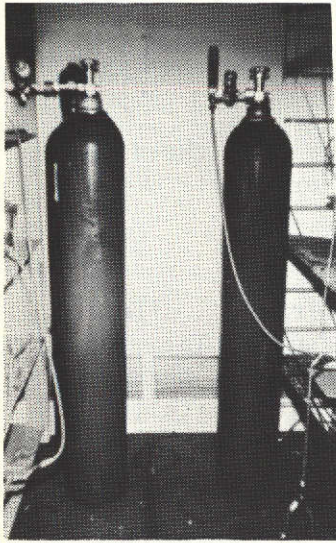
no stimulus other than the mild warmth of a room at about 20-22°C. The steps in making an animal hypothermic (slide 20) include exposure in the helox chamber (helium-cold chamber). After a couple of hours when the animal is hypothermic, and body temperature has reached 7-10°C, it is then placed in an ordinary household refrigerator and held at 7°C. At this temperature, it can be used for experimental purposes. Body weights are often recorded before and after; a wide variety of physiologic parameters can also be measured at this stage. It takes about 6-8 hours (slide 21) for a hamster to become hypothermic, and they can be maintained for several hours or even a day or two. Revival and rewarming take about 2 hours (1 1/2 to 2 1/2 hours, depending on the animal). Hypothermic hamsters were used to ascertain their radiation resistance potential and to make comparisons with ground squirrels (slide 22). Dose rates used did not go higher than 3000 and 5000 rads because at this point percent survivals were all about the same whether they were hypothermic or active. The curve on the right represents a series of hypothermic hamsters; each data point consists of 20 animals, the standard error of the means are given
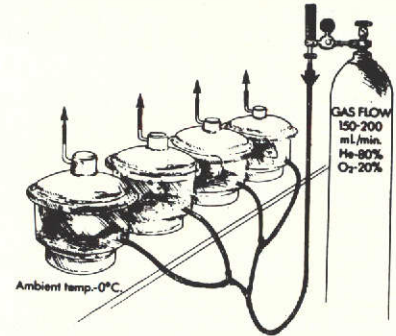
SLIDE 17

SLIDE 18

HYPOTHERMIA INDUCED BY EXPOSURE TO HELIUM + COLD **
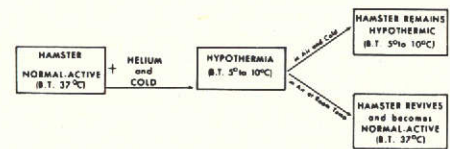
SLIDE 19
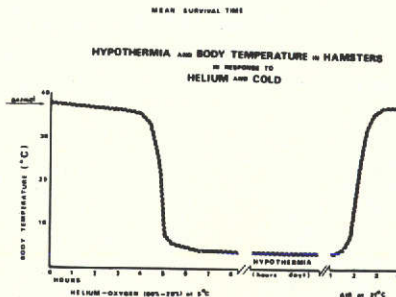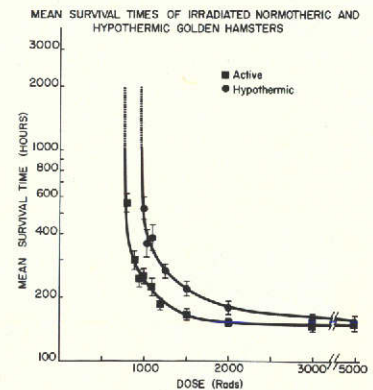
SLIDE 20

SLIDE 21*

SLIDE 22**

*In Depressed Metabolism, eds. Musacchia, X. J. and J. F. Saunders, Elsevier, Publ., N. Y.: 588, 1969.
**Rad. Res. 46: 353, 1971.

TABLE 1. LD$_{50/90}$ VALUES FOR ACTIVE AND HYPOTHERMIC HAMSTERS EXPOSED TO ^{60}Co. WHOLE BODY IRRADIATION.

HAMSTERS		RADS
GOLDEN	ACTIVE (37°C)	875 ±15
		P<0.01
	HYPOTHERMIC (6-10°C)	1067 ±24
ALBINO	ACTIVE (37°C)	867 ±34
		P<0.01
	HYPOTHERMIC (6-10°C)	1143 ±99

SLIDE 23

SLIDE 24

also. We've used golden hamsters and albino hamsters; each variety showed increased radioresistance due to hypothermia. The experiment is a parallel to that described for hibernating ground squirrels. In this case, the hypothermic hamsters are irradiated and then permitted to awaken. The LD , 875 rads for normothermic hamsters (slide 23), is significantly increased to well over 1000 rads for the hypothermic animal. We will use this dose, 875 rads, for later studies. The potential increase in radiation resistance in hypothermic hamsters (slide 24) is not fully appraised. We have not done everything we want to do with hypothermic hamsters. For example, we still want to make a more thorough comparison with hibernating hamsters.

The hamster is a fortuitous selection (slide 25) because for the first time we can raise questions concerning hibernation and hypothermia in the same animal. Previously, when we compared hibernating ground squirrels and hypothermic hamsters, one could offer a reasonable criticism concerning the validity of the comparison. However, if hamsters are hibernated and compared with hypothermic hamsters, then we are truly approaching a more valid comparison between a natural phenomenon and the artificial laboratory phenomenon. In the first study of this type, the results show an excellent similarity between hypothermic and hibernating hamsters. Compared with active animals, there are significantly higher percent survivals in animals in depressed metabolic states. To date, we have used only one dose, 1000 rads. We are now doing a dose-response curve with hibernating hamsters. However, despite the fact that this is the first of the studies that we planned, and because of the highly promising results, I did want to present them today.

What else have we done with the hypothermic hamster? We have begun an

SLIDE 25*
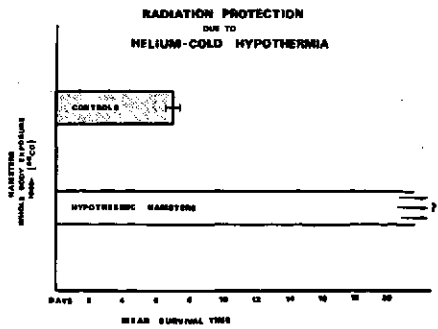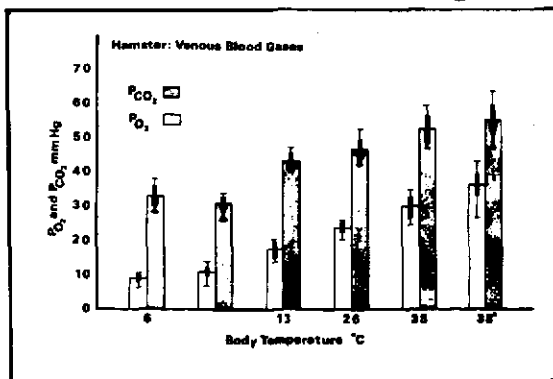
SLIDE 26**

* FASEB, Proc. Internat. Symposium: 179, 1972
** Am. J. Physiol. 219: 919, 1970

investigation of biochemical and physiological parameters of the helium-cold phenomena. There are several advantages in utilization of the helium-cold hamster. For instance, body temperature can be controlled at a variety of levels. The data in the next slide (22) shows a measure of blood pO_2, pCO_2, at a variety of body temperatures in the hypothermic animal. The response at T_b 6°C will obviously be much different than that in the normothermic animal at T_b 38°C. I want to show you something else that we thought was interesting (slide 27). In that same study of blood gases, we made comparisons of blood pCO_2 in long term hypothermia, short term hypothermia, and hibernation. Consider Lyman's data (1951) for hibernating hamsters: he reported pCO_2 values of 32.4 + 1.3 mm Hg. I would say these are wonderfully close values. It is, therefore, reasonable to suggest that in this type of hypothermia we have found a condition which may mimic, or may even compare physiologically with hibernation. Incidentally, short term hypothermia applies to animals that are at low body temperatures for about 30 minutes, whereas in long term hypothermia the periods are about 16-24 hours. We did hemoglobin dissociation curves in hypothermic hamsters and obtained predictable results (slide 28), namely, a shift to the left in the cold animal. There is a considerable hemoglobin binding capacity in the hypothermic hamster.

One of the characteristics of hibernation and hypothermia is cold exposure; it is an experimental factor which one has to consider in making an animal hibernate or in making it hypothermic. In the next series of experiments cold exposure was examined as a controllable experimental parameter combined with varying levels of radiation exposures. Not only are we interested because cold is one of the features used to induce hypothermia or hibernation, but also because other radiobiologists have been interested in the effects of cold exposure and radio-sensitivity. The general contention has been that the combination of cold exposure and radiation exposure increases radio-sensitivity. With the exception of Ghys (1963) who reported that cold exposure in the rat followed by irradiation results in improved radio-resistance. He seems to be the only one who takes a different point of view.

In our experiments, hamsters were subjected to various combinations of cold exposures, viz. cold exposure before and/or after irradiation. Some animals were maintained at normal room temperature (herein referred to as "warm" exposure) and then returned to the same room, others were acclimated, T_a 7°C, for various periods, 3 weeks up to 8 weeks. In slide 29 several of the experiments are summarized to illustrate the kind of result one can expect with cold exposure before or after irradiation in hamsters.

BLOOD PH AND P_{CO_2} IN HYPOTHERMIC AND HIBERNATING HAMSTERS

EXPTL. CONDITION	PH	P_{CO_2}
LONG TERM HYPOTHERMIA	7.35 ± 0.01(SEM)	35.7 ± 1.0(SEM)
SHORT TERM HYPOTHERMIA*	7.39 ± 0.03	33.3 ± 3.7
HIBERNATION**	7.44 ± 0.01	32.4 ± 1.3

*VOLKERT AND MUSACCHIA, 1970
**LYMAN AND HASTINGS, 1951

SLIDE 27*

HAMSTER: Hb-O₂ DISSOCIATION CURVES
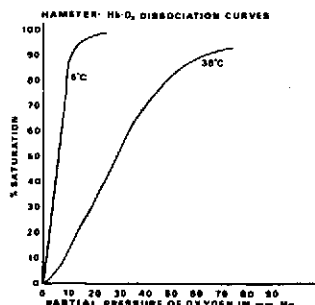
SLIDE 28 **

*Am. J. Physiol. 221: 1774, 1971.
**Am. J. Physiol. 219: 919, 1970.

EXPERIMENTAL CONDITIONS
PRE-IRRADIATION   POST-IRRADIATION
C= COLD ACCLIMATION, 7° C (WEEKS)
W= WARM 22-24° C (ROOM TEMPERATURE)

1200 RADS

% SURVIVAL

W → W
C(3wk) → W
(N=10)

C(3wk) → C
W → C
(N=10)

1100 RADS

C(8wk) → N
W → W
(N=12)

C(8wk) → C
W → C
(N=17)
(N=19)

1000 RADS

C(3wk) → W
W → W
(N=15)

C(3wk) → C
W → C
(N=15)

DAYS

SLIDE 29 *

HAMSTER: COLD ACCLIMATION and IRRADIATION (^{60}Co, RADS)

EXPERIMENTAL CONDITIONS
PRE-IRRADIATION and POST-IRRADIATION
C= COLD ACCLIMATION, 7° C (WEEKS)
W= WARM, 22-24°C (ROOM TEMPERATURE)

1200 RADS

W → W
C(3wk) → W
(N=10)

C(3wk) → C
W → C
(N=10)

DAYS

1100 RADS

SLIDE 30 *

In the next slide (30) the top group
of data show that between 7 and 10
days there is a cascading death re-
sponse to 1200 rads. In this instance,
it made little difference whether the
hamsters are cold or warm prior to
irradiation. The next slide (31, 32) show the same kind of result with
1000 and 1100 rad exposures. There may be a little hint of a shift to the
right. This slight improvement in survival occurs in practically all the
populations, suggesting that we're approaching the LD_{50} level. To extend
this experiment more logically, we used radiation doses that were very close.
to the LD_{50} dose (slide 33); after all we had been working at high levels
of irradiation to see if we could push radio-resistance or radio-sensitivity
one way or another. Therefore, we designed experiments with exposures
slightly less (850 rads) than and also slightly greater (900 rads) than
the LD_{50} (875 rads) for the golden hamster. The experimental protocol
was comparable to that described above. In addition we increased the
numbers of animals in each population. The results showed that when
hamsters were exposed to the cold prior to irradiation and then brought
out into room temperature, there seemed to be some increase in the
animals' ability to withstand levels of radiation which are close to the
LD_{50}. This is the first experiment we have done in this area and we want
to repeat it to make doubly sure that the results are statistically
significant.

SAUNDERS: This is strictly a cold temperature effect? No heating?

1100 RADS

C(8wk) → W
W → W
(N=12)

C(8wk) → C
W → C
(N=17)
(N=19)

SLIDE 31*

1000 RADS

C(3wk) → W
W → W
(N=15)

C(3wk) → C
W → C
(N=15)
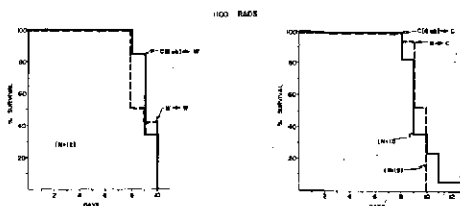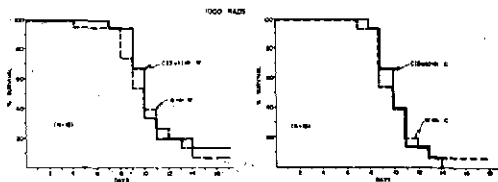
SLIDE 32*

* FASEB, Proc. Internat. Symposium: 182, 1972

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MUSACCHIA: Right.   This is just strictly exposing animals to cold, T_a 7°C. If you want to look at applications of this sort of thing, these are synergistic effects of cold and radiation.   Consider the tragic possibility of a massive and widespread radiation exposure, people and animals are not going to be nestled in nice, warm rooms and houses anymore.   Shelter, as we know it, may not exist and natural exposure to cold or heat could be commonplace.   Knowledge of synergistic effects of environmental extremes and radiation exposures can be useful in predictions and expectations.

Slide 34 presents a comparison of <u>dose reduction factors</u> from a variety of typical experiments.   I would like to point out that the best radiation protective response that has been reported is by Hornsey in 1957.   She used mice made hypothermic to 0-1°C and the dose reduction factor was about 2.8. Hornsey used the Gaja technique for hypothermia (a combination of hypoxia and hypercapnea) and in this case there is definite tissue hypoxia. Studies have been made at these low T_b's on spleen and testis.   In animals hypothermic at those levels, the results show tissue oxygen content of only 1-2mm Hg pO_2.   It must also be noted that in order to revive these animals some form of direct heat must be applied to the praecardium and also very often respirators must be used.   In none of our hibernating or hypothermic animals do we have to revive the animals or do anything more than simply bring them out into room temperature.   The dose-response factor that Ghys reported in his animals was 1.6 and this is quite good.   Our dose reduction factors, i.e., "protective factors", are 1.2 up to about 1.4.   They are modest, but are very solid.   We don't know yet what will be obtained with the cold-acclimated animals.

Our recent interests have turned to measurements of a variety of biochemical and physiological parameters or characteristics.   We have found a few interesting things.   For example, (slide 35), in measuring the oxygen uptake of the hypothermic hamster from the time it reaches a T_b 7°C, we noticed that during the first hour or two there is a continuous decline in oxygen consumption (Phase 1).   Then it proceeds into a steady level of oxygen consumption for periods of anywhere from 10-20 hours (Phase 2). Prior to death, the animal exhibits a series of gasping reflexes (Phase 3) and at this point the observer can predict to within an hour or two when

in oxygen consumption (Phase 1).   Then it proceeds into a stable level of oxygen consumption for periods of anywhere from 10-20 hours (Phase 2). Prior to death, the animal exhibits a series of gasping reflexes (Phase 3) and at this point the observer can predict to within an hour or two when

SLIDE 33 *

SLIDE 34 *

the animal is going to die. For the time being, we have identified these three periods as: Phase 1, Phase 2, and Phase 3. Another useful and readily measured physiologic characteristic in hypothermia is the heart rate (slide 36). The heart rate is normothermic active animals is 300-400 beats/min. In the hypothermic animal, it is greatly reduced to 17 beats/ minute. As you can see from these studies, physiologic parameters can be measured and used to provide indices of functional capacity.

Our next area of interest has been the improvement of the helium-cold method and the broadening of its use to include other animals. In addition, we have now looked at the possibility of shortening the induction time and lengthening the viability, i.e. the hypothermic survival. We became aware of an inverse relationship between induction time and survival time: viz. the shorter the induction time the longer the survival time in an experiment in which both heat- and cold-acclimated hamsters were subjected to hypothermia(slide 37). The hypothesis was that a cold-acclimated animal would become more resistant to undergoing hypothermia and the heat-acclimated animal was going to have less resistance to the induction of hypothermia.* We found that if an animal was kept at room temperature, 20-22°C, the time required to induce hypothermia is anywhere from 5 to 8 hours. Animals that had been heat-acclimated for a week or more at T_a 34-35°C required only about 2-3 hours to induce hypothermia. In marked contrast, animals which had been cold-acclimated for a week or more at T_a 4-5°C required the longest periods for induction of hypothermia, 8-12 hours and some animals never did go into hypothermia within a 12 hour period. One cold-acclimated population never did become hypothermic. In short, our conclusions were that heat-acclimation will shorten the induction time; and cold-acclimation will increase induction time.
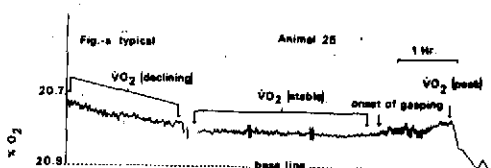
SLIDE 35
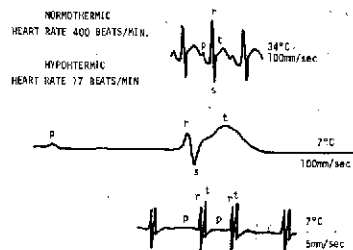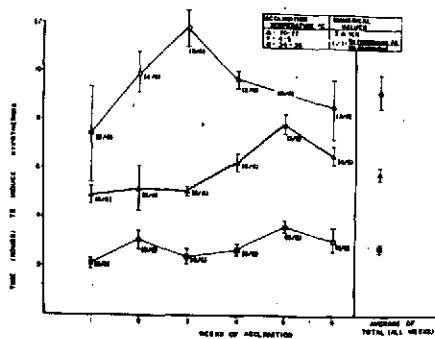
SLIDE 36

SLIDE 37 **

As a sort of bonus to the heat and cold acclimation study (slide 38) was the fact that we learned that these animals had altered survival periods while in hypothermia. The warm-acclimated hamsters had greatly increased survival times, e.g. periods of between 50-60 hours. The cold-acclimated hamsters required a long time to become hypothermic and had the shortest periods of survival in hypothermia. Most of the control i.e., room temperature maintained animals survived in hypothermia about 24 hours. In these studies, we used siblings, so that litter mates were used simultaneously for heat-acclimation, cold-acclimation and normal room temperature exposures.

The relationship between shortened induction time and lengthened duration of hypothermic survival was now clearly evident. We decided, however, to test the concept of shortened induction time a little further (slide 39). Heat acclimation is long and requires a long occupancy of cages. Was there another way we could shorten the induction? Was there another way we could modify temperature regulation? Our first approach was to shave the animals; this was a way to effectively remove some of their insulation. The open bars represent unshaved animals and the speckled bars represent the shaved animals. The induction time is shortened considerably in the shaved animals, about 4 hours as compared with about 8 hours in the unshaved hamster. The survival time is almost doubled for the shaved hamster, viz. 24 to 40 hours. Having now found another way of shortening the induction time and increasing the survival period, we began to look for various metabolites that could be implicated. As you know, two readily measured constituents are plasma glucose and liver glycogen. Our initial thinking was that there may be a sparing action of some metabolite in the fast induction method which could be related to prolonging survival in hypothermia. Some initial data are presented in the next slide (40). The plasma glucose in control animals was about 100 mg%. With the slow induction procedure, there is a marked depletion in blood glucose levels. With the fast procedure, the levels are reduced somewhat, but significantly different from those in the slowly induced animals. Our initial deduction is that there is some sparing action. If we look at the terminal stages of these animals, i.e. anywhere from 16 to about 24-28 hours later, there is a further reduction in plasma glucose and, in fact, all the animals are markedly hypoglycemic. Thus, even with the fast induction procedure, in which there appears to be some sparing action on glucose, after they remain hypothermic for a long period of time there is a slow decrement in the plasma glucose levels. Liver

TIME UNTIL DEATH IN HYPOTHERMIA
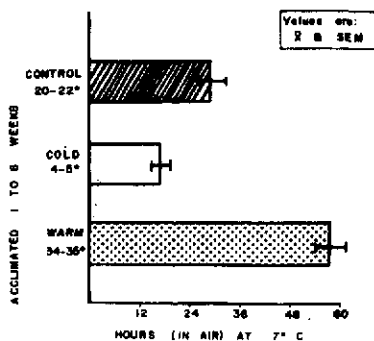
SLIDE 38 *
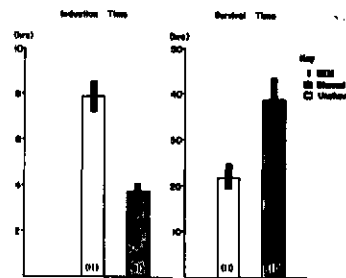
* Am. J. Physiol.222: 495, 1972

SLIDE 39
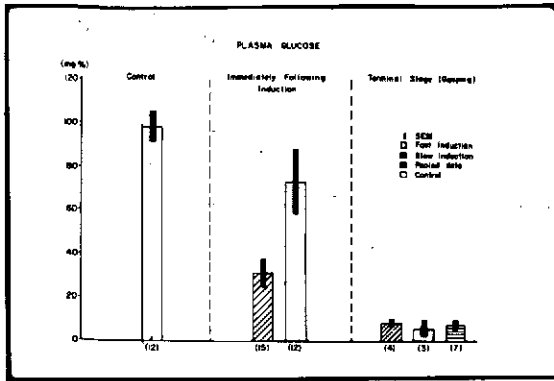
SLIDE 40

glycogen is also depleted in relation to the dura-tion of in-duction and to the extent of hypother-mia. The next slide (41) shows data from animals which reached the gasping stage and there is a complete depletion of liver glycogen. This was viewed as an expected result.
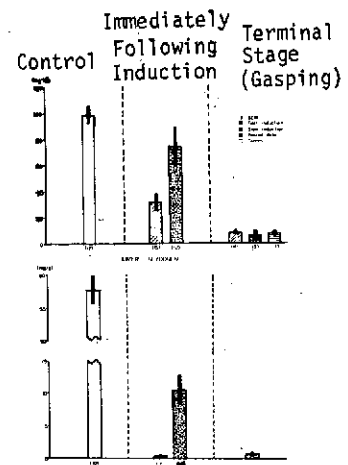
SLIDE 41

A comparison was made of the various types of induction techniques that we've used (slide 42) and some interesting correlations began to emerge concerning the average induction time, and the average survival time. The average induction time is given in hours; survival time is in days. Generally speaking, the initial trials of helium cold hypothermia (80:20 helox) required from 6.5 to 8 hours for induction and survival was about one (1) day. Now when we used a combination of 90% helium and 10% oxygen, the induction was shortened and the survival time increased, in these experiments we used the 90:10 helox only long enough to make the animal hypothermic, then we switched to 80:20 helox and then moved the animal into the cold room air. When we used 80:20 helox and introduced a small amount of halothane, about 2-3%, hypothermia was induced in about 1 1/2 to 2 hours (maximum) and hamsters remained hypothermic from 3 to almost 4 days. Halo-thane is particularly interesting since once it is removed, the animal can blow it off and remain hypothermic. Shaven animals take about 2.5 hours to go down and they remain alive for about 1 1/2 days. Lastly, if we take hamsters down with ordinary 80:20 helox, as we had been doing, but ventilate these animals using a tracheal cannula, they can be maintained in hypothermia at T_b 7°C for 4 days even though it required almost 8 hours to induce hypothermia. This is the only experiment that gives us a "divergent" result in the sense that despite the fact that it takes a long time to go down, we are able to keep them hypothermic the longest. However, we do respire them, in other words, they are artificially maintained during that long period of time (4 days). These experiments provide a most important clue. A clue which may help to identify the limiting factors involved in hypo-thermic maintenance, namely, respiratory failure. In addition, there may well be concomitant exhaustion of carbohydrate energy sources.

Our research efforts with helium-cold hypothermia in mice and rats will be reviewed in brief. It takes about 55 minutes, less than an hour, to make a C3H mouse, a small 25-30 gram mouse, hypothermic to T_b of about 13°C (slide 43). It takes about 2 hours for a mouse to rewarm, from 1:15 to 3:15 p.m. Temperatures are monitored with deep rectal thermocouples. There is nothing unique or bazaar in these results; we can take mice down

SLIDE 42

SLIDE 43

SLIDE 44

and we can warm them up. The data in slide (44) is divided into 2 slide
presentations. We did 2 completely different series of experiments.
Series 1 and Series 2 and used the same kind of grouping, Group A and
Group B. The data show induction time and body temperature. The next slide
(45) shows results of our initial experiments: 29 animals, required about
55 minutes to become hypothermic to T_b of about 14°C. In the next series
they averaged about an hour, (56 minutes) to become hypothermic and T_b's
reached about 12°C. We repeated the same type of experiment, (slide 46)
and reduced T_b's down to 12-14°C, and the awakening responses were comparable
to those in Series 1. Mice can be maintained hypothermic for varying periods
of up to 20 hours. The general survival time is about 10 to 16 hours,
however, several have gone to 20 hours. We concluded, therefore, that the
C3H mouse has the capacity to remain hypothermic for long periods. How much
we want to experiment with these, how much we want to further define these
periods of survival is really a matter of having time, personnel and helox
equipment available.

The Sprague-Dawley rat was another animal used in these comparison studies.
Animals were purchased, maintained for several days under standard labor-
atory conditions and then used in helium-cold experiments. In the first
series (slide 47), we used 130-180 gram animals and T_b reached 14.5-17.2°C;
it required 160 minutes (2.5 to 3 hours) to rewarm. One interesting
observation was that if they were maintained at T_b 16-17°C, they often
spontaneously rewarmed. Rats at T_b 13°C (slide 48) survive and are viable
for periods of anywhere from 5 to 20 hours. There is a great deal of
variation in the viability of these animals. However, when the ambient
temperature is lowered to about 13-14°C there is no spontaneous rewarming.

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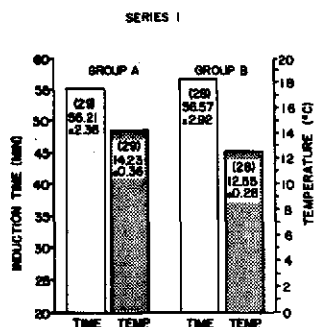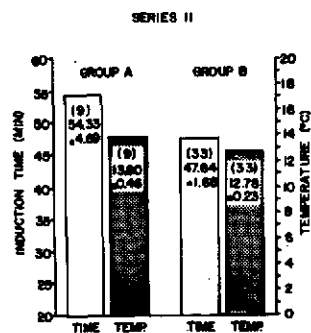
SLIDE 45

SLIDE 46

HELIUM-COLD HYPOTHERMIA IN WHITE RATS

(VALUES=MEANS AND RANGES)

NO. OF RATS	INDUCTION TIME (MIN.)	HYPOTHERMIA T_B °C	REWARM* TIME TO 20-25 °C (MIN.)
17	170	14.5-17.2	160
(130-180 GM)	(110-230)		(80-240)

*MAINTAINED AT 16.5 °C T_A SPONTANEOUS REWARMING OCCURS

SLIDE 47

VIABILITY OF HYPOTHERMIC WHITE RATS

(VALUES=MEANS AND RANGES)

NO. OF RATS	INDUCTION TIME (MIN.)	HYPOTHERMIA T_B °C	VIABILITY IN HYPOTHERMIA (HOURS)
17	205	13.4-15	5-20
(160-216 GM)	(160-250)		

T_A=13.5-14 °C

SLIDE 48

SUMMARY

I have reviewed 4 major areas of research which has occupied our attention
for the last 6-7 years. Chronologically, the depressed metabolism, hiber-
nation and hypothermia, and irradiation work has been underway for the
longest period, it takes a long time to gather that kind of information.
In the last 2 sequences you have seen our directions with the improvement
of the helium-cold technique and the broadening to include other species.
A great deal of effort is now focused on physiological characteristics of
helium-cold hypothermia and in seeking methods of shortening the induction
time and improving the survival time. In the last 2 sequences you have
seen our directions with the improvement of the helium-cold technique and
the broadening to include other species. This has been underway for about
1 1/2 years. There is another major series or works which are collabora-
tive projects with Dr. Cecil Entenman and he will report on those.

