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DEPARTMENT OF THE ARMY
OFFICE OF THE SURGEON GENERAL
WASHINGTON, D.C. 20315

IN REPLY REFER TO:
MEDPS-PO

16 January 1970

Isotopes Branch
Division of Materials Licensing
U. S. Atomic Energy Commission
Washington, D. C. 20543

Gentlemen:

The Commanding General, Fitzsimons General Hospital, has requested AEC License Number 05-00046-15 be amended to indicate the use of the following isotopes in lower animals by personnel of the U. S. Army Medical Research and Nutrition Laboratory (USAMRNL) located at the Pikes Peak facility:

Byproduct Material	Chemical Form	Amount
a. Potassium^{42}	Chloride	10 millicuries
b. Sodium^{24}	Chloride	10 millicuries

These studies will be under the supervision of the Radioisotope Committee, Fitzsimons General Hospital and the Radiation Protection Officer, USAMRNL.

Protocol of the planned study is inclosed.

Sincerely,

James E. Anderson

1 Incl
as

JAMES E. ANDERSON
LTC MSC
Preventive Medicine Division

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August 1969

USAMRNL
PHYSIOLOGY DIVISION
PROTOCOL

PROJECT NO.	3A104501B71R	Research in Biomedical Sciences
TASK NO.	05	Environmental Medicine
WORK UNIT NO.	082	Metabolic Effects of Altitude

STUDY NO. 13: Effects of Altitude on Body Fluids

I. INTRODUCTION

Numerous studies (1-5) over the last 75 years have shown altitude exposure to be associated with a marked reduction in plasma volume. As shown by the experiments of Gregg, Lutz and Schneider (6) the onset of this reduction occurs within minutes of the onset of hypoxic exposure. It persists, as shown by Reynafarje (5) and Hannon (6, 7), for at least 2-1/2 months and perhaps longer. This response, furthermore, is not limited to humans. It is seen in many lower mammals including the rat (8), rabbit (2, 9) and dog (10, 11), yet it is apparently not seen in ruminants such as the sheep (12) and goat (13).

The factors responsible for plasma volume loss are not known. In part, it may be due to a transfer of blood from the systemic to the pulmonary circulation (14-16). It may also be related to the increases in lymph flow, primarily from the viscera which accompany hypoxic exposure (17, 18, 19). The loss of plasma volume is not due to simple dehydration (7, 20). Rather, it is associated with a proportionate loss of extracellular and interstitial volume. The latter has been seen both in humans (20-22) and rats (23) but not in sheep (12). In humans and rats the fluid loss from the extracellular space is almost exactly balanced by a gain in intracellular fluid volume (20, 21, 23).

H^3, Br^{82} K^{42} S^{35} Na^{24}

14305

The shift of fluid from the extra- to the intracellular space is accompanied by a marked loss of extracellular ions, particularly sodium, chloride and bicarbonate (28). Presumably, these ions are deposted in the intracellular space, but this has not been directly established (28).

For many years it has been known that the metabolic rate of a tissue has a marked influence on its state of hydration (24-26). If the metabolic rate is depressed, or more specifically if the capacity for oxidative phosphorylation is depressed (27), tissue hydration increases. Hypoxic damage to cells, furthermore, has a much more pronounced effect on oxidative phosphorylation than on oxygen consumption, e.g. it uncouples oxidative phosphorylation (28). The increases in tissue hydration under these circumstances are attributable to increases in intracellular osmotic activity (29, 30).

II. , OBJECTIVE

The purpose of this study is twofold: first, to define the specific sites within the body where water is transferred from the extra- to the intracellular space and second, to delineate, if possible the specific functional defects which are responsible for this transfer. Several experiments will be required. Four of these are described below.