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DISCUSSION BY PARTICIPANTS

This part of the discussion (between asterisks) took place in the middle of the speech while the projector bulb was being replaced. The tape recorder was turned on after several questions had been asked so that the discussion is not complete. Someone apparently questioned Dr. Musacchia about the temperature level in his hypothermic animals.

*"ANONYMOUS": Is T_b 7°C the precise temperature of the hypothermic hamsters?

MUSACCHIA: It would be unfair to say that the animals were at precisely 7°C because there is a slight amount of variation, for example $\pm$ 1°C.

POPOVIC: Is it correct that the animals hibernated after irradiation showed a reduced percent survival as compared with animals who were awakened from hibernation? I would have expected something quite different.

MUSACCHIA: I tend to agree with you, since that is the general contention in published views about hibernation and irradiation effects. All I can really say is that these are the results we obtained and they are consistent in repeated experiments. The differences may be due to any variety of features: others have used X rays, we use a ^{60}Co source; we don't disturb our animals before or after irradiation, and the ^{60}Co source is just a couple of feet away from the cold room, we give them a total body exposure, others may use different quality of exposures. Also there are differences in the way the animals are maintained. Let me emphasize, we never touch the animals for 30 days after they have been irradiated regardless of whether or not they are active or hibernating. We give them food and water but we don't handle them even to change the bedding; they are checked every day sometimes 2-3 times a day.

POPOVIC: The idea being that in hibernation, the metabolic rate may be only 1% of the normal rate. Would you expect then, a much longer survival?

MUSACCHIA: This is what I would have expected, but that's not the way it turned out.

ENTENMAN: Have you done any bacteriology on them?

MUSACCHIA: No.

ENTENMAN: That may be one of the reasons for death after irradiation.

MUSACCHIA: Are you thinking of wide spread septicemia which may accelerate death following intestinal damage from irradiation?

ENTENMAN: You may not have intestinal damage at those high levels. You may still get a bacterial growth in your controls which would also push up the deaths in the control group.

MUSACCHIA: That's if you're going to compare my controls against the ones that were put back in the hibernaculum. But if you compare my controls with the animals that were irradiated while hibernating and permitted to awaken, you've got the same ball game.

SAUNDERS: To extend Cecil's point a little further, what about radiation-resistant species of bacteria that you may not be knocking out and are potential pathogens.

MUSACCHIA: But they are pathogens in both types of animals, the hibernating ones and the warm ones; unless those species of bacteria are protected while they are cold, that's possible.

ENTENMAN: Don't you have different bacterial flora in the body of the animal that has been hibernating for some time vs. one that has not been hibernating at the start of radiation?

MUSACCHIA: The only answer I can give you is reference to work that Ella Barnes did in McKenna's laboratory at the SSRC. I provided her with ground squirrels similar to those we use in these studies, and as I recall, there is a fall in total numbers, not so much a difference among the quality of bacteria, simply a quantitative drop. As I recall from discussions with her, the only thing one sees in the hibernating animal is a fecal or rectal plug which has the same kind of bacteria that are found in the active animals. Incidentally, she points out that what other people had done could be quite misleading. They often measured the bacteria in fecal pellets that had been passed, this means pellets in contact with contaminants in the nest and bedding. She used an improved method of isolating colon bacteria in the hibernating animal and under anerobic conditions.

POPOVIC: Are you talking about hamsters?

MUSACCHIA: No, ground squirrels.

POPOVIC: Z. Petrovic in Belgrade found a big difference between hibernating and nonhibernating ground squirrels several years ago.

MUSACCHIA: But where did he get the fecal pellets from?

POPOVIC: I'm not sure.

MUSACCHIA: I suspect this could make quite a difference, you see?

OYAMA: How about the tissue composition? Isn't the hibernating animal loaded with fat, isn't he more obese as contrasted to the hypothermic animal? The nutritional state of the animal after irradiation could be very critical, you could have accelerated pathogenesis.

MUSACCHIA: Yes, there are all sorts of possibilities and it is awfully difficult to look at the "total" picture.

JORDAN: Did you try any other gas beside helium, say argon or something, to make the animals hypothermic?

MUSACCHIA: We have not tried argon, we have tried nitrous oxide. We've anethetized animals, kept them in the cold, and they wake up; we have put them down with ether and the body temperatures fall low, and sometimes they don't wake up. We've tried a variety of anesthetics. We've not tried neon although we have thought about it, however, it's too expensive; we've not tried argon, I think it's also expensive.

SMITH: If you have some cold-acclimated rodents given a lethal, say $LD_{50:90}$, dose of irradiation, what time after irradiation would you expect the peak of deaths?

MUSACCHIA: I showed you the results with cold-acclimated hamsters, and the peak of deaths are during the first week. I would say that for rats, if you accept what Ghys says, then you might improve the survival time slightly. I would predict that if you are irradiating at levels higher than the $LD_{50:30}$, e.g. 1000 rads and using a rat or mouse, regardless of any prior acclimation, they will die just as rapidly as the noncold-acclimated animal. From our experimental data, I would say that only if you were irradiating close to the $LD_{50:30}$ levelwould you possibly find a slight increase in survival. One is dealing in periods within the first 15 days, where most of the deaths occur, and after that one might see a slight improvement in survival. But that's about it.

SMITH: In the gut, you see these things in about 8 days, is it?

MUSACCHIA: In the gut, you'll see an intestinal response after day 5 or 6. They say intestinal response as though it is going to happen on a given day. That's not the way it works. Damage occurs from day 1; because the intestinal cellular turnover is about 1.2 to 1.5 days, less that 2 days for the cycling of the gut mucosal cells. Thus the effects really starts at day 1. The reason one sees responses after day 5 or 6 is that this is the time for the most obvious results. There is a diminution and a sloughing off mucosal cells and the damage is truly visible. A rat or an animal that has been irradiated with a dose that's very close to the LD_{50} will often become anorexic soon after it's been irradiated. There are often behavioral clues, for example, a reduced food intake and a lethargy.

OYAMA: I have several questions. One is with respect to hibernation and the hypothermic response, I presume that if an animal is right for hibernation and if you subject that animal to cold, it will undergo hibernation. Is that right?

MUSACCHIA: Yes.

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OYAMA: So that your comparison between the hypothermic and hibernating animals must have occurred at different times of the year in your studies. Is that right?

MUSACCHIA: I can make the hamster hypothermic anytime of the year.

OYAMA: Without inducing hibernation?

MUSACCHIA: I can make the hamster hibernate or I can make it hypothermic, either one. The ground squirrel is different, it will hibernate in the wintertime--true hibernation, or it can be stressed into hibernation in summertime; but it's not a natural state of affairs. The animal is stressed. There are some who say that you can't make them hibernate, but you can force them into hibernation, i.e. if you define hibernation with the usual characteristics of a decrease in body temperature, decrease in metabolism, the ability to wake up--etc. I can do all that to a ground squirrel in the summertime, but it takes longer. If I start out with 10 animals, I may get only 5 that will hibernate; some of them resist. It is a stressful procedure in the summertime. This is one reason for going over to hamsters which 1) you can make hypothermic any time of the year and 2) although one must wait 3-4 weeks, they too will hibernate.

OYAMA: Second question. I'm very much interested in your blood glucose and the respondability. Have you tried to starve the animals before you irradiate them so that you, in essence, stimulate perhaps the gluconeogenic mechanisms and perhaps this may drastically alter the survivability of the animals.

MUSACCHIA: I haven't yet.

OYAMA: These are all fed animals when you irradiate?

MUSACCHIA: Yes. They are feeding up until the time that they undergo hibernation. Then they stop feeding. They are not particularly hypoglycemic when they are hibernating.

ENTENMAN: I don't believe it.

MUSACCHIA: Well, I won't say that these animals are not hypoglycemic, but the arctic ground squirrel is certainly not hypoglycemic. I've measured blood glucose levels in those animals.

SOUTH: The marmot is not hypoglycemic.

ENTENMAN: Which animal does Twente use?

MUSACCHIA: He uses the Citellus lateralis, Cecil.

JORDAN: The trouble is that with any of those steady-state measurements, you don't know anything about turnover rate, about gluconeogenesis . . .

MUSACCHIA: Right. Any of the blood glucose studies that have thus far been reported have not been done repeatedly on the same animal.

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POPOVIC: Rene Agid did in France.

MUSACCHIA: On the same animal?

POPOVIC: Yes, he used cannulated ground squirrels. He was the first one to use them.

MUSACCHIA: And what did he find? What was the blood glucose?

POPOVIC: It was very low. He's doing it now.

SOUTH: We've being doing cannulated marmots.

POPOVIC: I'm not talking about marmots, I'm talking about ground squirrels at the moment. I'm talking about Agid's results and those of his students because they are working on this problem a great deal. I'm talking about a ground squirrel that is hypoglycemic during hibernation and wakes up after reaching extremely low values like about 25 mg%.

MUSACCHIA: You're right. Agid's laboratory is an exceptionally good laboratory. They are probably working with Citellus citellus; they also work with dormice.

POPOVIC: What is the dormice doing?

MUSACCHIA: This is interesting. Their animals are "hypoglycemic:" whereas some of the species that we've seen on this side of the Atlantic are not necessarily hypoglycemic. I have absolutely no idea as to why there are such differences.

OYAMA: Last question. Do hibernating animals exhibit a rhythm, a diurnal rhythm?

MUSACCHIA: They exhibit a rhythm but I don't know if it's diurnal. They exhibit rhythms of awakening and hibernation.

OYAMA: When you irradiate, do you select a specific time of the day to irradiate?

MUSACCHIA: With the hibernating animal, I haven't been as fussy or particular as with the other animals. In general, we have been specific about time of day, because radiation sensitivity varies depending on the time of day. We probably have irradiated most of the hibernators, mostly by chance, in the morning. I don't recall specifically all the ones we have irradiated and whether they were all in the morning or afternoon. The one thing we do at all times is a paired experiment, in which we irradiate a control with a hibernating animal or a hypothermic

animal. But, definitely, with the hamsters we have been very particular about the time of irradiation. Several years ago Ron Barr, and I did a 4-hour irradiation study over 24 hour periods. We would irradiate every 4 hours over a continuous 24-hour period. After dozens of animals, when we had no more room left and the animal room was filled with irradiated hamsters, we scored survival. There was a cyclic death curve which could be related to the time of day when irradiation was done. I think that is in a very early NASA Status Report.

HORWITZ: Did the results correspond with metabolic peaks or metabolic lows?

MUSACCHIA: I don't recall at this moment. The only reason we did the experiment was because we wanted to eliminate variables. One investigator, Pizzarello, had done some experiments with mice and he said that there is a radiation sensitivity related to the time of day in which you irradiate mice. He removed all known distinguishable clues. However, we should consider that Frank Brown says there are clues no matter what one does, and I agree with him. We were repeating a type of experiment that Pizzerello had done using the hamster. We found diurnal variations and all it told us was that everytime you irradiate, stick to the same time of day for the control and experimental animal. We now routinely use this protocol as a precautionary device.

POPOVIC: I wonder if you are aware of Hajdukovic and Harve who study survival time in dogs in terms of radio-protection and hypothermia?

MUSACCHIA: I haven't mentioned all investigators in this field.

POPOVIC: You can see the point I am making.

MUSACCHIA: Yes, I am aware of other papers, so let us say there are a few more in the literature.

SMITH: Is Harvey Patt still in the business? Patt did frogs many, many, years ago, and they keep quoting those as being hibernating animals. I get a little annoyed with the radiobiologists because I go to the Radiation Research Society meetings and they are still holding onto a couple of archaic concepts; they literally won't give them up. He's still in the business, I guess.

JORDAN: Do you plan to use other gases?

MUSACCHIA: I'd like to use neon, I really would, but let's face it, it's much too expensive. Neon might be a good one to use, I would guess. Liquid fluorocarbons would be interesting; Wynn Volkert was planning to use some. The gaseous halothane is probably very good with small animals;

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I don't know if it would work with large animals since there are a number of side effects. So far I can't say that we have had any particular side effects with halothane.

HOLTON: What about freon?

MUSACCHIA: Why freon? Remember that I used helium strictly for its heat conductive qualities.

JORDAN: That's why freon would be kind of interesting.

MUSACCHIA: What does it do physiologically?

JORDAN: I don't know much about it except that it's not toxic. It's used as a coolant in refrigerators and in air conditioning units. It just seems like it would be a darn nice thing to use.

ENTENMAN: They also use it as a coolant for quick-freezing foods.

JORDAN: It's not toxic. At least, several of that family are non-toxic.

MUSACCHIA: Do you know of any reports in the literature?

JORDAN: It's been used as a breathing gas. I've thought of using it as another gas in our system.

MUSACCHIA: That may be a good idea, Pat, but I don't know anything about it; however, I'm willing to learn.

HORWITZ: Relative to your blood glucose levels, can you extend the survivability of your animals?

MUSACCHIA: We're trying that. The biggest problem with that is that you have to infuse the animals with cold glucose. I don't know why it would not work except that the metabolic machinery for the use of the glucose is greatly reduced.

POPOVIC: I tried it several years ago with the rat and it just did not work. The glucose level doesn't mean much for the animal in terms of survival.

MUSACCHIA: You know, I'm surprised that you guys haven't criticized me on something that has bothered me, and I'm going to get to it, but it just takes time. I've been using 7°C throughout all the holding temperatures; what would happen if I were to go up to 10°C or just a little higher? We might be able to turn on metabolic machinery at slightly higher temperatures.

HOLTON: You said you get your best response at 12°C?

MUSACCHIA: That was the hibernating animal and it was 13°C in the radiation experiments.

POPOVIC: It seems to me that you have to choose one temperature in the beginning to get your initial values and then you can move up or down.

HORWITZ: The extended survivability you get when you artifically respirate, does that mean that they die after 8 days while they are still being respired?

MUSACCHIA: Yes, but they die after 4 days.

POPOVIC: This is not permanent survival. They are dying in hypothermia.

MUSACCHIA: Right. But if I take them out of the cold at any time prior to reaching the gasping stage, and even during the gasping state, they will survive. I differentiate between clinical survival and biological survival.

SAUNDERS: You're just testing the length of time you can keep them in heat balance.

MUSACCHIA: That's strictly clinical survival. But biological survival is when I can take them out of the cold room, put them on the table, and they rewarm and become active. I can do that any time up to and into the gasping stage. The gasping stage is a good warning to me. We believe they die from a respiratory failure because they attempt to respire. (In some ways it even looks like a hibernating animal attempting to respire). Since it fails to adequately maintain respiration, it dies.

HORWITZ: What is the respiratory rate of your hypothermic animals as compared to your artificially respired animals?

POPOVIC: 30-40?

MUSACCHIA: Yes, we've got respiration rates and during that long period, I'd say about 30/min. or less.

HORWITZ: And the artificially respirated?

MUSACCHIA: We drop them down to about 20-40/min. We're down at that level.

POPOVIC: What is the tidal volume you are giving them? 0.5 or 1cc?

MUSACCHIA: I don't know. With the artificial respirator we were using, I don't know what it is. Volkert and Anderson have been working with the respiration project. They are pushing survivals to 3 and 4 days.

POPOVIC: It appears that for you a shorter induction time is better. Why don't you decrease the external temperature?

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MUSACCHIA: We use 0°. I could go to minus temperatures.

POPOVIC: Then you would decrease the induction time. It might be better.

MUSACCHIA: Possibly. Possibly.

SAUNDERS: You wouldn't want to go minus.

MUSACCHIA: It can be done. LeBlanc has done this; he has gone to minus $20^{o}C$ external temperature. It's like putting the animal in ice water or colder. It's a possibility.

HOLTON: I was surprised to hear that if you cold-expose your animals that it takes them longer to go into hypothermia.

MUSACCHIA: That's right, after they have "learned" to increase heat production, they can resist the external cold for longer periods.

HOLTON: There is a paper about one of the quickest ways to make animals hypothermic is to expose them to the cold, I think it's 10 minutes for 8 hours a day, and by the third day you can get them in nothing flat.

MUSACCHIA: What kind of animals?

HOLTON: It was rats.

MUSACCHIA: That's why--it was in rats. The hamster probably has better compensating mechanisms for temperature regulations.

POPOVIC: The way I see it is that we are talking about a temperature of $0^{o}C$ (the exposure temperature for warm adapted animals) and something like maybe minus $5^{o}C$ for cold-adapted. But that temperature is misleading because it doesn't mean the same physiologically for cold groups of animals.

MUSACCHIA: Except that physiologically the warm-adapted animals literally try to do all the things that a warm animal would do--they attempt to expose as much as possible to cool off, behaviorally they attempt to cool while they are being warm-acclimated. So, they are warmer. Physically, they are "well aware" of the heat exposure. I'm sure that the cold adapted animal is building up cold resistances and he's laying on fat. In fact, the weight changes; we had a whole series of studies of weight change. The cold animals gain more weight than the others. The others sort of maintain a weight level; they lose a little at the beginning but then they come back. The cold animals really tend to gain weight.

POPOVIC: Maybe another way to extend survival would be to preadapt the animals to hypothermia. Expose them to hypothermia once or twice.

MUSACCHIA: No, we've done repeated hypothermia and they build up a resistance. They resist induction of hypothermia.

POPOVIC: Then you have to decrease the external temperature.

MUSACCHIA: I can try that.

JORDAN: It would be interesting to find out if you could take one that you're having trouble putting down, on the basis of temperature, and sock him with a heavy diluent gas and see if you can put him into a state where he will fall into a hypothermic state.

MUSACCHIA: What diluent gas?

JORDAN: A zenon:nitrogen mixture maybe.

MUSACCHIA: I've done it with halothane, although I realize halothane is not a diluent gas.

HOLTON: Have you ever tried pentobarb?

MUSACCHIA: Yes, but they either die or wake up. It is difficult to determine dosage under these conditions. They wake up--their body temperatures are lowered but often they wake up when you least expect it. The anesthetics just don't seem to be consistent.

HOLTON: You mean that in the cold room they wake up?

MUSACCHIA: Yes. We don't get good reproducible results with anesthetics. Wynn Volkert has screened just about anything that comes in a bottle. I'm glad that Popovic reported on the liquid fluorocarbons because this made us change immediately to halothane.

OYAMA: Do you have any ideas about why you have increased survivability the shorter the induction time?

MUSACCHIA: Well, I don't think that the animal is as metabolically exhausted. With the shortened induction time, the plasma glucose is at an intermediary level, and there is still some liver glycogen left.

POPOVIC: What do you plan to stress more in your future work, radioprotection or your method of induction?

MUSACCHIA: The radio-protection I'll extend to a dose-response curve for the hibernating hamster. We'll do some more work with the radiation protection and also with the cold, but for the most part I think we are going to swing into an improvement of the methodology and making a better hypothermic hamster, and investigating specifically parameters characteristic of hypothermia. To make the animal hypothermic and not know what is going on physiologically and biochemically at this stage would be ludicrous. We've got to learn more about the conditions that we have imposed on these animals and how readily they survive and come back out of it.

SOUTH: There is something that bothers me. Maybe I don't understand my physical chemistry, but freon works in the gaseous state. Now, in equilibrium it is no more conductive and probably less conductive than most gases. The only reason that freon is used is that it goes from the liquid to the gaseous state readily and uses up a lot of heat and makes the environment cool. This is no reason for using freon as a conductor or a conductive gas like helium. As a depressant or a diluent, maybe, but it

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has no conductive properties that I know of. I don't know what its conductive properties are but I know that it's nothing like helium.

JORDAN: It's a much bigger molecule. It has a molecular weight of 380 some odd.

MUSACCHIA: Weren't you suggesting it as a diluent?

JORDAN: Yes, the same thing with some of the heavier ones, like a zenon: nitrogen mixture; not because it is a good conductor but because it slows metabolic rate so that you'd be using 2 components to bring you to the same state. The question really is, in my mind, at what kind of temperature could you hold an organism in a kind of suspended animation with a combination of a depressant gas and lower temperature.

SOUTH: I don't know that the price of zenon is now but you ought to be able to get a few liters of it. It used to be $80/liter at 1 atmosphere.

SAUNDERS: Really, Frank.

SOUTH: Yes.

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Intermediary Metabolism During Brief and Prolonged Low Tissue Temperature

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As you probably know, the work that I have been doing has been done, for the most part in Berkeley. The tissues that I have been working on for the most part have been derived from Columbia, Missouri, in collaboration with Dr. Musacchia. We set out to find out more about the intermediary metabolism of the depressed metabolic state, mainly in the hypothermic hamster, and the hibernating ground squirrel. He has already shown you the methods of induction of hypothermia in hamsters. First, I want to show you an outline of the experiments that we have done (opaq. 1). I'm afraid it is actually a little excessive for you to sit through all these things at this late hour. This is the outline of the ten experiments that we have undertaken. We first started out with the in vivo uptake of acetate-2-^{14}C into the hypothermic hamsters. The first experiment was 18 hours long; the second experiment continued for a 7-hour period. The acetate was injected intravenously 1 hour before sacrifice. The effects of different time periods on the metabolism of acetate were studied. Then we turned to the in vitro metabolism of acetate on hamster tissue. I went to Columbia to carry out these experiments and carried the tissues back to Berkeley for analysis. We repeated the experiment with hibernating hamsters and hibernating ground squirrels. The seventh experiment is a study of palmitic acid metabolism in the hypothermic hamster. Palmitic acid metabolism in the hibernating ground squirrel was studied next.

OPAQ. 1

JORDAN: Do you retain most of your label?

ENTENMAN: You retain more of your label with acetate-2-^{14}C than you do with acetate-1-^{14}C.

JORDAN: I haven't been able to demonstrate it in vivo with that length of time, although theoretically you should. When you go much longer than 20 minutes or so with it in vivo, have you been able to demonstrate that you can hold on to it better?

ENTENMAN: We haven't done any time studies.

JORDAN: Theoretically, of course, it should, since there is an acid to it, retain more of it, shouldn't it?

ENTENMAN: I'll give you a look at the slides that I have and we'll see a little later.

In experiment number 8, we went to the palmitic acid-^{14}C in the hibernating ground squirrel. In experiment 9, the study was carried out at Berkeley using rat liver slices incubated in various temperatures in vitro. For the last experiment, we used some radioactive intermediates with the hypothermic hamster at two temperatures.

In the first experiment (Slide 1) that Dr. Musacchia carried out at Columbia, an indwelling catheter was placed into the hamsters, hypothermia was then induced, and they were injected with acetate-2-^{14}C, intraveneously. The animals were maintained at 7°C. Hamsters were killed at 30 minutes, 1 hour, 4 hours, 8 hours, and 18 hours after acetate injection. The data show the difference in the 7°C uptake into the total lipids of hamster tissue over the period of time. This is sort of a cumulative thing because ^{14}C was present in the blood throughout the 18-hour experimental period. You can see the differences in uptake by the tissues. Note the scales that were used. Lipids were not synthesized to any great extent. You can see that there is a lowering in some of them after the initial rise, but the rise was pretty fast in all tissues after the injection of the acetate. In the next slide (2), the tissues which synthesize lipids at a greater rate are shown. Again, lipid synthesis is fast at the beginning, but you see that there are some tissues, such as the brain, at 7° which reach a maximum level at 30 minutes and level off; other tissues like the kidney reach it later. In the small intestines and in the liver at 7° the acetate incorporation keeps increasing and I would say that this may be due to the continued availability of acetate for the synthesis of lipids. This is the only published work that I will report on, the rest is unpublished.

The data on second in vivo experiment that Dr. Musacchia carried out are shown in Slide 3. As I said previously, a 7-hour time period was used.
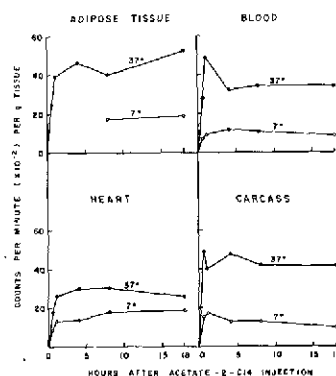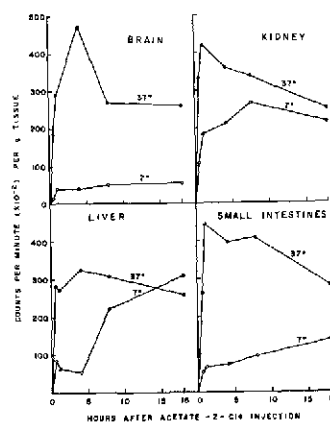
JORDAN: Cecil, where is this published?

SLIDE 1 *

SLIDE 2 *

* Proc. Soc. Exp. Biol. Med. 137: 47, 1971

ENTENMAN: In Proc. Soc. Exp. Biol. Med. in May of this year (vol. 137, 47-51, 1971).

Since, in the first experiment, the peak was reached rather early we thought that it would give more meaningful data if the hamsters were sacrificed early after the injection of the radioactive ^{14}C. So, Joe induced the hypothermia and after 7 hours of hypothermia (7 hours after the body temperature had reached 7°C), he intravenously injected the acetate-^{14}C and sacrificed the animals, in all cases, 1 hour later. A rapid uptake of ^{14}C into lipids was noted during the 1-hour period rather than a slower accumulation of ^{14}C into lipids over a period of time such as was found in the previous 18-hour experiment. I'll tell you what happened and then the slide will come on so you can see it. In this experiment, as with practically all the experiments that I've done, and all the different isotopes I've used, the kidney and the small intestines have been the most active tissues in the oxidation of the substrate and in the synthesis of lipid in most cases. Not all, but most. Another thing I want to say now is that especially in the in vitro work, you have to resist the temptation to compare the amounts, the absolute amounts, of radioactivity that appear in the carbon dioxide or in the lipids when you are comparing tissues. I think this is somewhat valid, but not exactly because of the differences in pool sizes in the different tissues. You can compare, I believe, crossways, that is within a tissue, but I don't think it is quite valid to compare tissues. Since you will find that I do it, you have to realize that isn't strictly the way it should be done. So in this second in vivo experiment you can see that there is a marked temperature effect on all the tissues (Slide 3). At 37°C, the kidney is very active (the values are as total lipid-^{14}C counts/min/g tissue) and the activity is still pretty high at 7°C. The small intestine activity is appreciable at 37°C and still fine at 7°C. This is after 7 hours of hypothermia and 1 hour after the injection of acetate-^{14}C. There is very little temperature effect on the adipose tissue as you can see.

The third experiment (slide 4) was carried out to see if the length of hypothermia changed the capacity of the tissues to synthesize lipids in the hypothermic hamster. We could have, but we didn't at that time, set up to measure oxidation so we have no in vivo data on the oxidation of various substrates. We only have the synthesis of total lipids. Again you see that kidney and the small intestines have the great synthetic

In Vivo Incorporation of ^{14}C from Acetate-2-^{14}C into Total Lipids of Tissues of Hypothermic and Normothermic Hamsters

Tissue	No. of Hamsters	Incubation Temperature (°C)	Average Total Lipid-^{14}C (cpm/g tissue)	Standard Deviation
Adipose	5	7° / 37°	7,768 / 7,312	±7,418 / ±922
Blood	5	7° / 37°	930 / 2,306	±408 / ±346
Brain	4	7° / 37°	8,075 / 31,456	±993 / ±18,750
Heart	4	7° / 37°	8,375 / 21,732	±792 / ±997
Kidney	5	7° / 37°	18,865 / 21,475	±8,310 / ±5,580
Liver	3	7° / 37°	6,352 / 26,729	±626 / ±614
Small Intestine	4	7° / 37°	8,444 / 81,847	±2,570 / ±18,780
Spleen	5	7° / 37°	2,832 / 9,570	±1,995 / ±5,447

Hypothermic hamsters were kept at body temperatures of 7°C for 7 hours prior to sacrifice. Normothermic hamsters were maintained at room temperature (26°C) and had body temperatures of 37°C. All hamsters received acetate-2-^{14}C intravenously 1 hour prior to sacrifice (ILR 69-50).