III. JUSTIFICATION

There is an ever increasing amount of evidence that the debilitating symptoms of acute altitude sickness may be related to alterations in water and electrolyte metabolism. Stampfli and Eberle (31) and later Ullmann (32) observed oliguria in subjects experiencing high altitude sickness and polyuria in subjects not

afflicted by this malady. Ullmann also found (32) acute altitude sickness to be associated with sodium retention (32). Schaltenbrand, Michelson and Thompson (34) and later Singh et al. (35) attributed the symptoms of mountain sickness to an increase in cerebrospinal fluid pressure. Ullmann (32) observed sodium retention in subjects experiencing altitude sickness and such a retention is also suggested in the work of Waterlow and Bunje (36). Singh et al. (35) reported increased body hydration and cerebral edema were responsible for the symptoms of mountain sickness. In contrast to this last report we found (unpublished data) a negative correlation between body hydration and certain symptoms of mountain sickness. Serum sodium levels and extracellular volume were also correlated with certain symptoms, but insufficient data were available to determine whether intracellular volume or other electrolytes were correlated.

IV. METHODS AND MATERIALS

A. Experimental Design

1. Experiments involving actual altitude exposure will be conducted on New Zealand White rabbits and mongrel dogs, using the laboratory in Denver as a low-altitude site and the Pikes Peak Laboratory as the high-altitude site. All such studies will be conducted on groups of 10 animals, the number of groups being dictated by the particular experiment being conducted. Animals will be randomly assigned to these groups, and at both high and low altitude sites they will be housed in individual cages. They will be given standard laboratory feed and water ad libitum. Consumption will be recorded. Data accumulated in these experiments will be analyzed by analysis of variance, paired t tests or

other statistical techniques as appropriate. The specific objectives, environmental conditions and methodology attendant to the first of these experiments are described below. The design and details of later experiments will be dependent upon the outcome of these initial efforts and hence will be developed and described later.

2. Certain experiments directed at the mechanisms underlying the alterations in water and electrolyte metabolism under hypoxic conditions will be conducted at this laboratory. Again, groups of 10 animals, individually housed and ad libitum fed and watered will be used. Mice, rats, guinea pigs, rabbits, and dogs will be used in these experiments as the objectives of each particular investigation dictates. In some instances the intact animal will be used in these experiments, while others will employ in vitro techniques. Hypoxia will be induced by exposure in the environmental test chamber or with low oxygen gas mixtures. Details concerning the methodology to be used in these experiments will be described below.

3. Short-term (1, 3, 7, 14 days) and long-term (>30 days) exposure to high altitude will be studied.

Experiment 1. The determination of total body water, extracellular and intracellular water.

In this experiment total body water and extracellular space will be monitored to determine whether the two species (dog and rabbit) show the same alteration in water distribution at altitude that has been shown previously in man and rat. Secondly, conventional chemical and radioisotope dilution procedures will be used to

determine the reliability of the radioisotope procedure to reflect the two body water compartments. Antipyrine and 30μc of ^{3}H (as $^{3}H_2O$) will be used to monitor total body water. Thiocyanate, 3μc of ^{82}Br (as $Na^{82}Br$) and/or 10μc of ^{35}S (as $Na^{35}SO_4$) will be used to monitor extracellular water. After serial serum sampling (37, 38) urine and serum will be assayed for the presence of chemicals by appropriate techniques of Huckabee (39) and Bowler (40). To monitor radioactivity, from each sample of blood and urine tritiated water will be sublimated and counted according to the method of Cooper et al. (41), bromide or sulfate will be separated in an ion exchange column with Amberlite-IRA-400 and counted according to the method of Rovner and Conn (38).

Experiment 2. The determination of intracellular and extracellular water compartments of various organs.

In this experiment the extracellular and intracellular water distribution in various tissues will be ascertained. Thirty μc of ^{3}H (as $^{3}H_2O$) and 10μc of ^{35}S (as $Na^{35}SO_4$) or 3μc of Br (as $Na^{82}Br$) will be injected intravenously.

After sufficient time for equilibration, the animal will be sacrificed, various tissues will be excised, homogenized and radioactivity of the supernatant will be monitored as described in Experiment 1. The tissues to be examined are: brain, heart, kidney, striated muscle, blood, liver, spleen, pancreas, stomach, small intestine, large intestine, lung and urine.