SLIDE 3

In Vivo Incorporation of ^{14}C Activity from Acetate-2-^{14}C into Hypothermic (3, 6, 24-Hour) and Normothermic Hamster Tissues

Tissue	Time Hypothermic (hrs)	Hypothermic No. of Hamsters	Hypothermic CPM/g Tissue	Normothermic No. of Hamsters	Normothermic CPM/g Tissue
Blood	3	2	472	1	2,029
	6	1	898	2	2,799
	24	4	777	2	2,242
Brain	3	2	1,597	1	14,452
	6	1	4,309	2	13,786
	24	4	5,610	2	15,334
Heart	3	2	2,155	1	4,743
	6	1	3,138	2	4,194
	24	4	5,528	2	3,809
Kidney	3	2	28,472	1	47,714
	6	1	14,815	2	40,415
	24	4	16,408	2	32,124
Liver	3	2	2,488	1	14,863
	6	1	18,246	2	26,090
	24	4	15,348	2	20,053
Small Intestine	3	2	28,228	1	136,247
	6	1	43,154	2	193,274
	24	4	18,695	2	163,858

The hypothermic temperature was 7°C. The hours in hypothermia indicates the time each hamster's body temperature was 7°C, and does not include the period of time taken to cool the hamster to 7°C (ILR 69-78).

SLIDE 4

In Vivo Incorporation of [14]C Activity from Acetate-1-^{14}C into Hypothermic (3, 6, 24-Hour) and Normothermic Hamster Tissue Lipid Classes as Separated on TLC[1] (TLR 69-78)

Tissue	Hours in Hypothermia	Hypothermic							Normothermic						
		PL[7]	MG	FS	1,3-DG+LN	FFA	TG	SE	PL	MG	FS	1,3-DG+LN	FFA	TG	SE
Brain	3	38.53	-9	18.2	21.6	18.8	1.6	6.5	45.0	8.5	7.4	5.4	22.1	1.3	0.3
	6	32.13	-3	14.1	15.4	31.2	1.7	6.4	46.4	7.8	7.5	5.1	24.0	1.6	0.6
	24	33.63	-3	12.8	16.3	29.8	1.4	5.9	46.2	3.1	9.3	5.2	22.3	1.4	0.4
Kidney	3	35.2	10.2	13.7	16.2	18.8	2.8	3.1	60.0	4.8	9.2	1.4	18.4	3.6	0.6
	6	47.4	9.4	13.2	5.2	20.7	1.9	2.1	65.0	4.8	8.2	1.4	17.7	2.4	0.4
	24	29.6	7.0	15.7	26.3	15.6	3.6	1.8	61.3	5.0	7.4	1.9	15.0	7.2	0.5
Liver	3	42.x5	-3	22.1	5.8	23.7	—	6.4	32.7	7.3	7.0	3.2	28.0	3.3	1.3
	6	8.0	8.6	39.9	39.6	4.5	1.2	7.1	48.0	1.8	30.8	1.4	14.4	6.3	7.1
	24	7.1	9.5	38.4	33.4	4.2	0.9	6.2	51.4	4.1	8.4	4.8	17.6	9.8	2.2
Small Intestine	3	9.0	4.8	93.4	39.6	17.3	3.8	4.3	31.9	1.8	16.3	3.8	40.5	2.8	1.9
	6	7.5	6.2	24.5	35.8	16.4	1.5	8.5	32.6	4.5	16.2	3.4	39.3	2.5	1.7
	24	11.2	6.8	16.0	31.2	22.4	2.6	6.5	32.4	4.6	17.1	4.4	36.8	3.1	1.7

1. See text for treatment of hamster. The hypothermic deep body temperature was 7°C.
2. The following abbreviations were used: PL (phospholipid), MG (monoglyceride), FS (free sterols), 1,3-DG+LN (1,3-diglyceride + lanosterol), FFA (free fatty acids), TG (triglycerides), SE (sterol esters).
3. For the 3-hour hypothermic liver samples and all brain samples, the PL was not separated from the MG; thus, these values in the PL column are for both PL and MG combined.

OPAQ 2

In Vivo Incorporation of [14]C Activity from Acetate-2-^{14}C into Hypothermic (3, 6, 24-Hour) and Normothermic Hamster Tissue Lipid Nonsaponifiable, Saponifiable and Aqueous Fractions (TLR 69-78)

Tissue	Hours in Hypothermia	Hypothermic			Normothermic		
		%[14]C Recovered in			%[14]C Recovered in		
		Nonsap.	Sap.	Aqueous	Nonsap.	Sap.	Aqueous
Blood	3	3.5	60.3	47.0	8.1	54.3	37.6
	6	3.4	65.4	31.2	25.3	49.7	24.9
	24	10.1	22.8	67.1	8.1	56.6	35.3
Brain	3	35.0	39.8	38.2	21.6	60.6	17.8
	6	41.8	45.4	14.8	23.5	60.8	15.6
	24	42.0	28.1	27.2	18.0	45.5	36.6
Heart	3	8.4	27.6	67.0	8.3	65.8	25.9
	6	13.7	45.8	40.5	16.6	54.0	29.4
	24	11.4	38.6	50.0	8.6	52.4	39.0
Liver	3	18.2	28.9	57.9	15.5	51.9	32.6
	6	25.6	14.2	10.2	28.6	45.8	25.6
	24	67.7	17.6	14.7	9.3	30.2	60.6
Kidney	3	21.8	21.4	56.8	9.9	49.8	40.3
	6	10.6	30.1	59.2	10.6	57.0	32.4
	24	41.0	19.0	40.0	19.7	31.0	49.3
Small Intestine	3	64.8	23.0	12.2	20.6	72.4	7.0
	6	71.2	23.2	5.0	24.8	70.7	4.5
	24	53.2	81.8	15.0	21.8	71.2	7.0

SLIDE 5

capacity in the in vivo state in the hypothermic hamster. The brain is relatively active and shows temperature sensitivity. The heart is not so active, but shows less temperature sensitivity. The kidney and small intestines are the most active in the synthesis of lipids. When I say total lipids it doesn't mean fat synthesis in the sense that we ordinarily think of it. As you will see later at least in the in vitro situation, much of the label goes into glycerol and not into fatty acids. Data on the TLC separation of lipid classes are given in opaque 2.

The next slide (5) shows the distribution between saponifiable, nonsaponifiable and aqueous fractions after saponification of the purified total lipid extract in these various tissues. As I indicated, a fair amount of the activity goes into the aqueous phase which we believe is glycerol; we've done some tests on in vitro studies where we had more ^{14}C incorporation and it seems to be glycerol as isolated as the dimedon or tribenzoate derivative then counted. In this case, you will notice that there is a difference between the in vivo results and the in vitro results. In the in vitro work, to be presented later, it will be like this which is more typical; at the early part of the experiment, you get a fair amount of ^{14}C in the glycerol and at later times you get a fair amount of glycerol being synthesized and you also get a temperature effect with lower temperatures causing a greater proportion of the activity in the glycerol portion of the total lipids. The next slide (6) is still on the third experiment. The purified total lipids were again separated into the various fatty acid groups, the saturated, the monounsaturated, and the polyunsaturated fatty acids, using silver nitrate and TLC. Surprisingly, we have a fair amount of activity in the polyunsaturated fatty acids. This has been repeated in the most of the in vitro studies as well. Most of the activity in some of the tissue was in the saturates with a fair amount of activity in the polyunsaturates. The pattern between the hypothermic animal and the normothermic animal is different; you have more of the ^{14}C activity going into the monoenes and the polyunsaturated fatty acids in hypothermic hamsters than you do in normal hamsters.

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Tissue	Hours in Hypothermia	% ^{14}C Recovered in					
		Hypothermic			Normothermic		
		Polyunsat.	Monounsat.	Saturated	Polyunsat.	Monounsat.	Saturated
Blood	0	36.6	38.2	35.1	10.6	9.4	80.6
	6	27.1	73.7	49.3	6.5	10.3	83.1
	24	19.8	33.1	44.6	5.7	8.5	83.8
Brain	0	33.5	33.0	33.6	1.6	7.9	88.6
	24	24.6	28.9	35.1	4.8	9.5	86.7
Heart	0	43.5	18.9	37.6	13.3	6.6	80.3
	6	57.1	18.3	24.3	14.6	3.4	83.0
	24	86.6	18.6	43.8	21.8	10.9	68.1
Liver	0	40.0	19.9	40.2	11.6	14.2	73.9
	6	53.9	17.2	37.5	13.7	6.3	81.9
	24	47.1	—	32.5	10.4	—	89.2
Kidney	0	16.9	37.1	36.6	30.0	13.1	56.3
	6	14.6	30.6	35.5	23.0	9.5	27.5
	24	43.7	29.9	27.6	36.7	21.7	47.1
Small Intestine	0	24.2	23.5	53.6	6.5	14.7	77.1
	6	19.6	17.5	62.9	4.2	12.6	83.2
	24	20.6	27.6	61.6	9.9	10.6	87.6

Physiological State	Incubation Temperature (°C)	Adipose	Brown Fat	Diaphragm	Heart	Kidney	Liver	Small Intestine
Normothermic	7	110	900	1,240	7,170	1,260	138	760
	17	300	2,150	6,190	6,590	6,086	700	2,460
	27	870	4,150	12,200	15,880	16,780	1,460	7,020
	37	950	4,650	14,300	19,140	19,260	1,472	7,790
Hypothermic 1 hr	7	150	1,450	920	1,820	3,210	290	2,430
	27	990	1,260	8,800	13,700	12,480	1,350	9,240
	37	360	4,300	13,300	4,430	13,480	1,168	14,900
Hypothermic 12 hr	7	220	1,370	470	1,660	4,460	600	3,770
	27	400	4,160	11,800	10,480	16,480	1,034	11,200
	37	560	5,150	14,700	13,100	19,280	1,060	11,100
Hypothermic 18 hr	7	17	1,460	740	1,400	3,280	178	870
	17	160	6,550	4,840	5,400	13,760	468	5,970
	27	200	7,490	3,360	16,100	16,780	1,430	15,300
	37	190	7,790	11,200	18,100	19,900	680	11,300
Hypothermic 24 hr	7	120	2,170	1,270	3,300	3,150	270	1,940
	17	85	5,450	5,490	12,500	17,800	640	7,890
	37	330	9,480	15,400	19,000	23,300	1,300	9,700

1. The figures represent the average moles of acetate incorporated into carbon dioxide per gram of tissue per two hour incubation. In each instance, tissue was incubated at 37° as well as at one lower temperature of 7, 17 or 37°C (ILA BC-2).

SLIDE 6   SLIDE 7

Data from the first in vitro study that we carried out are shown in slide 7. We wanted to see whether the tissues taken from hamsters that had been hypothermic for 1 hour, 12 hours, 18 hours, and 24 hours, when incubated at different temperatures would give you results which would give some clue as to the viability of the hamsters at these prolonged hypothermic temperatures. The normothermic served as a control as in the in vivo situations. In all of these in vitro studies on hamsters and ground squirrels, I went to Columbia, Missouri, to carry them out. Joe and his technicians got everything ready. I prepared the buffer and put it into the incubation flasks and stoppered them with serum bottle caps after gassing the buffer, adjusting the pH and adding the radioactive substrate. The flasks were put in a 7°C room. We autopsied the animals which were at a 7°C body temperature (hypothermic) in the 7°C room and put the heart, adipose tissue, etc., at 2 different temperatures in each animal; the first animal would be incubated at 37°C and 7°C and the next would be incubated at 37°C and 17°C. The 37°C incubation was done in each animal so that we would have a basis for comparison. From slide 7 you can see that the oxidation of acetate by the normothermic animal increases when the incubation temperature is increased from the initial temperature of 7°C. When you look at the adipose values of the 1-hour hypothermic hamsters, the trend is not greatly different from the normothermic values. There does, however, appear to be a trend in that as the length of hypothermia increases, a little less of the acetate is oxidized. When you look at the brown fat values, you can see that it does pretty well during the first hour of hypothermia even though there is some variation, and that it does better as the period of hypothermia is increased. That is, the oxidation is greater and the temperature effect is less. The diaphragm after 1 hour of hypothermia at 7°C is about as good as the normothermic. Even after 24 hours of hypothermia the diaphragm still can oxidize acetate as well as normally. The same is true for the heart. As I interpret the data, acetate oxidation is essentially unchanged after the first 24 hours of hypothermia as compared to the normothermic animal.

The next slide (8) shows data on the total lipids in the same experiment. You can see that the brown fat synthesis of lipi drops off markedly with temperature. The synthesis of fats in this tissue increases with the length of hypothermia up to 24 hours. Remember that the oxidation of acetate was greater in brown fat as the time of hypothermia increased.

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There is nothing particularly remarkable about the other tissues. When compared to brown fat, synthesis of lipids by other tissues was rather small. In this case, I think that liver probably is next to brown fat in synthesis of lipids; I think this is true in most of the work that we have done, in vitro experiments at least. The synthesis of lipids is higher in the brown fat and the liver; you get appreciable synthesis in those 2 tissues, but in the other tissues you do not get much synthesis.

In the 5th experiment, we used hibernating hamsters in order to compare activities of tissues of such hamsters with the data previously obtained on tissues from hamsters in which the hypothermia was artifically induced. The tissues were obtained in the same manner at autopsy as previously described and were incubated for the same length of time (2 hours) at the same 4 temperatures as those used with the hypothermic hamster. The control animals were fasted overnight, 18 hours; this doesn't compare in fasting period with the hibernating animal, of course, but at least it is an 18 hour or overnight fast. In slide (9) you can see that the hibernating hamster tissues, when taken from the hamster at a body temperature of 7°C and incubated in a bath at 37°C, oxidize acetate at a rate that is quite comparable to the normothermic hamster. When the tissues are incubated at a lower temperature, the temperature of hibernation, you can see that again in some tissues the oxidation of acetate is about the same. In the brown fat, you have again a better rate of oxidation of acetate in the hibernating animals at 7°. The heart holds up pretty well as the temperature of incubation is decreased. The kidney also does pretty well and the intestines does rather well in oxidizing acetate. There seems to be a greater drop between the 17° and 7° incubation temperatures than there is between 27° and 37°C. And it seems that at various temperatures, rather comparable rates of oxidation of acetate by the hibernating and the normothermic hamsters are found. The data in slide 10 gives a quick way of evaluating this. The values obtained at 37°C which you saw in the previous slide were arbitrarily set at 100% and related to the values found at 27, 17, and 7°, so that at a quick glance you can get some idea of temperature effect. In adipose tissue in the hibernating hamster, you see that at 27°C the amount of acetate oxidized is 81% of that at 37°C; it drops down to 7% at 7°C. The heart maintains the oxidation of acetate as well at 27°C as it did at 37°C; it drops down at 17° and 7°C, but it's still not bad for it is not too awfully different from the oxidation by hearts from normothermic

The Effect of Duration of Induced Hypothermia on Incorporation of Acetate-2-14C into Total Lipids of Hamster Tissues During a 2-Hour Incubation[1]

Physiological State	Incubation Temperature (°C)	Adipose	Brown Fat	Diaphragm	Heart	Kidney	Liver	Small Intestine
Normothermic	7	14	52	8	12	35	16	14
	17	20	200	8	8	39	20	94
	27	90	600	66	47	117	129	367
	37	355	1,900	176	31	125	1,710	847
Hypothermic 1 hr	7	8	130	12	16	20	23	117
	27	51	530	35	36	90	55	312
	37	94	1,480	133	82	101	1,500	371
Hypothermic 12 hr	7	9	39	15	20	42	26	23
	27	45	2,525	36	19	100	53	183
	37	180	3,550	118	22	144	877	196
Hypothermic 18 hr	7	12	—	--	12	32	20	35
	17	14	340	16	35	35	82	113
	27	61	1,740	25	23	77	493	139
	37	26	5,460	125	33	152	461	236
Hypothermic 24 hr	7	18	64	6	12	23	24	40
	27	7	2,340	42	18	152	86	239
	37	198	5,140	141	20	135	342	166

1. The figures represent the average nmoles of acetate incorporated into total lipids per gram of tissue per two hour incubation. In each instance, tissue was incubated at 37° as well as at one lower temperature of 7, 17 or 27°C (ILR 69-78).

SLIDE 8

Oxidation of Acetate-2-14C to 14CO2 by Tissues from Hibernating and Normothermic Hamsters during Incubation at 7, 17, 27 and 37°C[1]

Tissue	Hibernating Hamsters				Normothermic Hamsters			
	7°C	17°C	27°C	37°C	7°C	17°C	27°C	37°C
Adipose	89.7	516	1,040	1,290	122	247	388	669
Brown Fat	1,220	5,470	5,300	7,760	475	2,740	11,100	8,220
Diaphragm	764	4,570	9,260	15,600	822	3,650	9,300	15,600
Heart	2,350	4,070	5,320	5,320	1,480	2,740	4,550	2,930
Kidney	1,140	5,240	9,440	9,440	1,390	6,080	12,600	18,100
Liver	250	1,820	2,670	3,370	223	551	1,065	2,870
Small Intestine	903	4,700	11,800	17,300	208	2,210	6,950	11,200

1. The values represent the nmoles of acetate-2-14C converted to CO2 per g wet tissue during the 2-hour incubation. The body temperature of each hibernating hamster at and during autopsy was 7°C. Body tissues taken from the other hamsters were rapidly chilled to 7° before start of incubation (ILR 93-4).

SLIDE 9

Oxidation of Acetate-2-14C to CO2 during in vitro Incubations of Tissues from
Hibernating Hamsters at 7°, 17° and 27° Relative to Oxidation at 37°C
(arbitrarily placed at 100)[1]

Tissue	Hibernating Hamsters			Normothermic Hamsters		
	7°	17°	27°	7°	17°	27°
Adipose	7	40	81	18	37	38
Brown Fat	14	70	68	6	52	135
Diaphragm	5	29	59	5	23	59
Heart	44	76	100	51	94	150
Kidney	12	56	100	11	40	90
Liver	7	45	79	9	23	45
Small Intestine	5	27	68	2	20	62

1. The values express the ratios of moles of acetate-2-14C incorporated
into CO2 at 7, 17, and 27°C to the moles of acetate-2-14C incorporated
into CO2 at 37°C (ILR 99-4).

SLIDE 10

Incorporation of Acetate-2-14C into Total Lipids by Tissues from Hibernating and Normothermic
Hamsters during Incubation at 7, 17, 27 and 37°C[1]

Tissue	Hibernating Hamsters				Normothermic Hamsters			
	7°	17°	27°	37°	7°	17°	27°	37°
Adipose	90	247	7,790	4,450	50	42	96	199
Brown Fat	54 (90)	212 (82)	891 (90)	3,940 (82)	13 (90)	39 (88)	367 (92)	747 (85)
Diaphragm	31 (91)	79 (91)	140 (85)	431 (85)	13 (94)	27 (94)	43 (88)	156 (84)
Heart	17 (91)	19 (84)	25 (81)	36 (76)	13 (87)	14 (84)	14 (75)	38 (75)
Kidney	33 (81)	66 (76)	144 (79)	193 (61)	74 (71)	69 (88)	134 (78)	188 (79)
Liver	13 (86)	24 (96)	142 (77)	1,660 (59)	7 (94)	19 (91)	71 (83)	836 (99)
Small Intestine	38 (90)	98 (99)	392 (76)	374 (31)	29 (90)	123 (98)	342 (79)	471 (76)

1. The values represent the nmoles of acetate converted to total lipid per g wet tissue during the 3-hour
incubation. The figures in brackets represent the esterified lipids present as a percentage of total
lipids. The body temperature of each hibernating hamster at end during autopsy was 7°C. Body
tissues taken from the other hamsters were rapidly chilled to 7° before start of incubations (ILR 99-4).

SLIDE 11

hamsters. The heart, to point out again, has less temperature sensitivity,
most of the time, than any of the other tissues.

Incorporation of ^{14}C into total lipids is shown on slide
that in hibernating hamsters, as in the hypothermic hamsters, the synthesis
of lipids by brown fat is superior to that in normothermic hamsters. The
amount of lipid synthesized by the heart is rather small. It doesn't change
much with the change in temperature, in fact, it changes less than any
other tissue. The small intestine doesn't change much, but after the
temperature is decreased from 37° to 27° lipid synthesis drops off
rapidly in most preparations. In slide (12) the 37° values have been
arbitrarily set to equal 100% for comparison. You can see the sharp
drop in adipose tissue synthesis and in brown fat lipid synthesis. Heart
lipid synthesis holds up pretty well as the temperature decreases. Liver
lipid synthesis decreases rather sharply as the temperature is lowered.

The synthesis of free fatty acids is shown in slide (13). We extracted
the tissue lipids and used TLC for lipid class separations. The free
fatty acids were scraped off the TLC plates and counted for radioactivity.
This slide shows that the fatty acid picture is pretty much the same as
that for total lipids. You can get some idea of the amount of activity
that has gone into free fatty acids. The liver synthesizes rather large

Synthesis of Total Lipids by Hibernating Hamster Tissues during Incubation at
7, 17 and 27° Relative to Synthesis at 37°C (arbitrarily set at 100)[1]

Tissue	Hibernating Hamsters			Normothermic Hamsters		
	7°	17°	27°	7°	17°	27°
Adipose	1	5	47	10	21	48
Brown Fat	1	5	16	4	5	123
Diaphragm	7	17	34	13	16	51
Heart	61	68	82	57	70	104
Kidney	13	31	79	15	38	86
Liver	2	2	11	3	2	9
Small Intestine	8	26	103	4	26	75

1. The values express the ratios of moles of acetate-2-14C incorporated
into total lipid at 7, 17, and 27°C to the moles of acetate-2-14C
incorporated into total lipid at 37°C (ILR 99-4).

SLIDE 12

Synthesis of Free Fatty Acids from Acetate-2-14C during Incubation of Hibernating Hamster Tissues at
7, 17, 27 and 37°C

Tissue	Hibernating							Normothermic						
	nmoles/g/2 hr[1]				% Rel. to 37°[2]			nmoles/g/2 hr				% Rel. to 37°		
	7°	17°	27°	37°	7°	17°	27°	7°	17°	27°	37°	7°	17°	27°
Brown Fat	3.5	36.3	57.2	706	1	5	8	3.1	4.7	176	135	2	3	130
Diaphragm	2.6	7.0	23.7	78.9	4	9	30	3.4	4.6	12.1	31.2	11	15	39
Heart	1.5	2.3	4.4	6.8	22	34	65	1.7	2.2	5.5	5.7	30	39	96
Kidney	2.1	12.2	42.5	70.5	3	17	60	2.1	6.8	29.0	37.7	6	18	77
Liver	1.1	1.3	38.4	603	0	0	6	1.2	1.7	14.3	336	0	1	4
Small Intestine	1.6	20.4	95.3	111	1	18	86	2.0	15.0	72.8	113	2	13	64

1. The values represent the nmoles of acetate-2-14C converted to tissue free fatty acid per g wet
weight/2 hr. The FFA were separated by thin layer chromatography (TLC).
2. The values represent the ratios of moles of acetate-2-14C incorporated into FFA at 7, 17, and
27°C to the moles of acetate-2-14C incorporated into FFA at 37°C (ILR 99-4).

SLIDE 13

PALMITIC ACID-I-^{14}C INCORPORATED INTO CO_2

—○—BROWN FAT
—●—KIDNEY
—▲—LIVER
—▲—SMALL INTESTINE

SLIDE 14

PALMITIC ACID-I-^{14}C INCORPORATED INTO CO_2

—◇—ADIPOSE
—★—DIAPHRAGM
—□—HEART
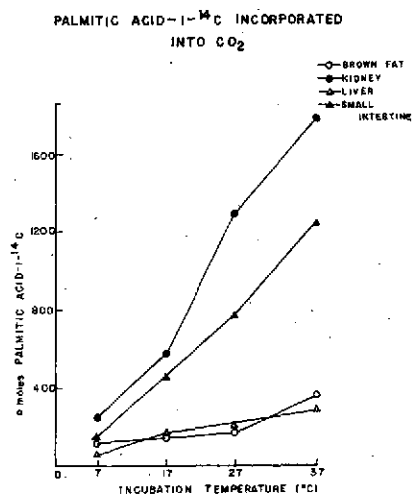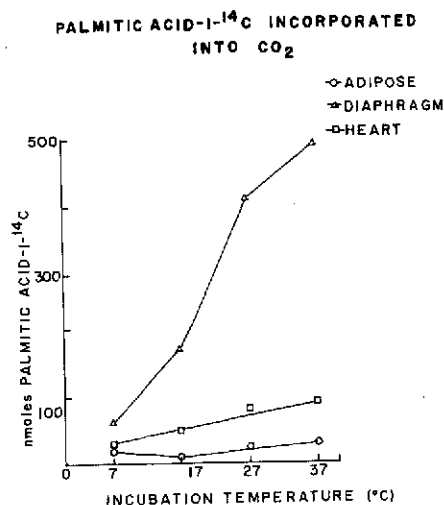
SLIDE 15

amounts at 37°C, but it seems to be a little bit temperature sensitive in the synthesis of free fatty acids, and very little is synthesized at 17°. A similar picture is seen for brown fat.

The first experiment (experiment 7) after switching from acetate was to study the metabolism of palmitic acid (slide 14). This work was on hypothermic hamsters. The kidney and small intestines have very high rates of palmitic acid oxidation at 37°, but they are rather temperature sensitive. In the liver and brown fat, the rate of oxidation of palmitic acid is not as high as in the other 2 tissues, but neither are they as sensitive to temperature.

JORDAN: Are those tissues homogenates? Is that what it was with the substrate in vivo of palmitic acid-1-^{14}C?

ENTENMAN: This is an in vitro study. The tissues were incubated with a palmitic acid complex with purified serum albumin. Tissue slices were used for liver, kidney and heart; brown fat and small intestines were minced; portions of adipose fat pads were used.

The next slide (15) shows the oxidation by the other 3 tissues. Diaphragm shows a rather sharp temperature sensitivity for fatty acid oxidation whereas adipose tissue and heart show little temperature effect.

In slide (16) the $^{14}CO_2$ values have been related to those at 37° arbitrarily set to equal 100%. At 27° most of the tissues are still oxidizing palmitic acid rather well. The activity falls off somewhat at 17° in most tissues, and it is even lower at 7°. The heart stays right in there in the oxidation of fatty acids much in the same manner as with acetate-2-^{14}C. You'll notice a little analytical problem with the adipose tissue. There were 3 animals that had more activity at 7° than at 17°. I have no real explanation for this.

PALMITIC ACID-1-^{14}C INCORPORATED
INTO CO_2

TISSUE	INCUBATION TEMPERATURE (°C)			
	7	17	27	37
Adipose	64	24	82	100
Brown Fat	27	34	47	100
Diaphragm	13	34	84	100
Heart	37	53	89	100
Kidney	14	32	73	100
Liver	21	55	75	100
Small Intestine	9	36	62	100

The values express the ratio of nmoles of palmitic acid-1-^{14}C incorporated into CO_2 at 7, 17, and 27°C to the nmoles of palmitic acid-1-^{14}C incorporated into CO_2 at 37°C.

SLIDE 16

The incorporation of fatty acid into esterified lipids is shown in slide 17. The lipids were separated on a TLC plate. All lipid-^{14}C except the free fatty acid activity were combined because in these experiments where large amounts of labeled palmitic acid were used in the incubation medium, the free fatty acids constitute most of the activity in the lipid extract. So, all lipid classes were separated on the TLC plate and esterified lipid-^{14}C values were combined. You can see that when you increase the incubation temperature from 7°, the hypothermic temperature, there is an increase in esterified lipid synthesis in the small intestine at 17° which falls down as the temperature increases further. The brown fat shows a sharp increase at 27° rather than 17°C as noted in the small intestines. The liver shows a gradual increase with the increase in temperature but not as dramatic as the increase in the small intestines or brown fat. The kidney shows little temperature sensitivity in synthesis of total esterified lipids. The next slide (18) shows synthesis of total esterified lipids in the tissues having a much lower rate of synthesis. As the temperature was increased from the hypothermic temperature to the normothermic temperature, the adipose tissue showed no effect until the temperature reached the normothermic value. The diaphragm has a different pattern but neither it nor the heart are markedly temperature sensitive.

In the next slide (opaq. 3), data on acetate-2-^{14}C utilization by tissues from the hibernating ground squirrel are presented. These data are to be compared to the previous work on the hibernating hamster or the hypothermic hamster. You can see that in the various tissues of the hibernating ground squirrel, the brown fat still synthesizes lipids pretty well at 7°. The heart is still right in there and doesn't appear to change a great deal at temperatures of 17° or above. The heart seems to do better than the brown fat. This may be an adaptation because, in the normothermic squirrel the oxidation is temperature dependent, whereas, in the hibernating ground squirrel, there is a maintenance of the capacity of the brown fat to oxidize

PALMITIC ACID-1-^{14}C INCORPORATED
INTO TOTAL ESTERIFIED LIPID
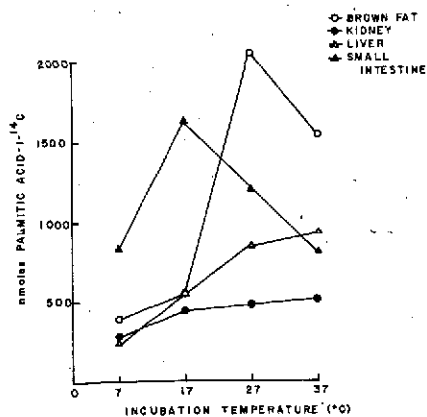
SLIDE 17

PALMITIC ACID-1-^{14}C INCORPORATED
INTO TOTAL ESTERIFIED LIPID

SLIDE 18

TABLE 1. Oxidation of Acetate-2-^{14}C to ^{14}CO$_2$ by Tissues from Hibernating, Normothermic and Awakened Ground Squirrels during Incubation at 7, 17, 27 and 37°C[1]

Tissue	Hibernating Ground Squirrels				Normothermic Ground Squirrels				Awakened Ground Squirrels	
	7°C	17°C	27°C	37°C	7°C	17°C	27°C	37°C	7°C	37°C
Adipose	59.6 ±34.7 (16)	87.9 ±8.1 (4)	172 ±52 (4)	385 ±79 (3)	52.1 ±30.1 (12)	62.5 ±15.6 (2)	212 ±98 (4)	291 ±58 (6)	38.8 ±9.8 (4)	504 ±219 (4)
Brown Fat	1459 ±677 (15)	3090 ±1790 (4)	4,740 ±590 (4)	6140 ±5420 (4)	248 ±175 (7)	718 ±185 (11)	1900 ±1080 (2)	3370 ±1460 (4)	1100 ±593 (4)	7410 ±5710 (4)
Diaphragm	932 ±174 (4)	3210 ±60 (4)	5590 ±1590 (4)	10,800 ±1300 (3)	915 ±220 (9)	3420 ±170 (4)	7010 ±2030 (2)	8680 ±2670 (4)	3030 ±88 (4)	12,400 ±1,600 (3)
Heart	2660 ±552 (8)	4630 ±80 (2)	3420 ±615 (2)	3750 ±646 (4)	2080 ±340 (6)	3420 (1)	4510 ±2290 (2)	2930 ±1260 (5)	2449 ±243 (2)	3280 ±1750 (2)
Kidney	273 ±46 (16)	1440 ±550 (4)	3590 ±940 (5)	5380 ±700 (17)	637 ±142 (12)	2680 ±60 (2)	6600 ±1270 (4)	6540 ±980 (6)	401 ±48 (5)	5,480 ±1,150 (4)
Liver	233 ±97 (16)	444 ±112 (4)	724 ±185 (5)	1650 ±500 (17)	251 ±97 (11)	489 ±41 (2)	1580 ±680 (4)	1270 ±540 (5)	259 ±66 (4)	1170 ±420 (4)
Small Intestine	431 ±91 (15)	1750 ±310 (4)	6310 ±290 (2)	11,900 ±7,300 (4)	474 ±86 (4)	2370 ±80 (2)	5650 ±2510 (4)	9670 ±2200 (6)	367 ±96 (4)	13,000 ±500 (4)

1. The values represent the nmoles of acetate-2-^{14}C converted to CO$_2$ per g wet tissue during the 2-hour incubation. The body temperature of each hibernating ground squirrel at and during autopsy was 7°C. Body tissues taken from the other ground squirrels were rapidly chilled to 7°C before start of incubations.

2. The numbers in parentheses represent the number of samples analyzed.

TABLE 2. Incorporation of Acetate-2-^{14}C into Total Lipids by Tissues from Hibernating, Normothermic and Awakened Ground Squirrels during Incubation at 7, 17, 27 and 37°C[1]

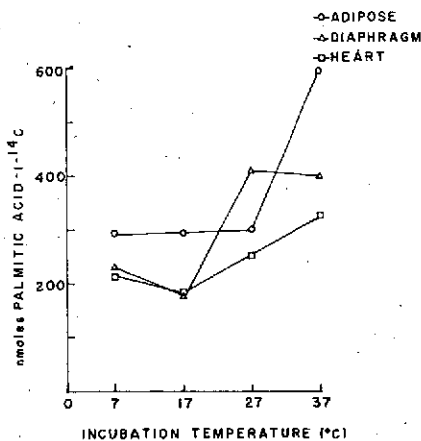| Tissue	Hibernating Ground Squirrels				Normothermic Ground Squirrels				Awakened Ground Squirrels	
	7°C	17°C	27°C	37°C	7°C	17°C	27°C	37°C	7°C	37°C
Adipose	26.4 ±7.9 (16)	32.6 ±9.3 (4)	61.2 ±3.4 (5)	131 ±82 (6)	36.3 ±11.0 (12)	60.5 ±11.0 (2)	61.3 ±40.3 (4)	93.6 ±24.8 (6)	17.2 ±3.2 (4)	158 ±70 (4)
Brown Fat	34.2 ±9.7 (15)	43.3 ±20.4 (6)	46.9 ±2.4 (4)	70.4 ±30.5 (9)	56.6 ±17.7 (10)	107 ±27 (3)	66.1 ±17.6 (4)	147 ±65 (5)	5.72 ±1.6 (4)	117 ±40.2 (6)
Diaphragm	49.2 ±11.8 (15)	48.2 ±6.0 (4)	56.7 ±7.6 (4)	89.1 ±7.2 (7)	67.6 ±18.4 (10)	69.8 ±21.3 (2)	83.6 ±6.4 (4)	114 ±24.6 (4)	27.4 ±3.5 (4)	73.8 ±5.7 (4)
Heart	28.5 ±6.4 (8)	56.5 ±5.0 (2)	39.4 ±47.9 (2)	50.9 ±10.3 (3)	50.2 ±9.8 (6)	54.4 (1)	47.4 ±17.2 (2)	59.6 ±15.6 (3)	21.7 ±9.8 (2)	42.5 ±5.7 (2)
Kidney	44.5 ±13.5 (16)	57.9 ±12.0 (4)	83.7 ±9.0 (4)	170 ±215 (7)	64.8 ±17.5 (11)	158 ±5.0 (2)	109 ±40.0 (4)	189 ±36 (6)	38.6 ±7.6 (5)	187 ±11.6 (4)
Liver	50.5 ±15.7 (19)	48.3 ±1.6 (4)	24.8 ±14.6 (5)	1255 ±270 (8)	84.5 ±39.5 (10)	131 ±8 (3)	97.7 ±9.0 (4)	1062 ±2354 (4)	47.9 ±4.2 (4)	271 ±106 (4)
Small Intestine	57.5 ±12.8 (16)	70.8 ±6.1 (4)	167 ±25 (4)	367 ±70 (12)	94.4 ±22.4 (12)	135 ±6.0 (2)	267 ±48 (4)	367 ±57 (6)	83.0 ±16.5 (4)	311 ±38 (4)

1. The values represent the nmoles of acetate-2-^{14}C converted to total lipid per g wet tissue during the 2-hour incubation. The body temperature of each hibernating ground squirrel at and during autopsy was 7°C. Body tissues taken from the other ground squirrels were rapidly chilled to 7° before start of incubations.

2. The numbers in parentheses represent the number of samples analyzed.

acetate at 7°C. In the other tissues, acetate oxidation rates are quite comparable to that in the hibernating and hypothermic hamster tissues. Thus, it is seen that hibernating and normothermic animals differ only in the brown fat and possibly the heart. Incorporation of acetate-2-^{14}C into total lipid-^{14}C by tissues of hibernating ground squirrels are shown in the next slide (opaq. 4). There is not a great deal of difference in these tissues. I've underlined each one that I feel shows some significance. In the heart, you get rather insensitive responses to the temperature change in both the hibernating and normothermic squirrels. The effect of temperature on liver is rather marked, but comparable in the hibernating and normothermic ground squirrels.

Data on the esterified lipids are given next (opaq. 5). The values do not change in the hibernating animal. Temperature sensitivity was not observed in either the hibernating or normothermic animal. There is no adaptation in the synthesis of lipids by hibernating ground squirrels. The next slide (opaq. 6) shows you ^{14}C activity distribution between the nonsaponified, saponified, and the aqueous fractions in adipose tissue lipids. I consider the latter to be glycerol. Although there is a fair amount of activity in the glycerol portion the proportions at 37 and 7°C are not greatly different. You may recall that in the hamster there was an increase proportion of activity in glycerol relative to that in the saponifiable and nonsaponifiable fractions. In the liver (opaq. 7) the proportion of ^{14}C activity in both the sterol fraction and the fatty acid fraction is lower at 7 than at 37°. It seems as though glycerol synthesis is affected less at the lower temperatures than is the synthesis of sterols or fatty acids.

TABLE 3. Newly Synthesized Esterified Lipid as a Percentage of Newly Synthesized Total Lipid in Ground Squirrel Tissues following Incubation with Acetate-2-^{14}C at 7, 17, 27 and 37°C[1]

Tissue	Hibernating				Normothermic				Awakened	
	7	17	27	37	7	17	27	37	7	37
Brown Fat	90	68	42	66	95	93	83	--[2]	90	90
Diaphragm	89	92	88	84	41	89	87	92	84	77
Heart	91	92	66	80	94	94	86	88	87	81
Kidney	91	94	72	27	96	90	95	90	93	83
Liver	90	96	93	71	94	88	87	95	91	85
Small Intestine	94	77	80	71	96	72	80	89	93	77

1. The values represent the ratio nmoles newly synthesized esterified lipid/nmoles newly synthesized total lipid produced during incubation for 2 hours with acetate-2-^{14}C. Total lipid values were taken from Table 2. Esterified lipid was calculated as TL minus FFA (Table 5).

2. The TG and FFA were not separated on the TLC plate.

TABLE 4. ADIPOSE TISSUE: Distribution of ^{14}C Activity in Nonsaponifiable, Saponifiable and Aqueous Fractions

Physiological Condition	Incubation Temperature	CPM in Aliquot Saponified	Nonsaponifiable		Saponifiable		Aqueous	
			% of Total CPM	nM/g/ 2 hr Incub.	% of Total CPM	nM/g/ 2 hr Incub.	% of Total CPM	nM/g/ 2 hr Incub.
Awakened Squirrels	7°	1,410	42.5	34.0	21.2	6.20	51.2	
	37°	11,200	1.5	2.59	25.6	6.77	72.6	94.1
Hibernating Squirrels	7°	1,500	7.4	1.96	40.6	46.2	52.0	15.4
	17°	3,552	3.4	1.41	30.7	11.7	65.8	15.0
	27°	3,452	2.1	1.12	19.8	10.6	78.1	41.6
	37°	7,580	1.8	2.59	33.0	19.8	65.7	91.7
Normothermic Squirrels	7°	1,850	4.5	1.23	39.8	10.8	55.7	15.1
	17°	4,762	2.0	1.36	22.7	15.0	75.8	47.4
	27°	4,690	2.5	1.41	17.6	10.0	80.0	45.6
	37°	6,770	2.4	2.56	20.9	22.6	76.8	83.7

TABLE 5. LIVER: Distribution of ^{14}C Activity in Nonsaponifiable, Saponifiable and Aqueous Fractions

Physiological Condition	Incubation Temperature	CPM in Aliquot Saponified	Nonsaponifiable % of Total CPM	Nonsaponifiable mM/g/ 2 hr Incub.	Saponifiable % of Total CPM	Saponifiable mM/g/ 2 hr Incub.	Aqueous % of Total CPM	Aqueous mM/g/ 2 hr Incub.
Awakened Squirrels	7°	4,024	2.3	1.09	19.2	9.17	78.5	37.5
	37°	21,632	24.4	20.2	42.4	122	33.1	95.1
Hibernating Squirrels	7°	3,822	2.7	1.53	18.0	10.7	78.2	44.1
	17°	6,382	1.1	0.45	18.6	7.87	80.3	33.0
	22°	6,060	13.2	10.0	14.8	11.55	72.0	54.6
	37°	79,964	9.1	112	79.9	980	11.0	155
Normothermic Squirrels	7°	6,586	1.5	0.95	11.2	7.29	87.3	56.6
	17°	7,218	10.4	18.7	21.6	39.0	68.0	133
	22°	10,212	1.4	3.08	15.9	14.8	79.7	74.2
	37°	77,652	7.4	69.4	75.6	707	17.0	150

OPAQ. 7

TABLE 6. ^{14}C Distribution in an Unknown Taken From the Thin Layer Chromatogram

Sample	Total CPM	Nonsaponifiable CPM	Saponifiable CPM	Saponifiable % of Total CPM	Aqueous CPM
MG	782	6	82	10.5	695
Unk	556	0	65	11.7	491
1,2-DG	394	2	97	24.6	295

A sample of normothermic 7° liver was separated on TLC, the MG, Unknown (Unk) and 1,2 DG sterol lipid classes were scraped off the plate and to each scraping were added 2 mg of nonradioactive cholesterol and trilinolein standards were added as carrier. Each were then saponified and fractionated. The values given are the distribution of radioactivity in the fractions.

OPAQ. 8

In this study, as in the hibernating ground squirrel, an unknown spot was detected between the monoglyceride spot and the sterol fraction. In the sterol fraction, by the way, in all my TLC work, I cannot separate out the 1,2-diglyceride from the sterols. So, we took the monoglyceride spot and the unknown spot and the 1,3-diglyceride, and prepared three fractions (saponifiable, nonsaponifiable, aqueous). We found considerable activity in the glycerol portion of the saponified monoglyceride spot and very little in the fatty acids (opaq. 8). The same is true with the unknown and with the glyceride. In other words, the activity from acetate in the mono-glyceride, the diglyceride, and the unknown fraction is in what we call the glycerol portion. The data in the next slide (opaq. 9) shows the synthesis of the unknown material by various tissues as related to the incubation temperature in the hibernation and normothermic ground squirrel. As you can see, in contrast to the other lipid spots, very little influence of temperature is noted on the amounts of the unknown material that is formed. I have no idea what the unknown material is.

The next slide (opaq. 10) shows a comparison of the acetate experiments on hamsters and ground squirrels. The experiments shown here refer to the oxidation or conversion of acetate to CO_2 in vitro. You can see that brown fat is quite active in acetate oxidation in the hibernating hamster, and quite probably in the hypothermic hamster and the hibernating ground squirrel. In the normothermic ground squirrel, it is quite low and the normothermic hamster is low. So, you get a tendency for maintenance of oxidation even at 7°C in the brown fat of the hypothermic or hibernating animal. The diaphragm has a temperature sensitivity. You don't get much difference in the oxidation of acetate by heart tissue from different animal preparations. In the kidney, you get some temperature effect, more so in the ground squirrel than in the hamster. The liver showed a temperature sensitivity, but the animal preparation did not differ markedly. The small intestine shows

TABLE 7. Synthesis of Compound Present in the Unknown Spot on the TLC Chromatogram[1]

Tissue	Hibernating Ground Squirrels 7°	17°	27°	37°	Normothermic Ground Squirrels 7°	17°	27°	37°	Awakened Ground Squirrels 7°	37°
Brown Fat	6.3	5.0	8.6	5.1	15.1	14.9	9.9	17.8	8.3	9.4
Diaphragm	9.5	30.5	17.0	17.6	15.6	12.7	15.4	14.6	6.4	9.4
Heart	7.3	7.8	7.2	8.0	12.4	11.5	10.5	9.4	7.0	8.1
Kidney	8.6	9.9	5.9	11.3	9.8	17.1	8.8	8.5	5.9	9.1
Liver	15.2	8.5	7.0	20.4	16.5	19.4	10.4	21.8	31.5	18.7
Small Intestine	10.0	20.4	9.4	13.2	16.4	12.2	12.1	13.4	17.8	15.8

1. The values are the nM unknown synthesized per gram tissue per 2 hours of incubation with acetate-2-^{14}C.

OPAQ. 9

Oxidation of Acetate-2-^{14}C by Hamsters and Ground Squirrels During in Vitro Incubations

Physiological State	Tissue	mμoles acetate converted to CO2/g/2 hr			
		7°	17°	27°	37°
Normothermic Hamster	Adipose	150	300	620	599
Hypothermic Hamster (18 hr)		12	160	290	190
Hibernating Hamster		98	516	1,040	1,299
Hibernating Ground Squirrel		60	59	172	383
Normothermic Ground Squirrel		52	63	217	291
Normothermic Hamster	Brown Fat	900	2,530	4,130	6,450
Hypothermic Hamster (18 hr)		2,450	4,350	7,490	7,780
Hibernating Hamster		1,220	5,470	5,960	7,760
Hibernating Ground Squirrel		1,459	1,990	4,240	6,140
Normothermic Ground Squirrel		248	718	1,900	1,370
Normothermic Hamster	Diaphragm	1,160	5,190	12,290	14,200
Hypothermic Hamster (18 hr)		768	4,840	6,350	11,200
Hibernating Hamster		984	4,570	9,260	15,600
Hibernating Ground Squirrel		932	1,210	2,390	10,800
Normothermic Ground Squirrel		915	1,420	7,016	6,600
Normothermic Hamster	Heart	2,170	6,990	13,080	13,600
Hypothermic Hamster (18 hr)		2,400	6,400	10,100	15,100
Hibernating Hamster		2,350	4,070	5,320	5,320
Hibernating Ground Squirrel		2,040	4,430	3,420	3,250
Normothermic Ground Squirrel		2,080	5,420	4,510	2,930
Normothermic Hamster	Kidney	1,260	6,080	16,900	13,200
Hypothermic Hamster (18 hr)		3,330	12,100	16,700	19,900
Hibernating Hamster		1,140	5,240	9,440	9,440
Hibernating Ground Squirrel		273	1,440	3,390	5,380
Normothermic Ground Squirrel		637	2,580	6,600	6,545
Normothermic Hamster	Liver	130	200	1,040	1,470
Hypothermic Hamster (18 hr)		370	840	1,020	980
Hibernating Ground Squirrel		350	1,520	2,670	1,120
Hibernating Ground Squirrel		233	444	724	1,630
Normothermic Ground Squirrel		264	889	1,250	1,270
Normothermic Hamster	Small Intestine	360	1,850	7,020	7,720
Hypothermic Hamster (18 hr)		394	1,970	10,260	11,500
Hibernating Hamster		983	4,700	11,800	17,500
Hibernating Ground Squirrel		411	1,740	6,210	11,200
Normothermic Ground Squirrel		674	2,370	5,650	9,930

OPAQ. 10

Total Lipid Synthesis in Tissues of Hamsters and Ground Squirrels Following Incubation with Acetate-2-[14]C

Physiological State	Tissue	mμoles acetate converted to TL/g/2 hr			
		7°	17°	27°	37°
Normothermic Hamster	Adipose	12	20	40	355
Hypothermic Hamster (18 hr)		12	16	61	28
Hibernating Hamster		20	247	2,290	4,930
Hibernating Ground Squirrel		26	53	61	131
Normothermic Ground Squirrel		34	60	61	94
Normothermic Hamster	Brown Fat	52	200	600	1,900
Hypothermic Hamster (18 hr)		—	360	1,240	5,460
Hibernating Hamster		36	212	391	5,940
Hibernating Ground Squirrel		34	63	46	31
Normothermic Ground Squirrel		57	107	56	147
Normothermic Hamster	Diaphragm	8	6	44	170
Hypothermic Hamster (18 hr)		—	16	23	125
Hibernating Hamster		11	79	180	421
Hibernating Ground Squirrel		40	48	56	90
Normothermic Ground Squirrel		68	70	84	176
Normothermic Hamster	Heart	12	8	47	31
Hypothermic Hamster (18 hr)		12	15	23	33
Hibernating Hamster		17	19	23	28
Hibernating Ground Squirrel		18	19	59	60
Normothermic Ground Squirrel		50	16	47	60
Normothermic Hamster	Kidney	35	39	117	125
Hypothermic Hamster (18 hr)		32	34	77	151
Hibernating Ground Squirrel		23	58	144	182
Hibernating Ground Squirrel		64	58	84	170
Normothermic Ground Squirrel		99	158	104	189
Normothermic Hamster	Liver	16	20	329	1,730
Hypothermic Hamster (18 hr)		26	52	493	461
Hibernating Hamster		28	44	162	1,450
Hibernating Ground Squirrel		50	44	75	1,215
Normothermic Ground Squirrel		84	137	98	1,962
Normothermic Hamster	Small Intestine	14	94	367	347
Hypothermic Hamster (18 hr)		15	111	139	236
Hibernating Hamster		30	98	392	374
Hibernating Ground Squirrel		53	71	161	272
Normothermic Ground Squirrel		48	121	161	367

OPAQ. 11

nothing of special interest except in that level of oxidation was relatively high at most temperatures. The interesting thing that I see here is the adaptation of brown fat to acetate oxidation and a decrease in temperature sensitivity with reference to acetate oxidation.

The next slide (opaq. 11) shows a comparison between the hamster and ground squirrel, the normothermic and the hibernating animal, as far as total lipid synthesis is concerned. In all tissues, except brown fat and liver, at normothermic temperatures, you get rather low lipid synthesis. At hypothermic temperatures, lipid synthesis was markedly decreased. The hearts seems to have the least temperature sensitivity in regard to lipid synthesis.

In the next experiment (opaq. 12), which was done at Berkeley, we studied the oxidation of palmitic acid and the synthesis of lipids from palmitic acid in rat liver slices. The data shows considerable activity in the total lipids - but this is primarily free fatty acids. At the lower temperatures, a marked decrease in the oxidation of palmitic acid occurs. This is in agreement with data on livers of ground squirrels and hamsters. The incorporation of palmitic acid into lipids was mainly into the triglyceride fraction as separated by thin layer chromatography (opaq. 13). When the purified lipids were separated into the saponified, and non-saponified, and aqueous phases, very little ^{14}C activity was found in the aqueous phase or the sterol fraction (opaq. 14). Most of the lipid-^{14}C was present in the saponifiable fraction (fatty acids). Appreciable amounts polyunsaturated fatty acids were not detected. Very little esterification of palmitic acid was noted. In slide (19) data is presented on the oxidation of palmitic acid-1-^{14}C by tissues from hibernating and normothermic ground squirrels. There doesn't appear to be any particular differences between the tissues from the normothermic and those from the hibernating animals. The brown fat from hibernating animals does oxidize the palmitic acid

TABLE 8. INCORPORATION OF PALMITIC ACID INTO TOTAL LIPIDS AND CARBON DIOXIDE DURING INCUBATION OF RAT LIVER SLICES AT 7, 17 AND 37°C[1]

Incubation Temperature (°C)	No. of Rats	Conversion of palmitic acid to[2]	
		Total Lipid	CO2
7°	9	2563 ± 180	22 ± 12
17°	9	2488 ± 133	75 ± 15
37°	9	2560 ± 258	198 ± 38

1. At sacrifice, the body temperature of the rats was 37°. The incubation period was 2 hours.

2. The data represent the mμoles of palmitic acid-1-^{14}C converted into total lipid (or [14]CO2) per g tissue ± 1 standard deviation.

OPAQ. 12

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TABLE 9. INCORPORATION OF PALMITIC ACID INTO LIPIDS IN RAT LIVER SLICES DURING INCUBATION AT 7, 17 AND 37°C[1]

Incubation Temperature (°C)	No. of Rats	nmoles of Palmitic Acid Converted to[2]					
		FFA[3]	MC	1,3 DG	1,2 DG	TG	PL
7'	9	2253	10	20	16	97	93
17'	9	2045	10	20	55	248	80
37'	9	1643	9	26	42	359	160

1. At sacrifice, the body temperature of the rats was 37°

2. The data represent the nmoles of lipid class formed from palmitic acid-1-14C per g tissue during the 2-hour incubation.

3. The following abbreviations were used: FFA (free fatty acid), MC (monoglyceride), 1,3 DG (1,3-diglyceride), 1,2 DG (1,2-diglyceride), TG (triglyceride), PL (phospholipid).

TABLE 10. CONVERSION OF PALMITIC ACID TO SAPONIFIABLE AND NONSAPONIFIABLE LIPIDS BY RAT LIVER SLICES

Incubation Temperature (°C)	Aqueous Phase	Nonsaponifiable Lipids	Saponifiable Lipids[1]			
			Total	Saturates	Monounsaturates	Polyunsaturates
7	1A	135	2563	2430	77	56
17	35	127	2485	2363	70	52
37	33	137	2363	2258	61	44

1. The data represent the nmoles palmitic acid-1-14C converted to the various components per g wet weight of tissue.

OPAQ. 13

Oxidation of Palmitic Acid-1-14C to $^{14}CO_2$ by Tissues from Hibernating and Normothermic Ground Squirrels during Incubation at 7, 17, 27 and 37°C[1]

Tissue	Hibernating Ground Squirrels				Normothermic Ground Squirrels		
	7°C	17°C	27°C	37°C	7°C	27°C	37°C
Adipose	8.2 ±1.4	3.9 ±1.5	7.0 ±0.9	15.8 ±5.1	6.0 ±1.8	4.8 ±0.5	12 ±1
Brown Fat	280 ±40	84 ±27	100 ±50	210 ±80	33 ±1	35 ±5	74 ±29
Diaphragm	120 ±10	100 ±20	170 ±25	280 ±80	140 ±10	140 ±20	210 ±30
Heart	87 ±19	69 ±8	135 ±20	125 ±30	57	74 ±21	80 ±30
Kidney	240 ±10	180 ±30	270 ±20	540 ±100	510 ±10	310 ±70	630 ±120
Liver	81 ±12	52 ±13	66 ±11	120 ±30	85 ±1	72 ±35	150 ±60
Small Intestine	490 ±80	260 ±10	550 ±40	720 ±60	170 ±10	340 ±100	450 ±270

1. The values represent the nmoles of Palmitic Acid-1-14C converted to CO_2 per g wet tissue during the 2-hour incubation. The body temperature of each hibernating ground squirrel at and during autopsy was 7°C. Body tissues taken from the other ground squirrels were rapidly chilled to 7° before start of incubations (ILR 100-4, 58).

SLIDE 19

OPAQ. 14

Oxidation of Palmitic Acid-1-14C to CO2 during In Vitro Incubations of Ground Squirrel Tissue at 7°, 17° and 27° Relative to Oxidation at 37°C (arbitrarily placed at 100)[1]

Tissue	Hibernating Ground Squirrels			Normothermic Ground Squirrels	
	7°C	17°C	27°C	7°C	27°C
Adipose	51	25	44	49	35
Brown Fat	132	40	49	45	47
Diaphragm	58	41	72	67	67
Heart	69	55	108	71	92
Kidney	44	34	51	49	48
Liver	26	48	54	57	49
Small Intestine	68	35	76	37	75

1. The values express the ratios of nmoles Palmitic Acid-1-14C incorporated into CO2 at 7, 17, and 27°C to the nmoles of Palmitic Acid-1-14C incorporated into CO2 at 37° (ILR 100-4, 58).

SLIDE 20

rather well at all temperatures. The small intestine of the hibernating animal, in this case, was doing rather well even at the low temperatures. The adipose tissue usually doesn't do very much, but that's not surprising because the hibernating animal is supposed to be liberating fat rather than storing it.