Experiment 3. The determination of total exchangeable Na, K and Cl.

In this experiment exchangeable Na, K and Cl will be monitored. Four μc of ^{24}Na (as $^{24}NaCl$), 12 μc of ^{42}K (as ^{42}KCl) and 3μc of ^{82}Br (as $Na^{82}Br$) will be intravenously injected. Twenty-four hour samples of blood and urine will be collected. Following ion exchange by conventional columns to separate Br, Na and K will be delineated in each sample by short-term differential counting (38).

Experiment 4. Effects of hypoxia on the in vitro distribution of tissue fluid and electrolytes.

Diaphragm tissue will be excised from adult male mice and incubated at 37.5 C in Erlenmeyer flasks containing phosphate buffered Ringer's solution as previously described (26). Initially, changes in tissue water content as a function of hypoxic exposure time will be determined gravimetrically. The gas phase in the flasks will contain either 100%, 50%, 21%, 15%, 10% or 5% O_2 (separate tissue preparations will be used for each of these hypoxic conditions). Subsequently, tracer amounts of, $^{3}H_2O$ will be used to verify the gravimetric data for specific hypoxic conditions, e.g., 10% O_2. In later series of measurements, tracer amounts of $^{35}SO_4$ will be used to assess changes in extracellular space and intracellular space will be determined by difference. ^{24}Na and ^{42}K will be used to measure changes in cation distribution as a function of hypoxic exposure. In these later measurements the effects of hypoxic exposure e.g., 10% O_2, will be compared to normoxic (21% O_2) or hyperoxic (100%) exposure. Finally, the relationships of tissue oxygen consumption to water and electrolyte metabolism as well as the influences of various substrates will be determined manometrically.

Other experiments.

As the foregoing experiments are completed, other experiments designed to delineate the cause(s) of water and electrolyte shifts that occur during high altitude exposure will be designed and submitted as an Addendum to Protocol.

V. HANDLING AND DISPOSAL OF RADIOISOTOPES

Precautions will be taken during the experiments to maintain radiological health standards.

The laboratory areas will be monitored daily with a Nuclear Chicago labitron ratemeter and a nuclear Chicago B-V survey meter. Animal carcasses, all excreta, radioactive samples and contaminated syringes and needles will be labelled and disposed of at USAMRNL as outlined in "Procedures for Use of Radioactive Material" (see Appendix E, Application for Renewal and Amendment to AEC Byproduct Material License No. 5-46-13(A66), dated June 1966). All rules, regulations, and limitations set forth by Army AEC and local authorities, including those embodied in AR 70-23; AR 40-35, Title 10, Part 20, Code of Federal Regulations "Standards for Protection Against Radiation"; and Handbook 69 of the National Bureau of Standards will be complied with.

VI. ADMINISTRATIVE

A. This will be a Physiology Division project, and the personnel, equipment and supplies as well as the analytical work will be supported by this Division. The animals and animal care facilities will be provided by the Pathology Division.

B. The project leader will be Dr. John P. Hannon. The

associate investigator will be Dr. Francis J. Sullivan, and two junior investigators.

C. COST

1. Salaries:

Dr. Hannon - 20% of time for 6 weeks	$561.00
Dr. Sullivan - 50% of time for 6 weeks	$816.00
Pvt Wooldridge - 100% of time for 6 weeks	$294.00
Pvt Schwalm - 100% of time for 6 weeks ...	$294.00

2. TDY (Experiments 1, 2 and 3)

2 investigators x 10 days x $16.00/day....	$320.00
2 junior investigators x 10 days x $16.00/day	$320.00
CQ, PPLF, 16 days x $8.00 per day	$128.00

3. Transportation

One GSA vehicle, 20 days x $8.00/day.....	$160.00
4. Supplies	$400.00
5. Radioisotopes	$1,200.00
Total	$4,493.00

D. Unless otherwise stated in this application or in our existing license, all rules, regulations and limitations set forth by the Army, AEC and local authorities will be complied with.

John P. Hannon

JOHN P. HANNON, Ph.D.
Chief, Physiology Division

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