The next slide (20) shows the customary way I've been comparing values by setting the 37° values equal to 100% and setting the other values relative to it. The brown fat oxidizes fatty acids very well. Some of the other tissues change but little. For instance, the heart does all right at 27° and is still doing rather well at 7°C. By a quick comparison of the values at 7° and 27°, you can see that there is little difference in the metabolism of palmitic acid by tissues from the hibernating or normothermic from the ground squirrel. The incorporation of palmitic-^{14}C into esterified lipids is shown in slide (21). The synthesis of esterified lipid is not altered to any great extent by temperature in the hibernating or the non-hibernating animal. There is nothing remarkable about the amounts of esterified lipids of the normothermic as compared to the hibernating animal. Slide (22) shows a comparison of the incorporation of palmitic acid into esterfied lipids and CO_2. It is clearly demonstrated that in the hibernating and normothermic animal more palmitic acid is esterfied than is oxidized. Thus, it implies that an animal in hypothermia, although presumably mobilizing fat, is still also reesterifying a fair amount of fatty acids.

The work that I have reported so far, has dealt with the oxidation of acetate which, when activated, would go into the formation of acetyl CoA and with the utilization of fatty acid, palmitic acid, which, when oxidized

Incorporation of Palmitic Acid-1-14C into Esterified Lipids by Tissues from Hibernating and Normothermic Ground Squirrels during Incubation at 7, 17, 27 and 37°C[1]

Tissue	Hibernating Ground Squirrels				Normothermic Ground Squirrels		
	7°C	17°C	27°C	37°C	7°C	27°C	37°C
Adipose	190	120	190	240	290	280	240
Brown Fat	170	240	220	200	470	150	270
Diaphragm	180	140	200	130	240	180	280
Heart	100	72	130	130	100	90	100
Kidney	200	150	110	200	280	180	250
Liver	980	830	870	890	430	390	550
Small Intestine	930	810	840	860	490	600	520

1. The values represent the μmoles of Palmitic Acid-1-14C converted to esterified lipid per g wet tissue during the 2-hour incubation. The body temperature of each hibernating ground squirrel at and during autopsy was 7°C. Body tissues taken from the other ground squirrels were rapidly chilled to 7° before start of incubations (ILR 100-4, 46, 72).

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Incorporation of Palmitic Acid-1-14C into Esterified Lipids and 14CO2 by Tissues from Hibernating and Normothermic Ground Squirrels during Incubation at 7, 17, 27 and 37°C[1]

Tissue	Esterified Lipids							14CO2						
	Hibernating				Normothermic			Hibernating				Normothermic		
	7°	17°	27°	37°	7°	27°	37°	7°	17°	27°	37°	7°	27°	37°
Adipose Tissue	190	120	190	240	290	280	240	8.2	3.9	7.0	16	6.0	4.3	12
Brown Fat	170	240	220	200	470	150	270	280	84	100	210	33	35	74
Diaphragm	180	140	200	130	240	180	280	120	100	170	230	140	140	210
Heart	100	72	130	130	100	90	100	87	69	185	125	57	74	80
Kidney	200	150	110	200	280	180	250	240	180	270	540	310	310	630
Liver	980	830	870	890	430	390	550	31	52	66	120	83	72	150
Small Intestine	930	810	840	860	490	600	520	490	260	550	720	170	340	450

1. The values represent the μmoles of Palmitic Acid-1-14C converted to esterified lipids per g wet tissue during the 2-hour incubation. The body temperature of each hibernating ground squirrel at and during autopsy was 7°C. Body tissues taken from the other ground squirrels were rapidly chilled to 7°C before the start of incubation (ILR 100-4).
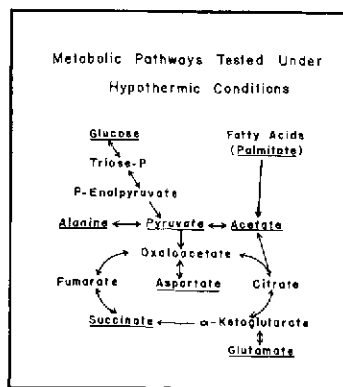
SLIDE 22

and metabolized through the TCA cycle, also contributes to CoA. Now, I am going to report on some preliminary experiments with other substrates (opaq. 15). We wanted to test the entrance of glutamic acid into the TCA cycle via the conversion to alpha-ketoglutarate. We wanted to test the metabolism of succinate as the example of a compound already in the cycle. We wanted to test the incorporation of alanine into pyruvate and then pyruvate into the cycle and conversion to acetyl CoA. We wanted to test glucose coming down through this portion of the glycolytic pathway. So, using the hypothermic hamster, we incubated tissues with various radioactive substrates which will be shown in the next two slides, beginning with opaq. 16. These results are very preliminary. I didn't use the right compound in all cases, but it will give you some appreciation of the activity. The succinic acid was labeled in the 2 and 3 position and incubated with hypothermic or normothermic hamster tissues for 2 hours. This slide pertains only to the oxidation. You can see that the oxidation of succinate doesn't proceed too well in some tissues; in other tissues, such as the kidney and small intestine, will oxidize succinate very well at 37°C. Whether the hamster is normothermic or hypothermic appears to make little difference in the oxidation of succinic acid. Oxidation of pyruvate by the kidney and the liver proceeds very well in both the normothermic and hypothermic animal. In this instance, it appears that pyruvate is better oxidized by the small intestine in the hypothermic animal than that in the normothermic animal. The diaphragm and the brown fat do a pretty good job. The liver is on the lower side at the lower temperature. The D-glucose-universally labeled-14C is not utilized to a very great extent by adipose tissue; the kidney at the lower

Metabolic Pathways Tested Under Hypothermic Conditions

OPAQ. 15

TABLE 11

Oxidation of Radioactive Substances by Hypothermic Tissues In Vitro

(The values are the μmoles converted per g tissue/2 hours.)

Labelled Substrate	Tissue	Hypothermic Hamsters		Normothermic Hamsters	
		7°	37°	7°	37°
Succinic 2,3-14C	Adipose	115	89b	126	1,188
	Brown Fat	1,340	5,662	1,044	6,468
	Diaphragm	776	8,470	328	12,382
	Heart	1,034	5,050	598	2,378
	Kidney	2,006	24,820	2,176	26,300
	Liver	864	9,140	34	11,498
	Small Intestine	768	30,560	304	29,000
Pyruvate-2-14C	Adipose	470	3,758	570	5,056
	Brown Fat	9,712	52,180	14,188	60,986
	Diaphragm	10,460	68,800	5,904	61,152
	Heart	13,464	34,760	17,216	29,794
	Kidney	8,972	205,200	10,544	267,400
	Liver	3,326	58,920	2,152	84,940
	Small Intestine	3,768	348,400	1,290	50,060
D-Glucose-U-14C	Adipose	158	650	144	352
	Brown Fat	732	12,568	292	5,044
	Diaphragm	2,474	17,006	258	10,808
	Heart	812	5,920	278	1,760
	Kidney	356	36,610	298	23,980
	Liver	330	4,936	336	7,594
	Small Intestine	776	28,440	252	16,718

OPAQ. 16

temperature doesn't utilize glucose but at normothermic temperatures, it is equivalent to diaphragm and brown fat. The liver is less. So compared to kidney, brown fat and small intestines, it doesn't utilize glucose very well. You get some differences between tissues of hypothermic and normothermic hamster in the utilization of glucose. Hypothermic tissues incubated at normothermic temperature oxidizes glucose well. The hypothermic diaphragm is no better than the normothermic, the kidney is about the same, the liver is about 4,900 as compared to 7,600 so it doesn't oxidize it as well at normothermic temperatures, but it's the same 336 vs 330 at low temperatures, and the adipose tissue is not doing so well. In the next slide (opaq. 17) the metabolism of alanine, aspartic acid and glutamic acid is shown. Alanine is converted to pyruvate, the aspartic goes to oxaloacetate, and the glutamate will go to alpha-ketoglutarate. The alanine is oxidized all right as it apparently is converted to pyruvate prior to going through the TCA cycle at normothermic temperatures, but at 7°C you get rather low metabolism of alanine to ^{14}C. At the low temperatures, the kidney and brown fat are the best with alanine.

JORDAN: Is this CO_2 measurement?

ENTENMAN: All data is on the formation of $^{14}CO_2$.   I haven't analyzed the tissues for lipid synthesis.

For the glutamate, you can see that at normothermic temperatures, in all the tissues, you get good utilization. In fact, the utilization and oxidation of glutamic acid is better than for any other substrate I'm reporting on. Although there is a temperature effect, you still get good oxidation of glutamic acid even in adipose tissue at the low temperature. There is not too much temperature sensitivity in the heart. Thus, the tissues seem to be able to oxidize glutamate better than any other substrate. I think I have one more slide (opaq. 18). This is a comparison of the different tissues in the normothermic and hypothermic animals with regard to substrate oxidation. The data are expressed as the percent oxidized at 7° vs at 37°. A remarkable agreement exists between the nor-

TABLE 12

Oxidation of labelled Amino Acids by Hypothermic Hamster Tissues In Vitro

(The values are the umoles converted per g tissue/2 hours.)

Labelled Substrate	Tissue	Hypothermic Hamsters		Normothermic Hamsters	
		7°	37°	7°	37°
L-Alanine-1-^{14}C	Adipose	242	1,002	366	1,248
	Brown Fat	1,532	12,038	886	7,522
	Diaphragm	--	9,650	1,026	10,796
	Heart	566	2,100	608	7,656
	Kidney	1,580	43,210	2,332	56,160
	Liver	660	32,840	368	30,260
	Small Intestine	764	17,992	436	10,398
L-Aspartic-U-^{14}C	Adipose	670	2,452	528	1,036
	Brown Fat	2,956	9,112	3,646	10,676
	Diaphragm	5,062	22,160	5,618	22,522
	Heart	7,064	14,286	7,116	16,998
	Kidney	5,202	141,440	8,058	145,580
	Liver	3,796	90,860	3,496	43,740
	Small Intestine	2,696	133,120	1,642	71,440
L-Glutamic-U-^{14}C	Adipose	8,004	10,172	6,854	11,860
	Brown Fat	7,648	14,376	4,778	14,958
	Diaphragm	14,396	25,260	14,718	28,560
	Heart	13,580	17,202	12,514	18,006
	Kidney	11,394	65,160	14,220	103,200
	Liver	8,396	31,588	8,216	29,360
	Small Intestine	8,476	66,560	5,792	41,400

OPAQ. 17

TABLE 13.   Incorporation of Substrate into $^{14}CO_2$ at 7° Relative to 37° for Normothermic and Hypothermic Hamster Tissues

Tissue	L-Alanine-1-^{14}C		L-Aspartic-U-^{14}C		L-Glutamic-U-^{14}C		Succinic-2,3-^{14}C		Pyruvic-1-^{14}C		D-Glucose-U-^{14}C	
	N	H	N	H	N	H	N	H	N	H	N	H
Adipose	28	35	17	19	62	25	11	13	13	16	24	55
Brown Fat	12	13	25	32	65	54	23	25	23	19	6	6
Diaphragm	10	--	25	23	52	57	3	3	10	15	2	16
Heart	17	19	42	69	70	79	25	26	61	46	16	16
Kidney	6	6	5	4	16	16	9	8	4	5	1	2
Liver	2	2	7	7	28	37	9	6	1	7	4	7
Small Intestine	6	6	2	2	14	19	3	3	3	1	2	1

OPAQ. 18

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mothermic and the hypothermic tissue's ability to oxidize the various substrates that we used. There are some exceptions as you see. This is just some preliminary work that I have done on the oxidation of these various intermediates. A lot of interpretation remains to be done to assess the value of the results. The substrates chosen were perhaps not the best. All of the substrates should have been of the uniformly labeled series.

In addition to the effect of temperature on intermediary metabolism, we're also carrying out studies at Berkeley using materials which enter the metabolic pathways at various points. For example, we will incubate a tissue with uniformly labeled radioactive glucose and include in the incubation mixture unlabeled fructose so that you can see if the nonlabeled fructose causes a dilution of the effect of the glucose. We are using this technique with various other compounds such as acetate, fructose, pyruvate, succinate, etc. We are trying to determine where there may be defects in the pathway of the metabolism of these compounds as the incubation temperature is lowered.

In summary, hypothermic hamsters seem biochemically to be in pretty good shape for up to 24 hours under the conditions of induced hypothermia used.

Oxidative metabolism seems to be dominant in the depressed metabolic state although synthetic reactions such as fat synthesis proceed in some cases at a faster rate than normothermic metabolism for the same tissues.

Fats from various tissues, notably adipose, seem to be the primary source of fuel during hypothermia and hibernation.

Fat oxidation provides material for energy and heat and the formation of ketone bodies which may be of great importance to such tissues as the brain in the depressed metabolic state.

Fat synthesis, mainly triglycerides, proceeds in all tissues studied in the depressed metabolic state. Brown fat and liver are especially active. Fat synthesis may be important in replacing endogenous materials lost from a given tissue for various functions related to the animal's survival.

Our studies have shown that at 7° temperatures, the TCA cycle is operative; acetate and fatty acids can be activated and glucose is poorly utilized.

Glycerol synthesis is relatively increased at low incubation temperatures. This reflects itself into an increased proportion of activity from acetate-^{14}C appearing in the glycero-lipids such that at 7°, most, if not all the ^{14}C activity is present in the glycerol moiety.

Enzymes for the synthesis of cholesterol seem to be more temperature sensitive (cut-out between 27-17°) than enzymes for fatty acid synthesis (cut-out between 17-7°).

Of the fatty acids synthesized, a greater proportion is present as monoenoic acids and polyenoic acids in hypothermia than in normothermia.

An unknown spot has been detected on TLC chromatograms that is not a glyceride but contains a high proportion of a water soluble component following saponification of the spot.

In rat liver slices incubated at 37°, 27°, 17°, and 7°:

Insulin at 37° and 27° increases glucose oxidation, conversion to lipid-glycerol and esterified fatty acids; at 17°, insulin still affects oxidation of glucose but no effect on fatty acid or glycerol synthesis; at 7° insulin does not affect oxidation, or fatty acid synthesis or glycerol synthesis.

Epinephrine at 37° and 27° enhanced oxidation, fat synthesis and glycerol synthesis; at 17° it enhances oxidation; at 7° it has no effect on oxidation, fat or glycerol.

There were no great differences noted in metabolism as defined by these studies between hypothermic and hibernating animals.

Incubation of normothermic tissues at various temperatures gives results which, in most cases, compare favorably to results obtained on hypothermic or hibernating tissues incubated the same various temperatures.

DISCUSSION

MUSACCHIA: Cecil, there is one point that keeps coming out that pertains to brown fat and Barbara had a question but she didn't want to interrupt you.

HORWITZ: In your hibernating ground squirrel, I saw something that seemed a little strange--the rate of oxidation of palmitate was greater at 7°C than at 37°C in the brown fat. What happened to the Q_{10} effect?

ENTENMAN: I don't know but, if you look at the data that I gave, the Q_{10} doesn't hold all the time in these experiments.

HORWITZ: You had the 37° rate as 100% and then at 27° and 17° it's about 50%, and then at 7° it was 132%.

ENTENMAN: You're talking about the brown fat in the hibernating animal. Remember that I prefaced my remarks on that by saying that these were very preliminary data, I don't have enough animals yet, and the results could be due to experimental variations.

JORDAN: Would you explain the protocol again? You took the animals and sacrificed them at the indicated temperature. Was the tissue also incubated at that same temperature?

MUSACCHIA: You can do a number of things. You can incubate it at that temperature, you can incubate it at 17° or at 27° but always with a matched sample going in at 37°C.

JORDAN: In most of these studies it doesn't indicate, for example the results of sacrificing the animal at 37° and incubating the tissues at 7°, 17°, etc.

OYAMA: Those are the normals.

SAUNDERS: They've never been made hypothermic, have they?

MUSACCHIA: Oh no, they're virgins, they're naive.

ENTENMAN: The controls for the hypothermic experiments were fasted beginning at the same time as induction of hypothermia was begun in their pair.

JORDAN: In the tables where you expressed the 37° animal at 100% was that on the basis of a 37° animal incubated at 37°?

ENTENMAN: The values were normalized for both hypothermic and normothermic tissues.

JORDAN: Do you think your glycerol incorporation data is really gluconeogenesis?

ENTENMAN: I think that we may find a greater synthesis of alpha-glycerolphosphate.

JORDAN: I just wonder that, as a tipoff, if the incorporation into carbohydrate in general as you measured it in glycogen for example would have been equally sky high?

SAUNDERS: Or is, Pat, the glycerol synthesized as a cryoprotective mechanism?

JORDAN: I don't know, but I'm wondering if it's just the result of carbohydrate synthesis in general.

ENTENMAN: I don't know, but there are experiments and reports for insects, particularly, that show an increase in glycerol synthesis in lower temperatures.

SAUNDERS: It's true that one of the better cryoprotective agents, intercellularly, is glycerol.

SMITH: When you have a hypothermic category at, say, 27°, you know that will go in vitro into a particular substrate and reaction.

ENTENMAN: In all cases though, Bob, in all tissues, we would take tissue slices, say from the liver, and we would put some of the slices in one flask at 7°C and some in another flask at 7°C, then we would place one flask in a 37° bath and the other flask at a different temperature, say 27°, 17°, or 7°. So all tissues were first placed in the buffer at 7°C, then warmed up to whatever higher temperature was used during the incubation.

SMITH: The hypothermic animal from which you got the liver was at what temperature?

ENTENMAN: The hypothermic animal's body temperature was at 7°C. We did the autopsy at 7°C. We usually sacrifice the normothermic animal outside the cold room, then took him into the cold room and put the tissues in cold buffer at 7°C as rapidly as possible and before placing the tissues in the radioactive incubation baths.

POPOVIC: Immediately after entering into hibernation, do you find an adaptation of the brown fat?

ENTENMAN: I don't know, but if you take the hypothermic tissue, you can show an increase in brown fat activity within hours after induction of hypothermia.

POPOVIC: Do you know if you would find this just before hibernation or in hibernation?

MUSACCHIA: There is no way of knowing.

POPOVIC: The reason I am asking that question is that for me an animal ready to enter hibernation is already a hibernating animal.

MUSACCHIA: An animal that is starting to hibernate goes through a lot of shivering and a lot of erratic heart activity before he becomes hibernating. Three hours after the beginning of hibernation, I think that you have a different animal.

SOUTH: I think you are splitting hairs on an artificial boundary. An animal which goes through hibernation comes back out after a while. When he prepares for hibernation, he is going through part of the hibernating cycle.

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The Role of Brown Adipose Tissue in Temperature Regulation

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For the present occasion, it was thought best that we review our work relating to some of the special functions of brown adipose tissue. To lend an historical perspective upon this tissue, one may turn to the marmot, figuratively and literally inscribed by Konrad Gesner in his great volumes issued in 1551-1558. In these tomes, one will find that Gesner was probably the first to describe the reddish brown fatty substance seen as a pad over the interscapular area. This is also well illustrated in the cold-acclimated rat as we shall see in later slides.

To understand the topologic distribution of brown adipose tissue in the adult, it is helpful to observe the fetal or neonatal stage (Slide 1) as it appears in a sagittal section through a fetal mouse. The areas of brown fat are marked here in black. These are distributed in partial cylinders around the vertebral axis and in a larger concentric internal arrangement around the thoracic organs.

Slide 2 gives a view into the thorax of an adult marmot where the brown fat overlies the azygous vein, and partially the aorta running counter-current.

Also we should note here that the marmot has quite a store of white fat, as seen below the diaphragm and here to the right of the brown adipose tissue. It is a point here, I think, that while all the known hibernators have brown fat, there are many mammals with brown fat that are not hibernators at all. The rat presents an example of this.
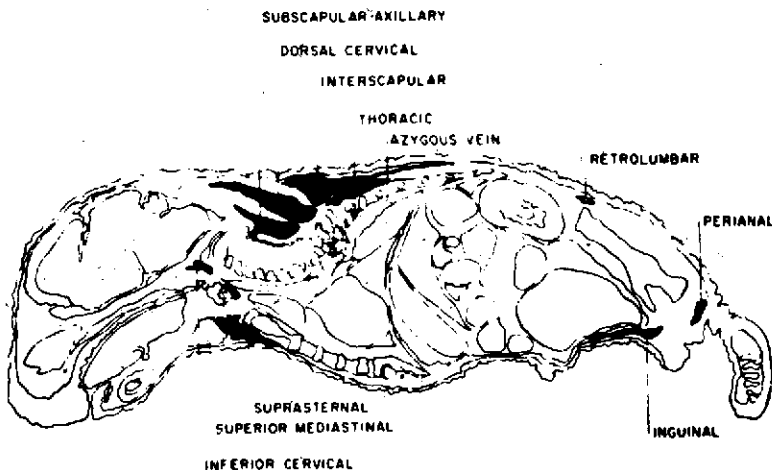
When we first brought out the proposition that the brown adipose tissue had thermo-genetic capacities, we

SUBSCAPULAR-AXILLARY
DORSAL CERVICAL
INTERSCAPULAR
THORACIC
AZYGOUS VEIN
RETROLUMBAR
PERIANAL
SUPRASTERNAL
SUPERIOR MEDIASTINAL
INFERIOR CERVICAL
INGUINAL

SLIDE 1

SLIDE 1*

had shown in vitro that the brown fat in the interscapular pad of the cold-acclimated rat displayed a qO_2 about twice that found in the control. Initially, however, it was thought that the brown fat was so small in proportion to the total body mass (Slide 3) as not to be of any thermal significance. This notwithstanding, by studying our replicas of the vasculature in the rat (Slide 4), it became apparent that we had a rather unique situation for the blood supplies to the interscapular pad. That is, the vascular drainage from the interscapular and cervical pads of brown fat deliver heat specifically to the vital organs of the thorax as well as to the cervical-thoracic spinal region, the significance of which we'll see later.

We are now observing a dorsal aspect of the interscapular fat pad of the cold-acclimated rat showing the brown adipose tissue (Slide 5). The vasculature has been injected with a dark latex that gives the spider-like appearance seen here. We can observe the central vein which goes down into the thoracic cavity. We've called this vein "Sulzer's vein", although it is not actually registered as such; in any case, it was described by Sulzer in 1774.

The next slide (6) shows the brown fat of the interscapular lobes as well as the bilateral cervical pads cephalad to the interscapular pad. We've resected the interscapular pad and you can see Sulzer's vein which terminates into the thorax at around T-4.

SLIDE 2

SLIDE 3

SLIDE 3*

Dorsal Cervical Veins
Left   Right

Brown Adipose
Dorsal Cervical   Interscapular

Vertebral Sinus
Left   Right

Sulzer's Vein

SLIDE 4

Brain

Azygous
Vein

Vertebral Artery (Left)

Thoracic
Aorta

Heart

Precaval Vein (Left)

C1 C2 C3 C4 C5 C6 T1 T2 T3 T4 T5

SLIDE 5

SLIDE 6

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SLIDE 4*

In the next slide (7) we have the brown fat in an adult cold-acclimated rat as it lies along the mediastinum deep into the thorax. Notably the brown adipose tissue partially covers both the azygous vein and the thoracic aorta.
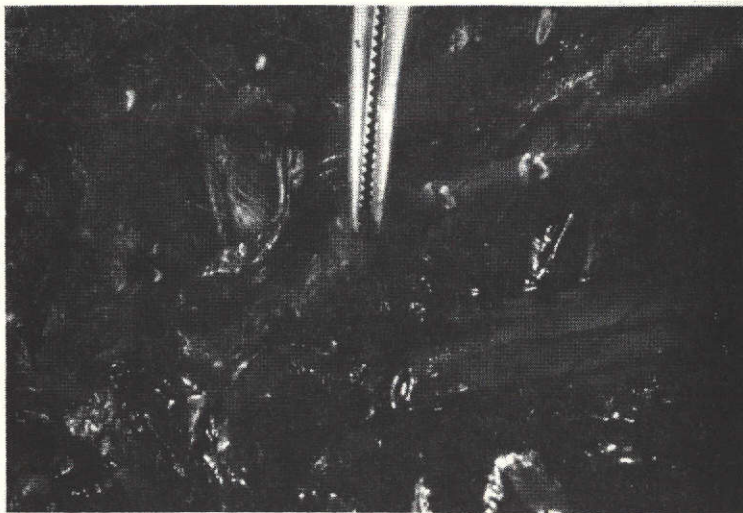
The next slide (8) demonstrates the extension of the brown fat under the diaphragm at the mediastinum, overlying the aorta and in the retroperitoneal space. Here we see brown fat at the poles of the kidney with the adrenals being embedded in the adipose tissue.
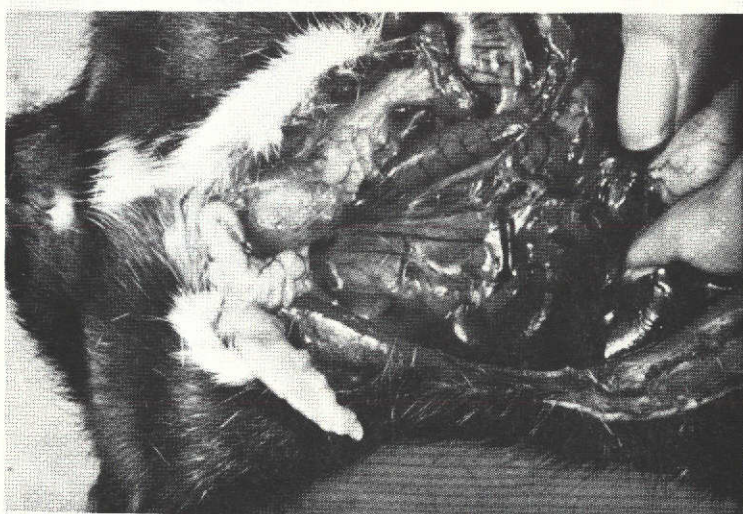
The next slide (9) is a table summarizing the rates of blood flow through brown adipose tissue. The values range from about 0.5% to about 20% of the cardiac output. The maximum and world's record is seen in the newborn rabbit stimulated by norepinephrine; this was shown by Heim and Hull in a very nice piece of work.

SLIDE 7

SLIDE 8

The next slide (10) is a diagram to give us some vascular orientation. This slide simply shows the classical parallel geometry with the main vascular loops to the respective visceral systems. The grey blocks depict the sites of brown fat from which blood may transport heat or engage in countercurrent exchange both within or at the periphery and by way of the axillae from the appendages. We might point out that the mass of the brown adipose tissue varies considerably according to the species as well as the age, season, and environmental conditions.

The next slide (11) shows the mass of the brown fat relative to the body weight. The neonates rank highest, having about 5.5% of the mass of the

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SLIDE 9

ANIMAL	ml/min/gm TISSUE	OF CARDIAC OUTPUT
WA - RAT (IN WARM)*	.67	--
	.51	.32
	---	.51
WA - RAT (COLD-STRESSED)	1.18	--
CA - RAT (IN COLD)	2.33	--
	1.46	1.35
CA - RAT (IN WARM)	.83	--
	---	.5
WA - RAT + NE	1.78	1.69
	---	.85
CA - RAT + NE	2.67	2.48
	---	2.25
NEWBORN RABBIT (WARM)	.87	7.05
NEWBORN RABBIT (COLD-STRESSED)	3.04	--
NEWBORN RABBIT + NE	3.60	20.13

* WA = WARM-ACCLIMATED; CA = COLD-ACCLIMATED.

SLIDE 10

10
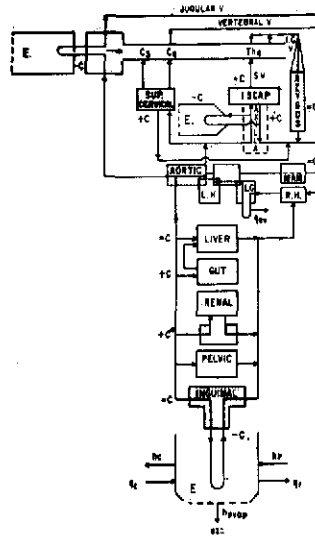
SLIDE 11

RELATIVE BROWN FAT CONTENT (% BODY WEIGHT)

SPECIES	BROWN FAT *LOCATION*	CONTROL	COLD-ADAPTED
LABORATORY RAT	T	.67	1.09
	-	.94	2.25
	I	.56	2.80
WHITE MOUSE	I	.65	—
DEER MOUSE (PEROMYSCUS)	T	.53	1.24
NEWBORN RABBIT	T	5.5	—
NEWBORN GUINEA PIG	T	2 - 2.5	—
HAMSTER	I + C	.64	1.59
HEDGEHOG	T	1.06-2.9	—
WOODCHUCK	T	2	—
CITELLUS LATERALIS	T	4.5	—
C. TRIDECEMLINEATUS	T	2.95	—
EPTESICUS FUSCUS	I	0.86-1.91	—
	I	5	—
	I	3.3	—
LEAST SHREW (CRYPTOTIS PARVA)	T	2.70	3.35
MASK SHREW (SOREX CINEREUS)	T	.63	1.29
TUPAIA CHINENSIS	T	.22	.24
MACACA RHESUS	T	.22	.21

* T = TOTAL BROWN FAT; I = INTERSCAPULAR; C = CERVICAL.

SLIDE 10*

animal. The hibernators also possess a large amount of brown fat - - ranging from around 2 to 4.5%. For example, in Citellus lateralis the brown fat constitutes about 4.5 - - 5% of the body weight.

As one would expect from a thermogenic tissue, brown fat undergoes trophic responses to changes in environment, notably to cold. To evaluate this response, we set up some experiments with rats, and the next series of slides contain the data from these attempts to standardize our cooling conditions.

The first slide (12) of the series is from an adult rat maintained at 26°C. In this low magnification view, one may see the islands of white (unilocular) fat cells. The brown adipose cells are the brownish colored cells (Masson's trichrome stain). The unilocular cells are particularly prominent around the trabeculae. Hence it is well to appreciate the fact that all of the tissue mass which looks like brown fat at a gross level may actually have as many as 60% white fat cells in the tissue. The next slide (13) is a high powered oil immersion from these control animals. In this section you see the typical multilocular brown fat cells with the numerous fat vacuoles per cell. When these animals are placed at 6°C, the tissue undergoes marked histological alteration. This can be seen in the next slide (14) which is a low power view of the tissue after 12 hours in the cold. Even at this time, the white fat cells are already greatly diminished.

SLIDE 12

The next slide (15) is an oil immersion view of the same area. I would like to make the point here that by 12 hours the intracellular fat vacuoles have largely disappeared. Now we have essentially an almost pure brown adipose tissue with only

SLIDE 13

SLIDE 12*   144

SLIDE 14

SLIDE 15

about 10% of the cells being of the unilocular type.

The next slide (16) shows the brown fat pad from a rat kept in the cold for 24 hours. The brown fat cells appear larger and the fat vacuoles in these cells seem to be repleted. At this time, the cells appear essentially as they would if taken from a rat exposed to cold for up to 60 days; i.e., after 24 hours, the cytological picture seems to reflect a steady state with there being practically no unilocular cells.

In the course of these studies, we made some measurements of the average cell area and the estimated size of the lipid vacuoles as functions of time in the cold (Slide 17). As is evident, by 6 hours there is a marked drop in the size of the lipid vacuoles and in the cells themselves. By 24 hours however, the cells have refilled with vacuoles and the average cell area seems to be back to control levels. These trophic responses of the brown adipose tissue (i.e., the loss of unilocular cells and acquisition of multilocular cells) represent a differentiation of the tissue, rather than the proliferation of a particular kind of cell. To follow these changes we used tritiated thymidine (H^3) and injected it into cold-exposed rats exactly one hour before sacrificing the animal. During this hour, we expected the H^3 to be incorporated into the nuclei of the cells that are about ready to divide; i.e., the DNA will pick up the H^3. Using autoradiographic techniques, we could then tell which cells were incorporating the H^3. Interestingly, we found that the label does not go into the brown fat cells or the white, unilocular cells; rather, it goes into the reticuloendothelial (R.E.) cells from the vascular system. The temporal sequence of this incorporation is seen in the next slide (18) which shows

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SLIDE 14*

SLIDE 16

SLIDE 17

RATS: COLD EXPOSED (6°C)
Interscapular Brown Fat

• Est size of lipid vacuoles (0-5)
○ μ² per cell

SLIDE 18

RAT INTERSCAPULAR BROWN FAT
DNA SYNTHESIS (H³)
IN COLD

SLIDE 17*

SLIDE 18*

that after 4 days or 96 hours, there is a maximum in the DNA-labeling of the cells. Moreover, when rats which had been exposed to cold for about 70 hours (while the H^3 incorporation was still high) were injected with H^3-thymidine, this radioactivity was found about 28 hours later to be moving into the multilocular cells. This gave us a pretty good argument, we think, that during this cold exposure, the brown adipose cells are developing from the reticuloendothelial cells which move into the multilocular sites in what might be called cytogenesis de novo. Thus, the significance of these data can be summarized as follows: (1) the autoradiographic technique demonstrates that the R.E. cells migrate into the areas of the multilocular brown adipose cells; (2) under the stress of acute or chronic cold exposure these R. E. cell progenators are mobilized and differentiated into multilocular cells; (3) the peak of the curve of the number of R. E. cells as a function of time in the cold allows us to predict the rate and time of mitotic events as well as that of the development of the mass of the tissue; (4) the response of the brown fat to cold is one of net enlargement and one which is hyperplastic rather than hypertrophic; (5) from the R.E. curve it appears that the number of brown fat cells approaches an asymptote at physiological limits of cold exposure.

The next slide (19) gives the relative masses of brown fat from the various sites in response to cold exposure. Reading up we have the cervical brown fat, the thoracic, the brachial, the renal, the interscapular, and finally the total amount of tissue. Between days 4 and 8 we found the maximum increase in brown fat. By day 8 we seem to be approaching a steady state since there appears to be little change in the total mass after this time. The mass of the brown fat seems to be limited to a given amount relative to the species and body size. So putting the animal into a colder environment may not get you anywhere, since the maximal mass of brown fat developed is not related linearly to the degree of cold exposure.

The next slide (20) is an electron micrograph of a brown fat cell. These cells display an extraordinary combination of cellular machinery which maximizes their capacity for processing substrate (oxidizing substrate) at a high rate. You will recognize the nucleus, the mitochondria, and the numerous vacuoles of fat. We can also see, we like to think, a tendency for the fat vacuoles to snuggle up to the mitochondria, a situation which may facilitate the oxidation of the substrate by
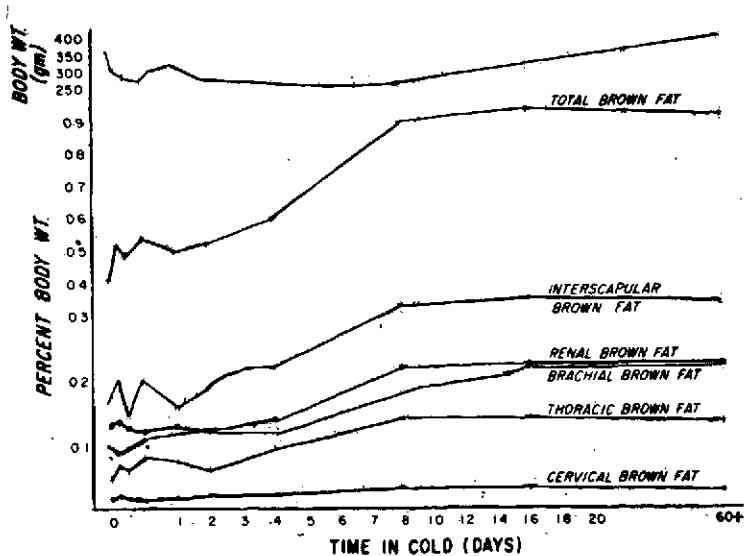
SLIDE 19

these particulates. This may explain the rapid removal of the vacuoles following cold exposure (i.e., within 6 hours). In the next slide (21) we see a "typical" mito-chondria as it appears in liver. Comparing this with the mito-chondion from brown fat in the next slide (22), we see in the latter, lamellated cristae which provide a tremendous surface area for activity, particularly the enzyme-substrate interactions. These account for the very rapid rate of substrate oxidation (and thus heat production) seen in the brown fat cells.
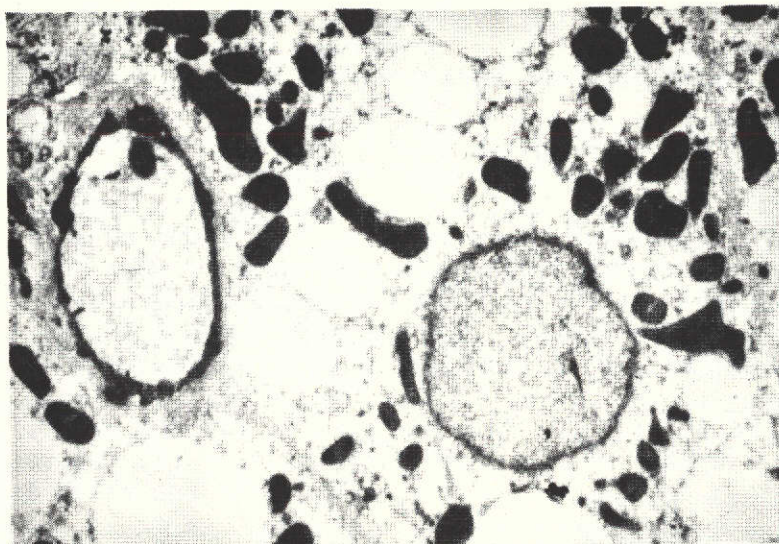
One of the most dramatic situations where brown fat thermo-genesis is observed is in the arousing hibernator. In the next slide (23) we see, the development of the evolution of heat in the arousing marmot from each of the brown fat areas. This ex-periment was carried out with Ray Hock at the White Mt. Research Station. The animal was taken from an hibernaculum at 6°C and exposed to the outdoor conditions of -13°C, snow, and a cold wind of about 25 knots. This cold stress stimulated the marmot to arouse, and as you can see in the slide, the rates of heating of various sites in the body are slightly different. Moreover, in the case of the brown fat, the steepness of the slope can be used as an estimate of the rate of heat generation.
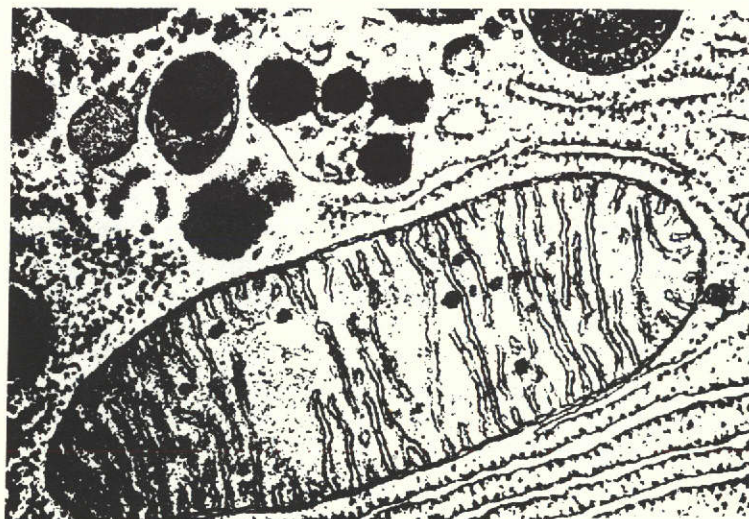
SLIDE 20

SLIDE 21

The next slide (24) gives us the same type of information as well as the respiratory pattern during arousal. Again, the brown fat areas are the warmest measured.

However, not only is the amount of heat produced by brown fat important, but in addition, the areas to which this heat is distributed are critical.

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SLIDE 20*

SLIDE 22

MARMOT NO. 2 - DEEP HIBERNATION

SLIDE 23

MARMOT NO. 5 - DEEP HIBERNATION
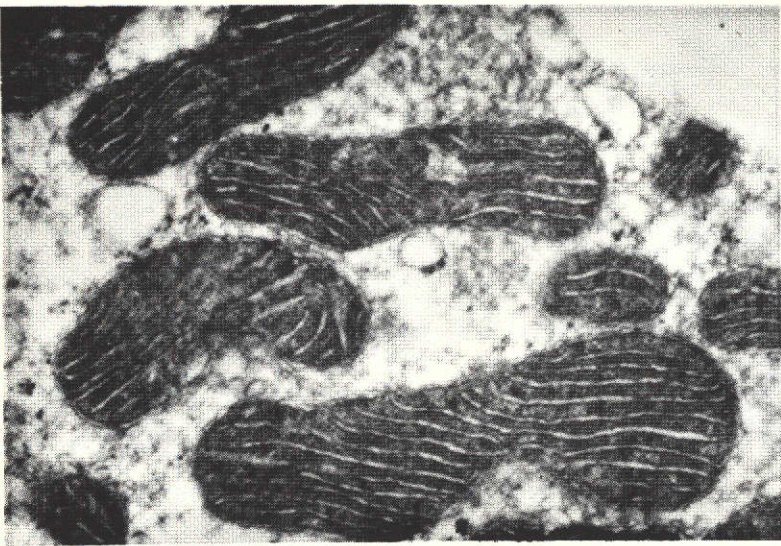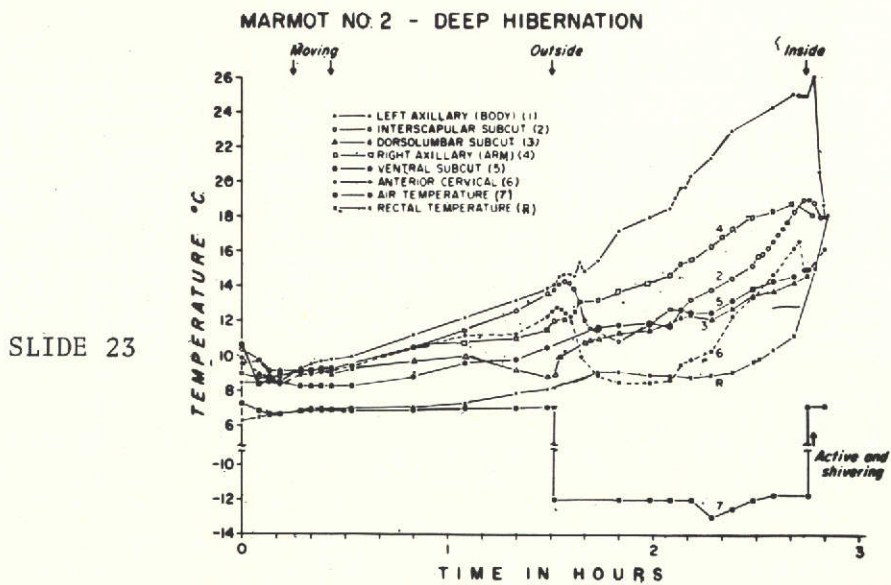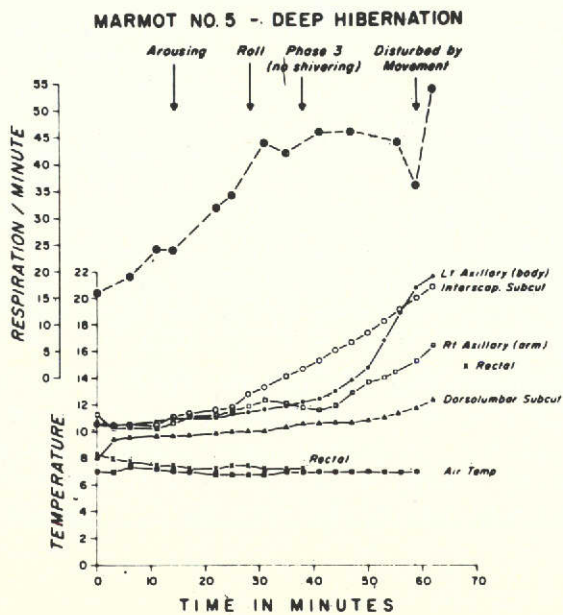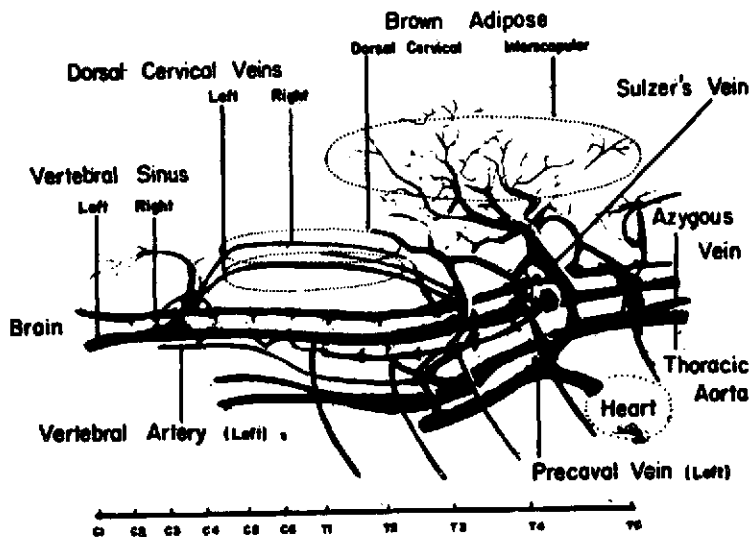
SLIDE 24

SLIDE 23*

SLIDE 25
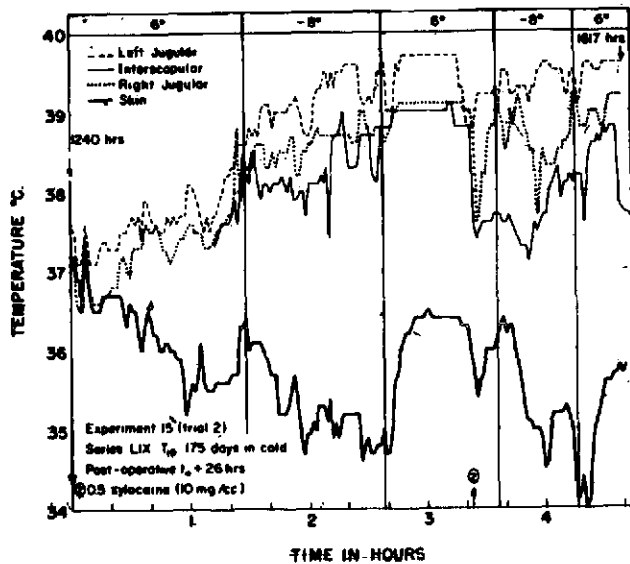
SLIDE 26

This can be seen in the rat where the venous drainage from the interscapular fat pad is asymmetrical. This vascular picture is depicted in the next slide (25). You can see that the major venous efflux from the fat pad goes via the fourth thoracic vein to the azygous, which in turn empties into the precaval near the jugular, on the left side of the rat.

That this asymmetry in heat delivery is indeed functional is indicated in the next slide (26). In this experiment, we implanted thermocouples in the left and right jugular veins at their confluences with the precaval veins and those from the interscapular fat pad. As you can see, when the ambient temperature was dropped from 6°C to -8°C, the temperatures of the brown fat and the jugulars rose. Moreover the temperature of the left precaval vein remained consistently above that of the level of the right side.

The importance of this specific delivery of heat is emphasized by the recent work in the newborn guinea pig by Kurt Brück in Germany. Some aspects of his data are summarized in the next slide (27). Here we see plotted the electrical activity as a measure of shivering, the oxygen consumption of the neonatal guinea pig and the temperatures of various areas of the body. When the guinea pig was placed at 20°C its oxygen consumption increased and shivering activity was seen. Experimental warming of the lumbar region of the vertebral canal with a thermode, had very little effect on the shivering activity even though the temperature of this part of the canal was raised to over 40°C. However, when

SLIDE 24**   150

SLIDE 26*

they warmed at a more
forward site - namely,
at the level of the
cervical vertebrae,
shivering was com-
pletely inhibited.
They thus concluded
that there may be
thermosensitive areas
in the spinal cord
which are involved in
regulating the onset
of shivering.

With respect to brown
fat, this finding
assumes importance be-
cause these postulated
thermosensitive areas
of the cord are locate
near the venous drain-
age of the fat pad -
i.e., C_6-T_4. Thus
brown fat can now be
directly linked to the
thermoregulatory
control of shivering.

In view of this
interaction, we decided
to examine the thermal
responses of primates.
This work was done at
Ames Research Center
with the pig-tailed
monkey, Macaca
nemestrina.

SLIDE 27

SLIDE 28

In the next slide (28)
we see the results from
an experiment in which the monkey was anesthetized, and thermocouples
were inserted in the area of the axilla (brown fat), under the skin,
and in the colon. The animal was then placed in a cold room and, as
is apparent, the temperatures of all areas fell rapidly. However, the
subcutaneous and colonic temperatures dropped at a faster rate than did
that of the axillary brown fat. This suggests that the brown fat is
producing heat, thereby slowing the cooling of the tissue. Such a
function of the tissue is further indicated in the next slide (29)
where we plotted the differences in temperature (Δ T) between the colon
and that of the axillary sites. Additionally, this slide illustrates
the close relationship between the thermogenesis of the brown adipose

SLIDE 27*

M. NEMESTRINA
646 2/4/70 ARC

SLIDE 29

M. NEMESTRINA
552 5/26/70 ARC
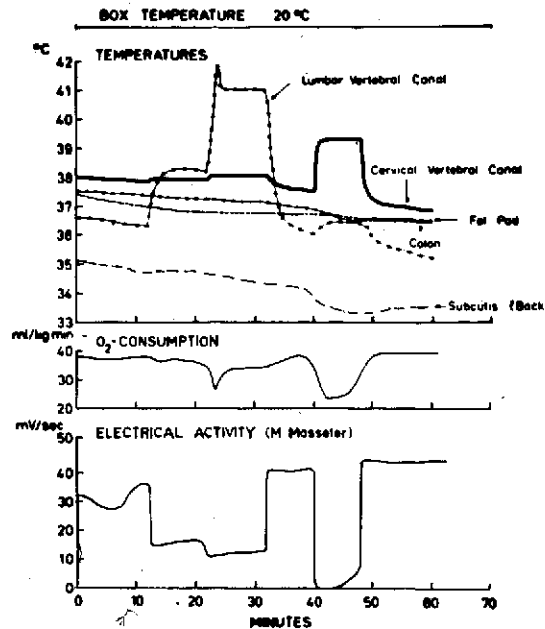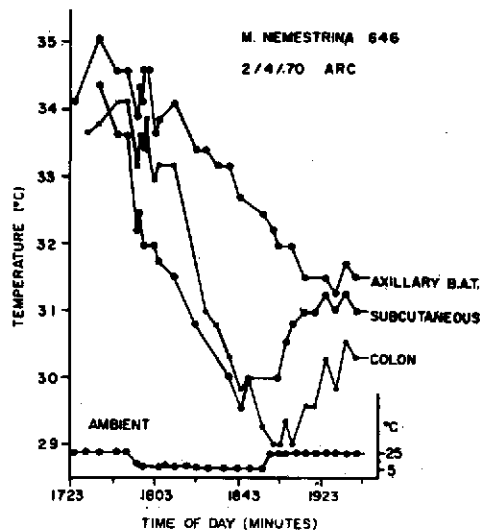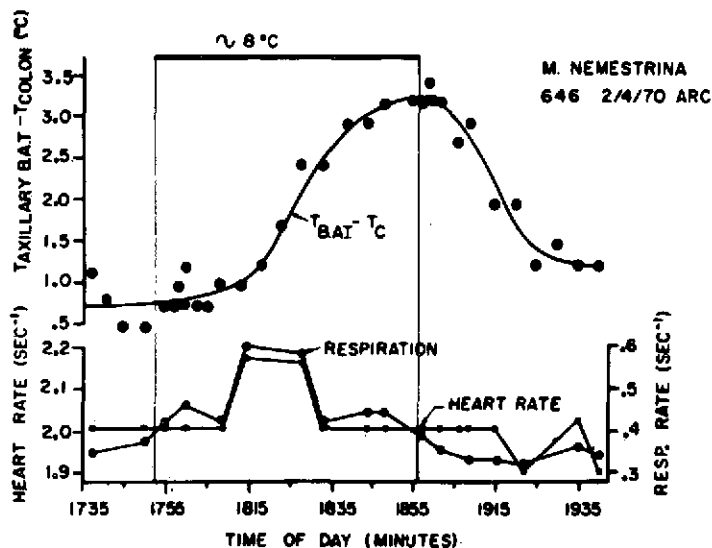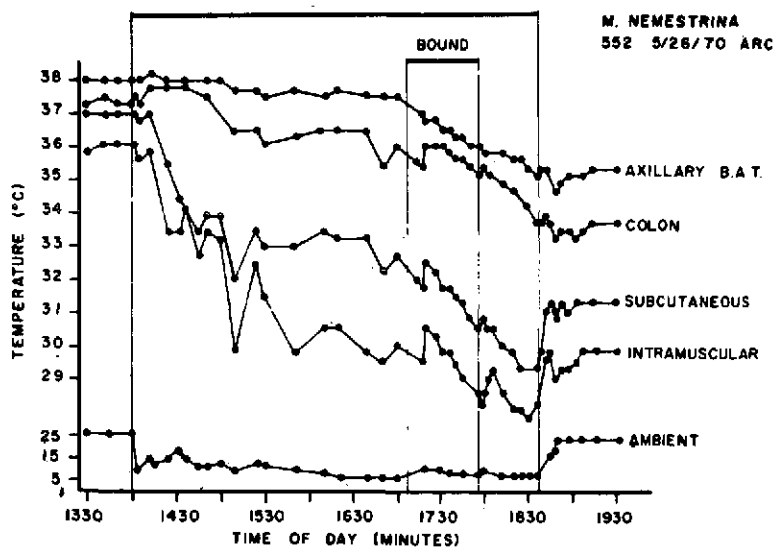
SLIDE 30

tissue and the highly coordinated, cardio-respiratory function. It is especially interesting that the inflection point of the warming curve of the brown fat (i.e., the point when the brown fat "starts into action") coincides with the maxima of the respiratory and heart rates during a period of one hour of cold exposure.

We further examined the role of brown fat in monkeys which were not under anesthesia when tested. In these cases, the animals were lightly anesthetized for the insertion of the thermocouples and fitting of a pectoral girdle. After recovery from the anesthetic, the monkey was placed in the cold room. As indicated in the next slide (30), this exposure to cold was followed by a small drop in colonic temperature with little change in the temperature of the brown fat, the latter always remaining higher than that of the core. Notably this exposure did not lead to a marked decrease in the temperatures of the core and brown fat as was observed in the anesthetized monkey (slide 29). That is, the unanesthetized monkey was able to maintain its body temperature in the face of the three-hour exposure to cold.

It is significant, however, that when the pectoral girdle was tightened ("BOUND" in the slides) the temperatures of the colon and brown fat began to fall. This decline continued throughout the period of restraint - about 1 hour.

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POPOVIC: Is this the same monkey that was previously anesthetized?

SMITH: No, this was a different monkey.

With respect to this fall in temperature following tightening of the pectoral girdle, it is of further interest that removal of the restraint was not sufficient to reverse this trend.   Rather, the temperatures did not begin to recover until the monkey was removed from the cold.   In the next slide (31), we have replotted these data as the difference between the axillary and colonic temperatures.   As indicated, the temperature difference between these two sites increased from about 0.5°C to about 2°C when the animal was exposed to cold.   Upon binding, the difference is similar to that seen at room temperature, but when the restraint is removed, the difference between the two temperatures reappears.   Thus, we can state that the cold exposure did not significantly depress the body temperature of the monkey until the thorax was bound; during such restraint, the animal appeared unable to maintain its body temperature in the cold.   Such a failure may be related to an effect on the venous efflux from the axillary brown fat -- a possibility which needs further investigation.

In our most recent work, however, we have turned to the control of brown fat heat production by the sympathetic nervous system.   This work, which is still in progress, is an attempt to examine the role of ion fluxes in the control of thermogenesis.

SLIDE 31

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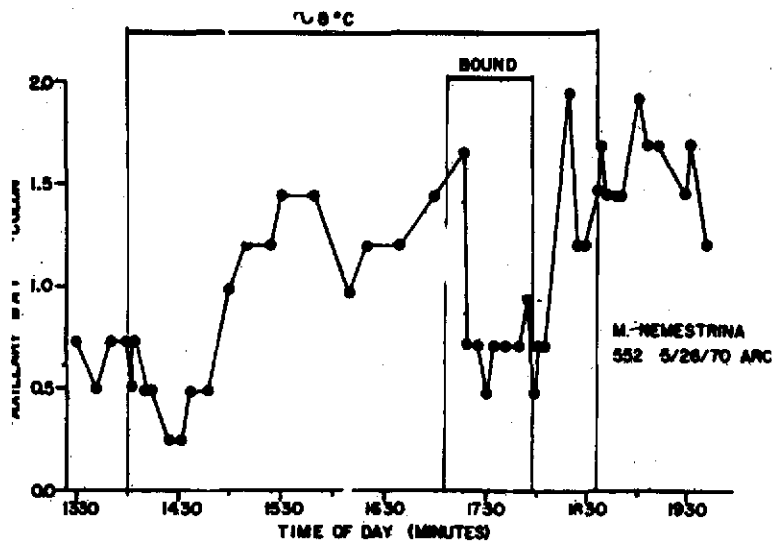
DISCUSSION BY PARTICIPANTS

POPOVIC: One of the slides you did show showed blood flow to brown fat in many different animals. What was the technique you used?

SMITH: The flow rates were picked up from the literature, most of the work using the ^{86}rubidium techniques. Do you recall the techniques, Barbara?

HORWITZ: I think only one was measured directly and that was in the newborn rabbit where flows were measured by cannulation.

SMITH: That was Heim and Hull (J. Physiol. 186: 42-56, 1966).

HORWITZ: Yes they measured the drainage directly. The rest is ^{86}rubidium, I believe.

POPOVIC: I think that with the drainage technique you could also easily measure oxygen consumption.

HORWITZ: They did.

POPOVIC: What was it? Was it high compared to other tissues?

HORWITZ: The value was very high - about 60 ml O_2/100 gm tissue per min.

POPOVIC: Couldn't you use the same technique on larger animals?

HORWITZ: In the newborn rabbit the blood from the brown fat drains into the jugular and they could thus cannulate the jugular up to the point of entry of the venous efflux from the fat pad. Then when they wanted to measure flow, they could clamp off the jugular in front of the entry point. In the interscapular area, the major drainage comes from a large vein that goes straight down into the spinal cord area. We've tried to cannulate it but haven't had any success. The major brown fat site in the rabbit is in the cervical not the interscapular region and the drainage is different.

POPOVIC: You prepare the animal, you find the piece of brown fat tissue with the small vein, all the blood will be draining from the tissue so you are measuring A-V difference and flow. Then you expose your animals to the cold. Do you know about the work that I did with Kenny Kent when I was in Ottawa some years ago? We measured blood flow and oxygen consumption of a group of small muscles in rats.

HORWITZ: Well, I have tried to cannulate the vein but I find that the drainage isn't consistent and when we try to stimulate we get erratic results. So, we haven't been able to get anything. Maybe I ought to come to Emory and try it.

POPOVIC: We'd like to see you again.

OYAMA: I'm rather curious about the use of the rat here as a model for brown fat thermogenesis. In Japan, there are a group of monkeys that live in the cold. Do you know of anyone that has worked with that particular group of animals that have acclimated themselves to extreme cold?

SMITH: Dr. S. Itoh has worked with some macaques that were brought to Hokkaido from Malaya and they had practically no brown fat. On the other hand, he found large amounts of brown fat present in monkeys born in the central part of Japan and reared in Hokkaido, the northern island.

HOLTON: I've always been fascinated by the fact that apparently in nonhibernating species, the fetus has a much larger brown fat content than does the neonate. Would this suggest, then, that the fetus is in a hypothermic environment?

HORWITZ: It does seem sort of paradoxical.

HOLTON: The fetus is so rapidly metabolizing that it is possible that the temperature of the fetus is considerably higher than that of the mother and therefore the temperature of the mother in this respect might be hypothermic even though the mother's temperature would be considered normal for an adult. It's just a thought.

POPOVIC: The question relating to the fetus is probably related to ontogeny, the brown fat is just something that exists during the early stage. It doesn't have any function.

HORWITZ: Not really. There are some Hungarian pediatricians who have autopsied premature and full term infants. They classified what the brown fat looked like in terms of how much fat was present and then tried to correlate the picture with the previous exposure of the newborn. For example, if the neonate wasn't incubated immediately, they found that the brown fat was almost completely depleted. So, apparently it can serve some function at least immediately after birth depending upon how the child is treated.

SAUNDERS: You said that if the infant isn't immediately incubated that brown fat is depleted?

HORWITZ: Well, what they do is to swaddle the child in blankets. If the child is not immediately warmed artifically and subsequently dies, they find at autopsy the brown fat content is less than in neonates which were swaddled or heated immediately.

SAUNDERS: Wouldn't you think the reverse of that? Rather than atrophy, the brown fat should increase.

HORWITZ: Well, one can argue that the tissue is using its endogenous fat stores which, with the cold stress, become exhausted. It's an environmental effect.

MUSACCHIA: I wonder if you would consider for a minute that the Nemestrina that was bound shows the hypothermic response and then when the binding is released, the temperature in various tissues and organs climbs. Isn't this the classical restraint hypothermia that is known in rabbits and is also known in another type of animal that we seldom talk about, the field trapped animals. If you trap a small mouse, for example, in a little box in the field and record its body temperature, you find that after a period of time, they tend to go hypothermic. So, isn't what you have here, essentialy restraint hypothermic?

SAUNDERS: Bob's being modest about this monkey business because when the situation developed with Bonny, we knew that Bonny was hypothermic. The controversy was why did he become hypothermic? Bob volunteered to look at the brown fat because he thought that the brown fat in the thoracic area could explain it. It may have been a triggering mechanism because Bonny was hypothermic but never gave any indication of any shivering. We didn't know what the thermoregulatory system was like at the time. Vojin, I know, and Bob, and I think you, Joe, all 3 came through with almost the same idea that this was an old situation dealing with classic restraint of an animal. He did one animal without the pectoral girdle and he did another with it. It turned out that this could have been the situation in Bonny because he was very uncomfortable, tightly bound and restrained, and probably forcing himself and cutting off the circulation as Bob has pointed out. It helped clarify at least part of the picture as to why Bonny behaved physiologically as he did while he was in orbit after the first couple of days of real good activity.

SMITH: There is another part to that same story. Fortunately no one cared about the brown fat and none of the pathologists had taken it from Bonny. So I got the axillary area from port to starboard and it showed extravasation on both sides, but the pathologist agreed that it probably wouldn't have been a cause of death. However, it means that there were contusions in that area. The thinking is that this occurred either immediately before or immediately after the demise. So, at splash down there could have been some physical forces.

SAUNDERS: Like about 25G. This package was not air retrieved. It hit clouds just as the plane had it in its sights and down it went into the pond. Perhaps if it had been caught in the air with the greatly reduced g forces, Bonny might have survived.

SMITH: There is another point that is just as important. I called the nurse who took care of the monkey. I asked her if they had given him catecholamines. They did give him, when he was having trouble, 1 cc of adrenaline which would be the standard dose. The critter died and the extravasation could have been due to that. We've killed enough rats with norepinephrine to realize that you can blow the whole capillary bed out. If we had contusions in that area, it could very well be the point of extravasation under that condition. That's all I can say.

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MUSACCHIA: I have one other question with regard to methodology. In your examination of the transition of one kind of cell differentiating into another type of cell, say going from unilocular to multilocular or reticuloendothelial cells to brown fat cells, I'm not sure what path you are going to or want to take. You look at different animals each time. Would it be possible to biopsy one animal, take the same animal put him in the cold, and 24-28 hours later do another biopsy? You have plenty of brown fat and a little biopsy shouldn't hurt.

SMITH: Yes, this would be possible.

POPOVIC: You could also use differentially labeled microspheres. You could inject them once and then several days later and then you could look and see what type of blood flow goes to the brown fat.

JORDAN: For us metabolic types, could you explain what makes the brown fat brown?

SMITH: That's been a question for many years. The best guess is a combination of 2 things. Most recently, Ball and Joel found a lot of cytochrome as did Hook years back; they ascribed the color to the cytochrome which no doubt represents part of the color along with the hemoglobin which is very plentiful.

ENTENMAN: Is there a lot of mucopolysaccharides in brown fat?

SMITH: I would say not very much.

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Methods for Study of Cardiovascular Adaptation of Small Laboratory Animals During Exposure to Altered Gravity

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Present knowledge about the effect of gravity on various physiological systems is rather limited. Today we know about some cardiovascular changes occurring during a free fall lasting several seconds and about some cardiovascular changes observed in astronauts during and after space flights lasting only a few days. It has been speculated, however, that long-lasting space flights may induce profound circulatory changes and that these changes might impair the performance of astronauts during the increased g forces encountered upon reentry.

Fig. 1. Rat with chronically implanted cannulas in the aorta and in the right atrium.
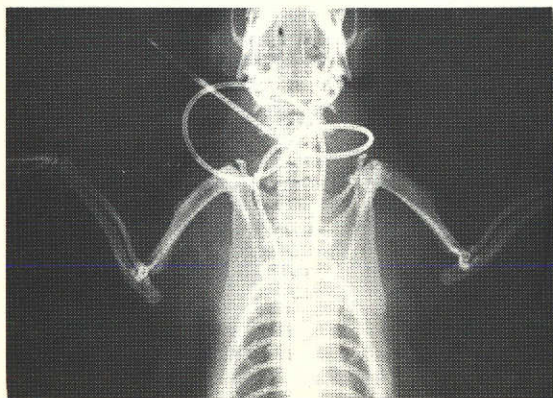
Fig. 2. X-ray photo of a cannulated rat.

Many of my associates contributed to the work that I am presenting to you today. I will summarize the results we obtained rather briefly. Please interrupt me at any time if you think I should expand my presentation.

In order to study circulation in small laboratory animals (mice, rats, hamsters, squirrel monkeys) we have developed in our laboratory several new techniques. The first slide shows a rat after his aorta and his right ventricle (or right atrium) were chronically cannulated and the cannulas exteriorized at the top of the neck (Fig. 1). The next figure shows an x-ray picture of a rat and position of the polyethylene cannulas (Fig. 2). The implanted cannulas stay patent for a number of months and often for the entire life span of the animal. The implanted cannulas permit direct measurements of blood pressures, intraventricular ECG and cardiac output (Fick principle) in unanesthetized, undisturbed, and unrestrained animals (Fig. 3 and Fig. 4). We have shown that reproducibility of the results obtained with out technique is very good. Figure 5 shows mean

Fig. 4. Schematic presentation of set-up explained in the previous figure.

Fig. 3. A cannulated rat is placed in a metabolism chamber where his oxygen consumption, body temperature, blood pressure, intraventricular ECG and cardiac output are measured.
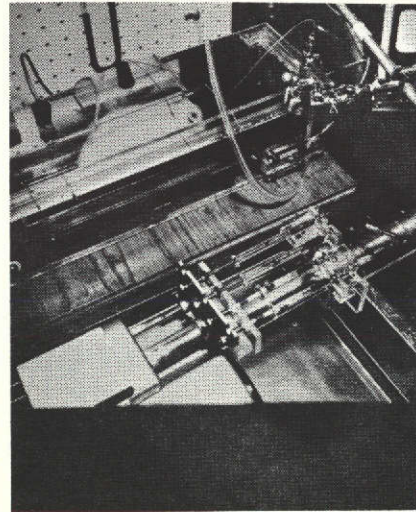

Fig. 6. A rat during forced exercise (treadmill, upper part) and the system for cardiac output determination, and determination of other circulatory characteristics.

Fig. 5. Cardiac index (cardiac output/kg) and mean arterial pressure measured in rats during a period of 120 days after chronic implantation of the cannulas.

arterial blood pressure and cardiac index of 96 rats during a period of 120 days after chronic implantation of the cannulas. In another work we have shown the effect of environmental temperature and of exercise on cardiovascular parameters of rats (Fig. 6), as well as the importance of temperature of adaptation of the animals for determination of real values of cardiac output.
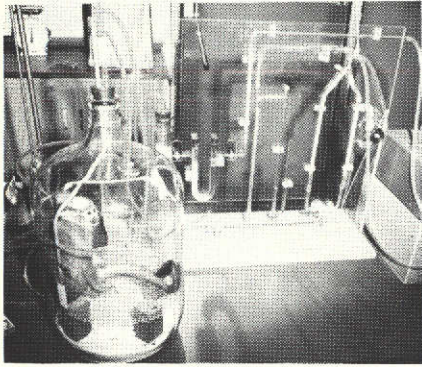
Fig. 7. A squirrel monkey during measurements of cardiac output.
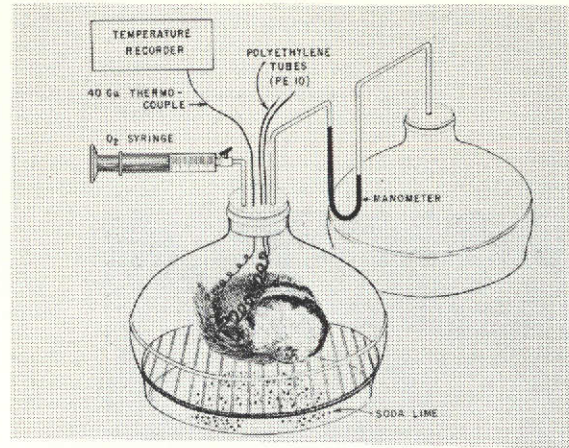
Fig. 8. System used to measure cardiac output in hibernating ground squirrels.
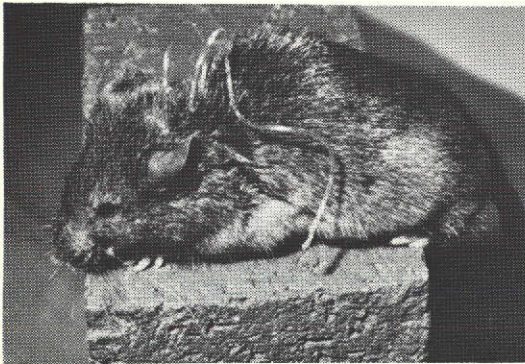
Fig. 9. C_3H mouse with chronically implanted aortic cannula.
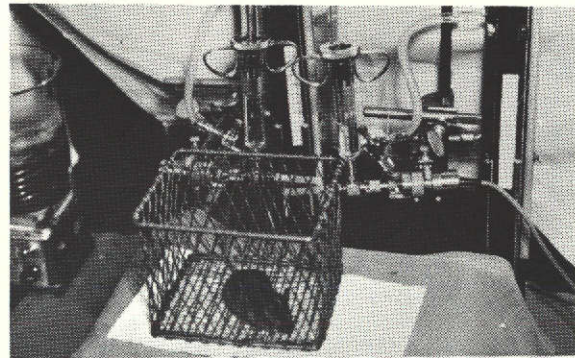
Fig. 10. A chronically cannulated mouse during circulatory measurements.

To measure cardiac output and other circulatory parameters in squirrel monkeys, the implanted cannulas was exteriorized at the top of the head and protected by a plastic crown (Fig. 7). A similar system was developed to measure the circulatory characteristics in hibernating ground squirrels (Fig. 8). Mechanical stretching of PE 10 cannulas permitted mice to be used for cardiovascular investigation. Fig. 9 shows a C_3H mouse several days after cannulas were implanted in the carotid artery and in the vena cava superior. The implanted cannulas stay patent in a mouse a shorter time than they do in a rat, hamster, or other small laboratory animals. Fig. 10 shows the experimental set-up used to measure cardiovascular characteristics of a C_3H mouse.

In order to study the eventual changes occurring only in the heart in animals exposed to long-lasting weightlessness and to be able to separate them from the changes that occur in the peripheral part of the circulatory system, we have developed a miniaturized membrane type heart-lung machine for small laboratory animals (Fig. 11-13). In order to avoid the effects of anesthesia and of surgery the large polyethylene cannulas (needed for these experiments) are implanted several days before the use of the miniaturized
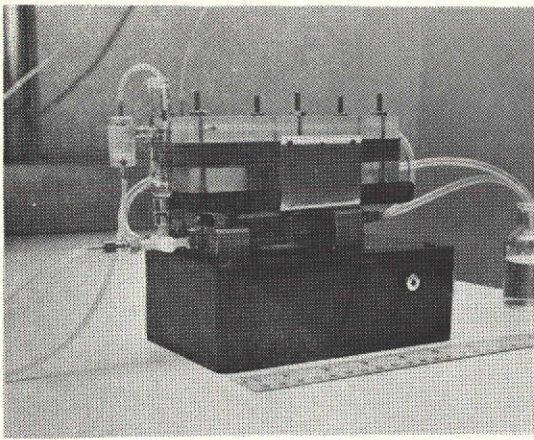
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Fig. 11. Miniaturized membrane oxygenator (heart-lung machine) for rats, hamsters or squirrel monkeys.
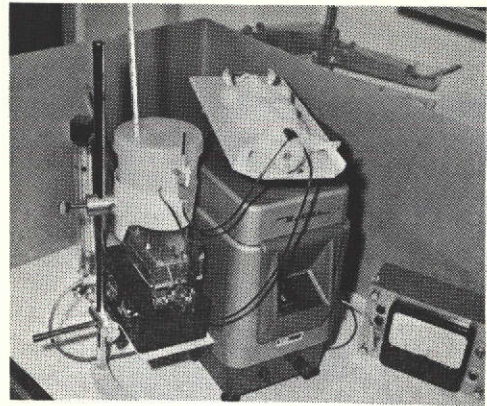

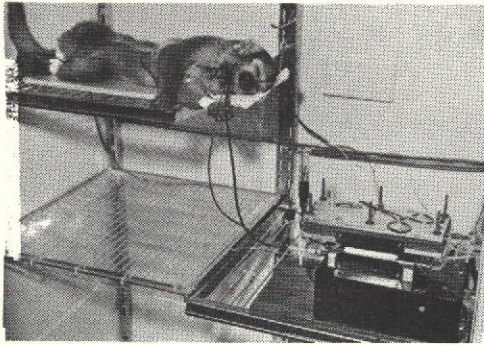Fig. 12. A rat during a partial cardiac bypass.

Fig. 13. A hypothermic ground squirrel during a complete bypass.

pump-oxygenator. The clotting in large cannulas is prevented by making a small shunt (PE 10 cannula) between the large arterial and the large venous cannula.

OYAMA: What type of anticoagulant do you use?

POPOVIC: We don't use any in this experimental set-up. It is not necessary since the blood is continually circulating through the shunt. The increase in the cardiac output induced by the shunt is very small, about 3 per cent.

OYAMA: What type of tubing do you use for your cannulas in regular non-shunt experiments?

POPOVIC: We use regular, commercially available polyethylene tubing (Clay-Adams). The volume of each cannula is very small so that there is little exchange between the fluid of the cannula and the blood. When the volume of the cannula is rather large (as mentioned in the last group of experiments) and because of the fact that the walls of the cannula are elastic, one sees an increased exchange between the fluid in the cannula and the blood and eventually such a large cannula plugs. This is why we developed the shunt I talked about a few seconds ago. Besides this miniaturized heart-lung system we developed also a miniaturized dialyzer (artificial kidney) so that we are now able to combine the heart-lung and kidney systems.

All these experiments and techniques that I have discussed with you were developed in order to learn as much as possible about circulatory physiology of small laboratory animals before we could even think about the possibility of evaluating the effects of long-lasting weightlessness on circulation. As

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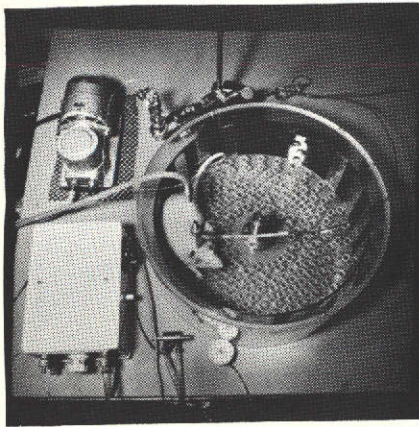
Fig. 14. Walking chamber
          for long-term
          cardiovascular
          measurements.
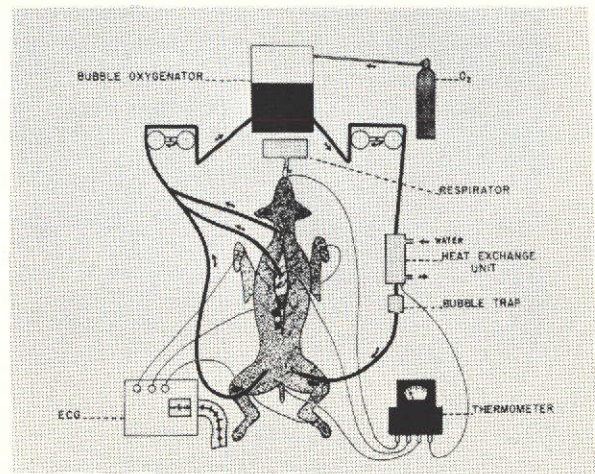

Fig. 15. Experimental set-up for
          profound (extracorporeal)
          cooling of dogs.

the next step toward this goal we built a prototype walking chamber for small
laboratory animals (Fig. 14) that would be exposed to a long-lasting space
flight. In this walking chamber, the rat is restrained with a collar around
its neck. However, the animal can walk and can exercise voluntarily by
turning the chamber around itself. Such a system could be used also to
measure cardiovascular parameters before, during an actual space flight, and
after the flight. The metabolic rate of the animal can be adjusted to a
"normal" level by increasing or decreasing the work load during exercise
(friction during turning of the walking chamber).

The second series of experiments that I would like to discuss with you deals
with hypothermia. We believe that this work is also related to NASA problems.
Exposure of an astronaut to a very low environmental temperature might lead to
a sudden decrease of his body temperature. One part of our work deals with
this problem: How to make recovery from profound short-lasting hypothermia
a safe procedure, even when such a profound hypothermia is associated with
complete cardiac and complete circulatory arrest. Because it has been
speculated already that long-lasting hypothermia might be the answer for
extended space trips that mankind will eventually undertake, we are
studying another problem that deals with body cooling: extension of safe
duration of profound hypothermia. We have used dogs in the first group of
these experiments. The dogs were cooled to a body temperature of 4°C (Fig.
15). During blood stream cooling (bubble oxygenator), respiration and then
the heart of the animal is arrested by the cold. When the body temperature
of 4°C is attained, the extracorporeal circulation is completely interrupted
for 1 to 2 hours. Clinically such a dog appears dead; there is no trace of
cardiac activity, mechanical or electrical, the arterial blood pressure has
the same value as the venous (around 0 mmHg). One to two hours after
interruption of extracorporeal circulation we restart the extracorporeal

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circulatory support. The dog is rewarmed until it reaches a normal
body temperature. In the beginning we encountered many problems. The dogs
were dying either during rewarming or during the first two days after re-
warming, usually because of congestion of the lungs or because of brain
damage. Now, after solving many problems, we are able to cool the animals
successfully. In the last group of twenty dogs that were cooled to a body
temperature of 4°C with one to two hours circulatory arrest we had one
hundred per cent survival. None of the dogs showed any impairment after
rewarming. Three of them are still pet animals in the households of some
of the Departmental personnel.

SOUTH: How does this differ from Frank Gollan's experiments?

POPOVIC: Several things are different. Fig. 16 shows our experimental
arrangement. We believe that all the factors listed in this figure are
important for successful survival of the dogs. Hypervolemia during re-
warming appears especially important. (In this respect it is interesting
to note that beneficial results with hypervolemia were also obtained in the
case of profound circulatory shock.) Another important aspect in our experi-
ments is the drainage of the left heart, to avoid congestion of the lungs.
Along with profound hemodilution (priming volume of the whole extracorporeal
system, about 1000 ml, is filled with glucose-saline only) we are using
small amounts of short-term anesthetic (Surital) needed to assure anesthesia
only during surgical implantation of cannulas. The cooling is very fast so
that physical anesthesia of cold soon replaces the chemical anesthesia of
Surital. During rewarming, at a body temperature of 32°-34°C, the dog
starts to wake up. During cooling the extracorporeal (arterial) blood
pressure is continuously decreased and stays below 50 mmHg in the arteries
of the cooled dog during the whole period of cardiac arrest. The positive
pressure artificial respiration is also continuously decreased during
cooling and eventually interrupted in the mid-inspiratory position when
circulatory arrest is induced. During circulatory arrest the extracor-
poreal blood volume is replaced with fresh blood. Rewarming is interrupted
when a body temperature of 30°-32°C is attained to permit the heart to
resume normal pumping activity. Then we complete the rewarming.

Profound hemodilution

Short-term anesthetic

Maximal venous drainage

Left heart drainage

Low temperature - low systemic pressure

Decreased respiration (rate and volume)

Circulatory arrest - maximum extracorporeal volume

Blood replacement

Rapid rewarming

Blood hypervolemia during last phase of rewarming

Reestablish circulatory functions with heart at 32°C

Fig. 16. Experimental components necessary
for survival of dogs cooled to a
body temperature of 4°C with 1-2
hours circulatory arrest.

SOUTH: How have you been able to sidestep fibrillation or do you just wait during rewarming of your dogs for a spontaneous sinus rhythm to appear?

POPOVIC: In our early experiments every dog fibrillated. But after we developed our system of cooling - rewarming, very few dogs fibrillated during rewarming and none during the cooling process. If the dog fibrillated during rewarming we did not do anything until a body temperate of 30-32°C. A single shock was then all that was necessary for the SA node to take over. The most amazing thing about these experiments was that as soon as an animal was rewarmed to a body temperature of 34°-35°C, the animal was wide awake and very vigorous (Fig. 17). One hour later the animal was drinking and walking and soon afterwards eating too. It was just fantastic how fast the animals fully recovered.

In order to measure circulatory parameters during submersion in fluorocarbon fluid or during experimentally induced "weightlessness" (in reality exposed to a negative weight, the fluid of immersion having specific gravity of 1.6), we used adult male hamsters with chronic cannulas. The hamsters were fully submerged for up to 24 hours (and later fully recovered) if their body temperature was decreased to 10°-12°C. At a higher body temperature the survival of the animals was much lower, however. I am not showing this figure here because Dr. Saunders used it yesterday in his presentation. The data that we collect now deals with measurements of central venous pressure, of mean arterial pressure, ECG, and some other parameters.

The last set of slides that I will show you deals with our cancer hypothermia work that we were kind of pushed into accidentally. During cooling of our rats to a very low body temperature to establish duration of safe survival at these low body temperatures we observed that the tails of our animals that were kept outside of the cooling chamber shrivelled after recovery of the animals and some of them even fell off. Immediately the idea arose that such an approach might be useful for the eradication of tumors; that
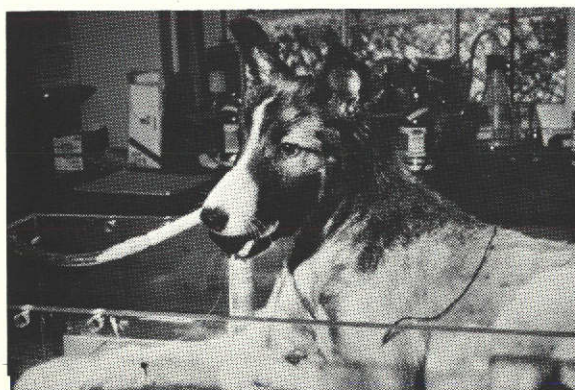

Fig. 17.   A dog after profound cooling and a 2 hour circulatory arrest. The photo was taken as soon as the body temperature of the dog rose to 34°-35°C during rewarming process.

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is, if the tumor were kept warm while the rest of the body of an animal was
profoundly cooled. In the first group of experiments we used Toolan
adenocarcinomas implanted in the cheek pouch of hamsters. The hamsters
were cooled to a body temperature of 4°C while their tumors were kept warm
(37°C) with electrical contact heating (Fig. 18 and Fig. 19). When such a
differential hypothermia was maintained for 10 hours and then the animals
rewarmed, the tumors of the animals regressed slowly and after 10-15 days
disappeared entirely (Fig. 20). When one tumor was kept cold together with
the rest of the body but the second tumor was kept warm for 10 hours, the
first tumor continued to grow after rewarming of the animal, while the
second regressed and eventually disappeared (Fig. 21). In order to
decrease the treatment time from 10 hours to a shorter duration and thus
make the differential hypothermia technique applicable for human clinical
work, chemotherapy was combined with cooling. The anticancer agent that was
used was 5-fluorouracil (FU), a drug often used in human tumor chemotherapy.
When 50 mg of this substance was injected (i.v.) into normothermic animals
with normothermic tumors, neither tumor size nor tumor growth were affected.
But when FU was administered in differential hypothermia lasting one hour only,
transplanted cheek tumors regressed and eventually disappeared (Fig. 22).
When one of the tumors was kept cold together with the body while the other
tumor was warm, the administered FU affected only the warm tumor (Fig. 23).

Differential hypothermia and chemotherapy were also more effective than
chemotherapy alone for chemically induced (DMBA) tumors in rats. During
the last five years we have been using C_3H mice with spontaneous mammary
tumors. This particular mammary tumor is known to be resistant to FU.
Still, even in this case, we have shown that combination of differential
hypothermia and chemotherapy is much more effective than chemotherapy alone.
Histological and histochemical examination showed that after treatment the
tumor cells undergo profound changes and that about 60 to 70 per cent of the
tumors regress profoundly (40 per cent or more of the pretreatment size).

While a uniform rewarming of small tumors in C_3H mice was possible with
an infrared heating lamp (Fig. 24), tumors in larger experimental animals
had to be rewarmed either by ultrasound or microwave energy. These new
heating devices that warm up the whole tumor mass uniformly were developed
in collaboration with the personnel of the Radar Branch of the Division of
Electronics, Georgia Institute of Technology. Figures 25-28 show the
ultrasond and microwave heaters. Fig. 29 shows the temperature of the body
and temperature of the tumor during one hour differential hypothermia.
Both systems work quite well for "surface" or for exposed tumors. The
difference between the systems is that one has to make contact (through
"colloidal water") between the ultrasound heater and the tumor, whereas with
a microwave heater the probe of the heater is placed about 1/2 inch above
the tumor. The size of the probes is adjusted according to the size of the
tumor treated. Recently we developed large probes for large spontaneous
tumors in dogs. We receive these dogs with spontaneous tumors (mostly
mammary) either from the Veterinary School of the University of Georgia or
from local veterinarians in Atlanta.

At this time of our work we turned our attention toward studying the
enhanced action of chemotherapy when combined with differential hypothermia.
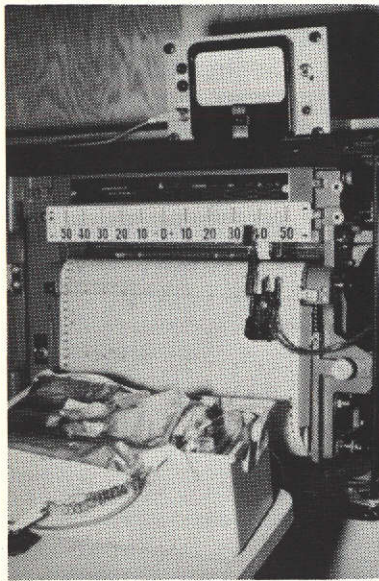We used ^{14}C-5-fluorouracil to follow the utilization of the drug after

Fig. 18. In differential hypothermia the body of an animal (hamster in this photo) is cooled to a body temperature of 4°-10°C while the tumor is kept normothermic.
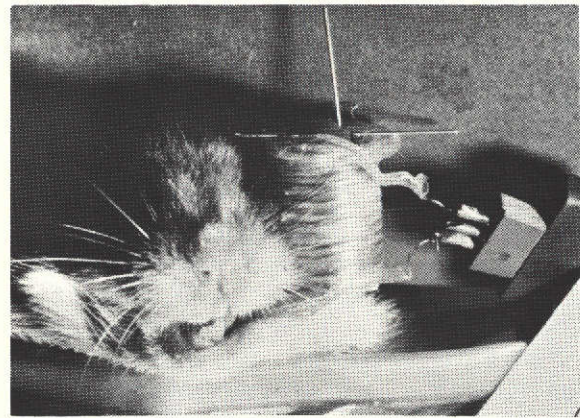
Fig. 19. Electrical servoheater which keeps tumor (transplanted in this case in a hamster's cheekpouch) at a temperature of 37°-38° while the rest of the body is deeply cooled.
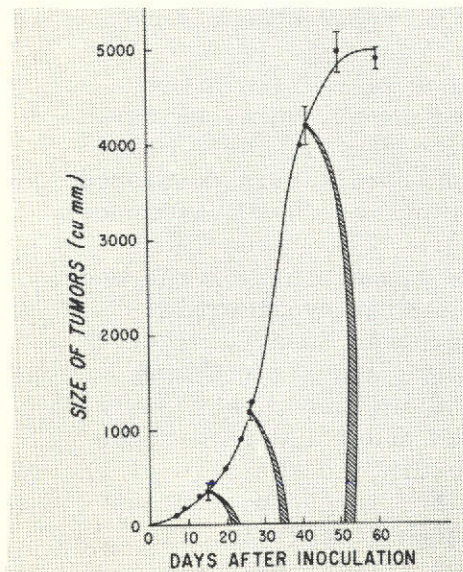

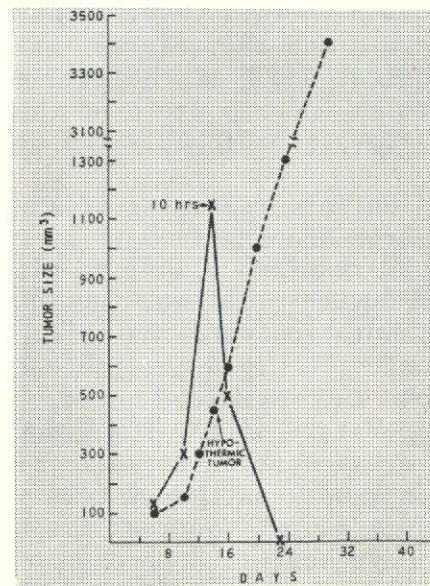Fig. 20. Growth curve of cheek transplanted Tooland adenocarcinomas in hamsters and their regression after 10 hour long differential hypothermia.

Fig. 21. The warm tumor regresses after 10 hour differential hypothermia in hamsters while the tumor kept cold during these 10 hours continues to grow.

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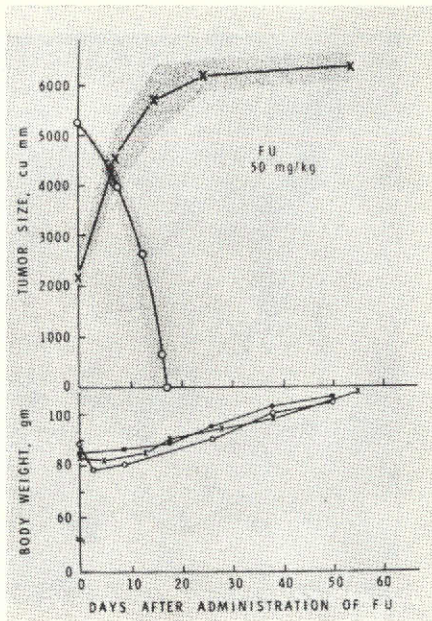
Fig. 22.   Regression of cheek
transplanted Toolan
adenocarcinomas
after combination
of one hour dif-
ferential hypo-
thermia and
chemotherapy (upper
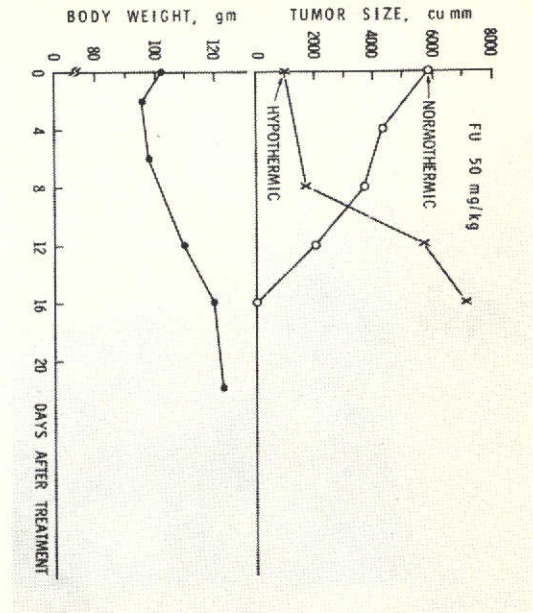curve: normal
growth).

Fig. 23.   Regression of a Toolan ad-
enocarcinoma tumor after 1-
hour regression hypothermia
and chemotherapy. Hypo-
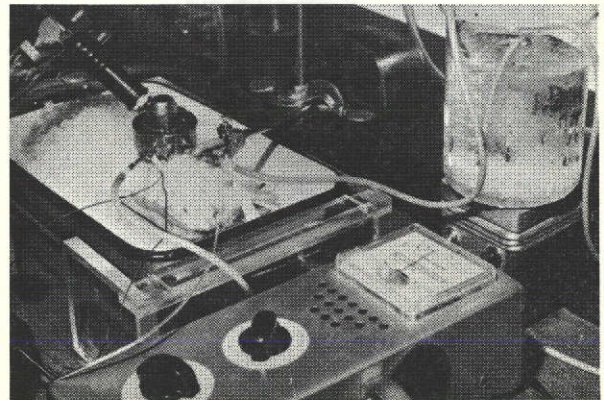thermic tumor continues to
grow.

Fig. 24.   A small spontaneous
mammary tumor in a
C₃H mouse can be uni-
formly rewarmed by an
infrared source.

Fig. 25.   Ultrasound heater for rat
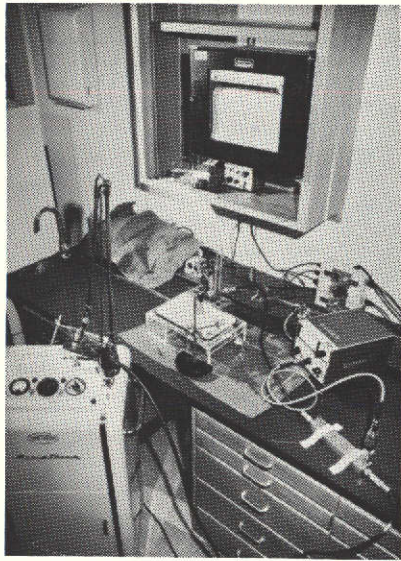tumors.

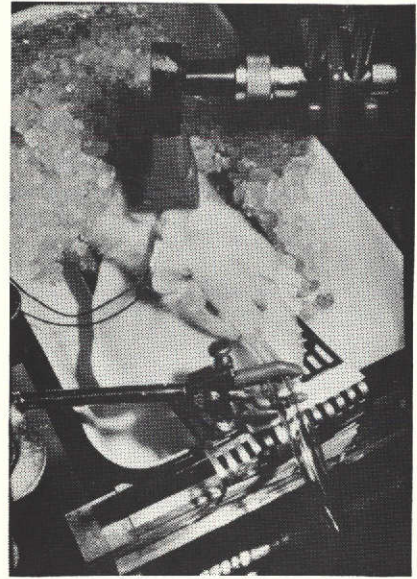Fig. 26. Microwave heater (with the wave guide) for localized heating of rat tumors.

Fig. 27. A close-up of the previous photo.
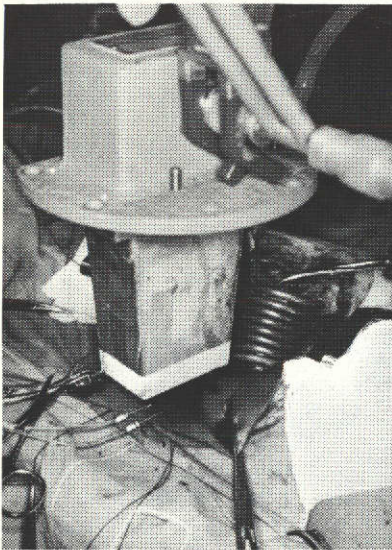

Fig. 28. Large probe of a microwave heater (with the waveguide) used to rewarm a part of the lung in open-chest dogs.
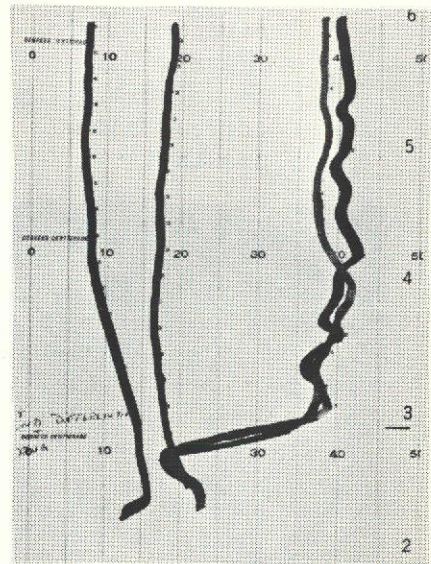

Fig. 29. Sudden rise of temperature of a tumor (lower part of the figure) after beginning of microwave heating. The tumor temperature 38°-40°C. Deep colonic temperature 8°C-10°C. The whole experiment lasted 1.5 hours.
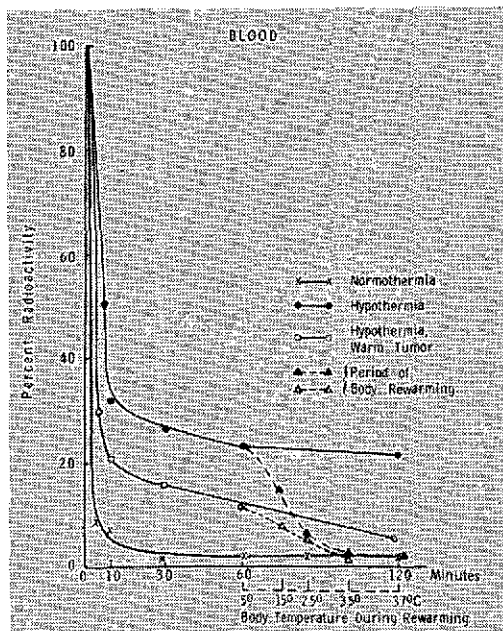
Fig. 30. Disappearance of radio-
activity from blood
after administration of
^{14}C-5-fluorouracil in
differential hypother-
mia (middle curve)
normothermia (lower
curve) and hypother-
mia (upper curve).

administration. In normothermic animals the drug was metabolized rather
fast while in cooled animals, during the whole hypothermic period, the
^{14}C-FU stayed at a high concentration in the blood. In differential
hypothermia the disappearance of the drug was slow but continuous. Two
hours after administration the level of blood radioactivity was as small as
in normothermic animals where many tissues were competing for the drug (Fig.
30). Thus it appears that the small rewarmed tumor used (in differential
hypothermia) as much of the drug as would be used in a normothermic animal
by all tissues including the tumor itself.

In addition to the increased metabolism of warm tumors, another possible
mechanism for enhanced action of chemotherapy in differential hypothermia
appears to be an increased blood circulation through the warmed tumor as
compared to the rest of the cold body. We studied this problem by using
several model systems. We used first a single lobe or a part of a lobe of
right lungs in dogs. In such (open chest) dogs, the right lung is completely
collapsed and then cooled to a temperature of 5°-10°C, while the left lung
is ventilated by means of a Carlens endotrachael cannula. The right lung
is cooled either by surface cooling (ice-cold saline) or by using refrig-
erated fluorocarbon fluid pumped through the right tube of the Carlens cannu-
la. After the desirable level of cooling of the right lung is reached, one
lobe of the same lung is rewarmed to a temperature of 38°C by microwave
heating. The labeled microspheres (usually ^{99}technetium) were administered
then through the bronchial artery of the right lung. On the basis of heavy

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concentration of radioactivity in the rewarmed part of the cooled lung (deep well technique), we estimated that circulation through the rewarmed part of the lung is 6-37 times larger than through the same lung that was cooled to a temperature of 5°-10°C (Fig. 31-33). Similar results were obtained when a hind leg was surface cooled in dogs to a temperature of 10°-12°C while the gracilis muscle was rewarmed (ultrasound or microwaves) to 38°C. After injection of labelled microspheres into the abdominal aorta, the tissues of the cold leg received little radioactivity while the warm muscles received 4-6 times more radioactivity suggesting an increase in blood flow. This high perfusion pattern through warm muscles in a hypothermic leg led to the idea of using ^{90}yttrium (a pure beta emitter) labelled microspheres for localized preferential embedding of microspheres and sharply localized internal irradiation without the use of superselective catheterization of small blood vessels (a task which is usually rather difficult and sometimes impossible). The damage done to the warm muscle cells of the cooled leg was proportional to the amount of administered yttrium. Administration of 10 millicuries (femoral artery) led to a complete disintegration of warm muscles leaving undamaged muscles of the same leg that were cooled (Fig. 34). It appears, therefore, that differential hypothermia might be a useful tool for localized embedding of radionuclides, as well as other biologically or medically important pharmaceuticals, without the use of superselective catheterization of small blood vessels.
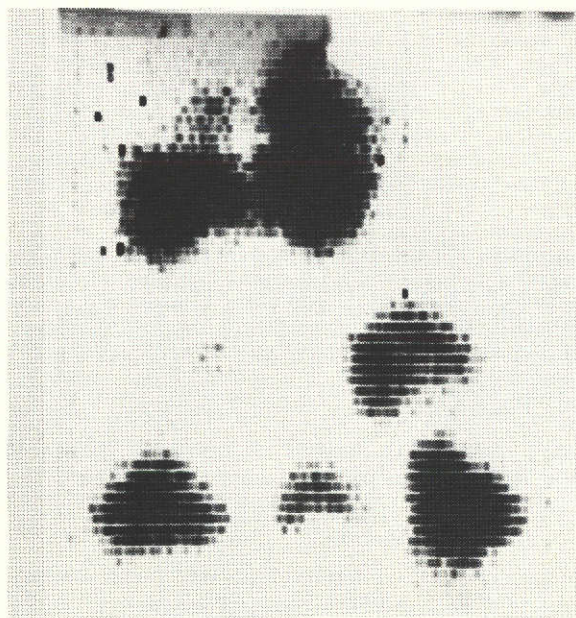

Fig. 31. Embedding of Technitium (T99m) labelled microspheres in right collapsed lung. Temperature of the lung: 37°C. (Left upper part: all lobes of the right lung scanned together).
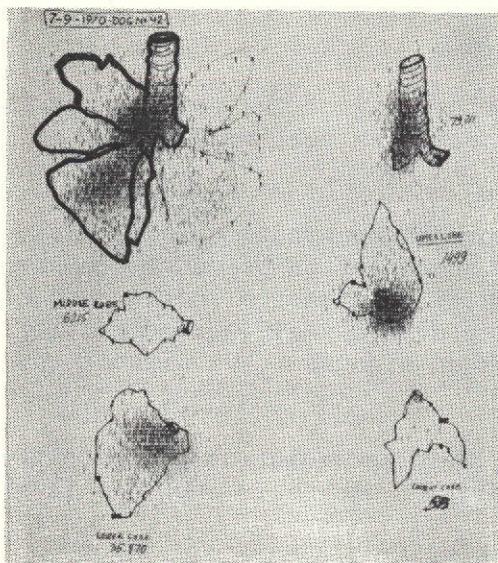
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Fig. 32. Embedding of Technetium (Tc^{99})^m microspheres in right collapsed lung. Temperature of the lung 4°-8°C. Lower lobe microwave rewarmed to a temperature of 38°C. Radioactivity in the lower part of the upper lobe is from blood in a larger blood vessel.

Fig. 33. Muscles from left hypothermic leg and from right normothermic leg after administration of (Tc^{99})^m labelled microspheres in the middle part of aorta. Muscle gracilis in the left hypothermic leg has the same temperature (37°C) as in the right normothermic leg but its embedding of (Tc^{99})^m is six times greater.

Fig. 34. Localized rewarming of gracilis muscle in the right hypothermic leg leads to preferential embedding of administered Yttrium microspheres and to damage and disappearance of the whole muscle.
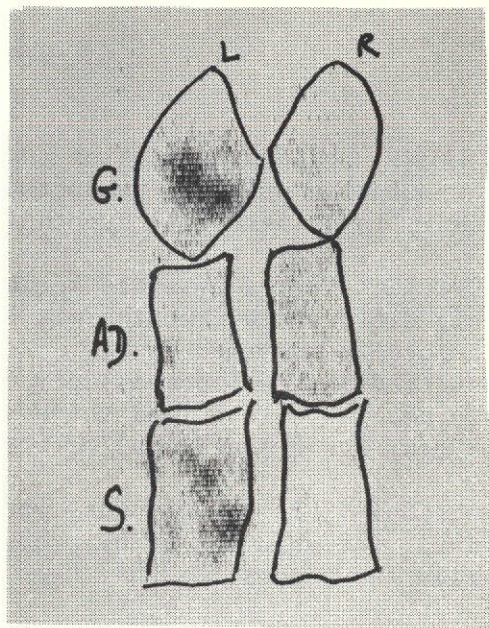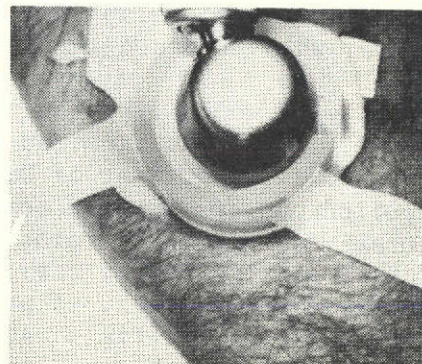

Fig. 35. Differential hypothermia in the treatment of actinokeratosis of the extremities (see discussion, p. 172).

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HOLTON: You didn't mention anything, I noticed, about the possibility that accumulation of waste products might be a mechanism for irradication of tumors in differential hypothermia.

POPOVIC: Yes, a possible mechanism of tumor disappearnace after 10-hour long differential hypothermia might be the accumulation of waste products in locally rewarmed tumors. A similar problem is observed in the case of assisted (extracorporeal) circulation, where even a small periodical reinstatement of blood flow through an organ or an organism (but without any oxygen) has a beneficial effect.

MUSACCHIA: In other words, you are saying that accumulation of waste products, accumulation of by-products of metabolism are there and this is what kills the tumors.

POPOVIC: That may be so. I'm talking here about tumor treatment with differential hypothermia but without chemotherapy.

JORDAN: If you accept the "garbage theory", you run into kind of a conflict if you want to move oxygen to the tissue that's warmed so that it will metabolize more rapidly and produce more end products. At the same time, you are carrying away some of the end products.

POPOVIC: We are talking here about two different mechanisms. First, we are talking about possible mechanisms of tumor eradication after 10-hour long differential hypothermia but without chemotherapy. Here is where accumulation of waste products might have some effect. But this does not apply when only one hour differential hypothermia with chemotherapy is employed.

JORDAN: You don't think chemotherapy is a combination of mechanisms.

POPOVIC: Chemotherapy and 1 to 4 hour differential hypothermia work much better when combined than either approach alone.

Briefly, I would like now to discuss some of the results obtained during human clinical application of our technique. In collaboration with Dr. Brown (Department of Dermatology, Emory University Medical School) we applied our approach to some skin cancers. Initially, we limited ourselves to actinokeratosis, a premalignant cancer disease that can be rather easily treated with topical application of FU when the lesions are on the face and chest of patient, but with difficulties when the lesions are on the extremities. After confirming, as we expected, that the temperature of the skin on limbs is much lower than the skin temperature of the trunk or of the face, we used topical FU with or without ultrasound heaters. Lesions that were heated three times per week for 4-6 weeks healed fully while similar lesions without heating were not affected by topical FU (Figure 35). Biopsies confirmed these findings.

Our technique has been used in human clinical practice in Japan during the last three years. This work was done by Dr. A. Nishimoto in the Department of Neurosurgery, Okayama University. Dr. Nishimoto applied our approach to recurrent gliomas of the brain, one of most difficult tumors to be treated. Dr. Nishimoto used the combination of differential hypothermia and chemotherapy after the brain tumors recurred for the third time (usually 4 to 8 months between each operation). Before starting the clinical application of our technique Dr. Nishimoto and his associates repeated our work using mice and later hamsters with virus induced brain tumors. They got results identical to ours with mammary tumors. Dr. Nishimoto's results, reported to the International Congress of Neuosurgery in Prague last year, were encouraging. Of all the patients treated only one patient died. Autopsy showed that even in this case the brain tumor was histologically and histochemically recessing and its cells were no longer malignant. This particular patient died because of other metastic growths in the body. All other patients are still doing fine. The first patient, a 12-year old boy, is attending school three years after the hypothermia treatment.

HOLTON: Did Dr. Nishimoto use differential hypothermia alone or in combination with chemotherapy.

POPOVIC: The Japanese team used the combination treatment. Sometimes they used one single anticancer drug, sometimes a combination of anticancer drugs, but always with differential cooling of the brain.

JORDAN: How long were the children cooled?

POPOVIC: Three to four hours. My associates and I are trying now to develop a similar approach for brain tumor treatment, using dogs as experimental animals. Contrary to the work of the Japanese team who cool the whole body of patients and therefore cannot descend below a temperature of 28°-30°C (surface cooling), we cool only the head of our animals while the rest of the body stays normothermic. The head is cooled by extracorporeal cooling of the blood of one of the carotid arteries (White's technique). When the brain temperature reaches 10-15°C, the area of the brain designated as "tumor" is rewarmed to 38°C by ultrasound or microwave heating.

MUSACCHIA: How is this cooling of the brain done? By external surface cooling?

POPOVIC: No, by extracorporeal cooling of blood in one of the carotid arteries. Initially the brain is cooled differently but when lower temperatures are reached, the temperature difference between one and the other side of the brain is only 1°-2°C. We are trying also to apply Dr. Wolfson's technique for a similar work. This technique consists of complete cessation of circulation for one hour after localized perfusion cooling of brain (ice cold saline, injected in the internal carotid artery) and simultaneous induction of ventricular fibrillation. In this case the brain is cooled more than the rest of the body, but both animals as

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well as a few patients survived this treament well. We are thinking of using the same technique with localized rewarming of brain tumors. We hope that such a treatment might be very effective either alone or when combined with chemotherapy. In the second case the escape of anticancer drugs into other vascular beds will be very small if any.

MUSACCHIA: Who is Wolfson?

POPOVIC: Sidney Wolfson, until recently Director of Surgical Research, Michael Reese Hospital, Medical Center, Chicago.

OYAMA: I'm absolutely fascinated by what you've presented this morning and I'm very impressed with your technical proficiency. How do you prevent an animal from biting off its external cannulas?

POPOVIC: It depends upon the animal species. In rats we have no problem, they don't care about the exteriorized cannulas and the cannulas never bother them. The exteriorized part of the cannulas is rather short. It is important that this part be kept short or the animals might chew on the cannulas. A similar technique is used for hamsters and for ground squirrels. In mice we use a different technique because they are more agile; they often jump suddenly and since they might easily pull the implanted cannulas while connected to the extension set of catheters, we place them usually in a chamber where they can turn and feel unrestrained but cannot jump much even if they want to. In the squirrel monkey we make a crown of dental acrylic material on top of its head with one hole in it so that one can pull out the cannulas during an actual experiment. Otherwise, monkeys chew their cannulas.

OYAMA: Have you run various hormone levels in the blood of these animals after prolonged cannulation?

POPOVIC: Presently our cannulation technique is being used in many laboratories. In our department several endocrinologists are using our technique to obtain blood samples exactly for this purpose: to obtain resting levels of hormones they are interested in.

OYAMA: And they have found no abnormal levels?

POPOVIC: It goes the other way. For example, lactic acid (which I have measured in rats before, during, and after exercise) has been found to be at a high level even during rest. This is because rats must be handled during withdrawal of blood. But with our cannulation technique we can determine the basic level of lactic acid. The return to normal values is only after 1 to 2 hours from the time the animal was initially disturbed (placed into the chamber).

OYAMA: What is the heart rate and respiratory rate of rats under these conditions?

POPOVIC: We have published this work in the "American Journal of Physiology " (207, 767, 1964). The heart rate of normal undisturbed young rats is about 420/min. The respiratory rate is, I think, about 60.

OYAMA: Are you recording body temperature in your animals?

POPOVIC: We continuously monitor body temperature. The body temperature of our cannulated animals is in normal limits. Naturally, one sees individual variations.

OYAMA: What do you feel is the most critical factor in recovery of the dogs cooled to a body temperature of 4°C?

POPOVIC: I think one must combine several things to have fast and full recovery of these animals. I have discussed them with you already. My impression is, however, that some aspects are more important since without them the dogs never recover. Drainage of the left heart, hemodilution and hypervolemia during rewarming, are probably the most important factors for full and fast recovery of deeply cooled dogs after 1-2 hours of cessation of circulation.

MUSACCHIA: Do you think that hemodilution has any role in preventing stasis in the capillary beds, emboli, etc.?

POPOVIC: I think that hemodilution has something to do with an increased circulation in the capillary beds permitting a large venous blood drainage. Otherwise, one cannot drain enough blood from the animal. The blood is obviously pooling somewhere in the venous bed.

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