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PHYSIOLOGICAL ADAPTATIONS OF MAMMALS OF THE ARCTIC SHORE. (U)
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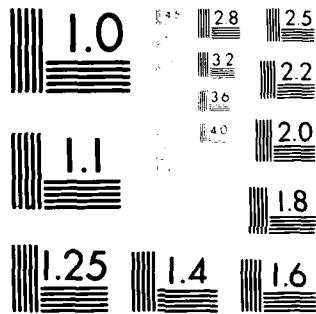
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ANNUAL REPORT, 1976-1977

THE ARCTIC INSTITUTE OF NORTH AMERICA
for 1976-1977

On Project

Physiological Adaptations of Mammals of the Arctic Shore

Contract Number: ONR 455 (amended)

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SELECTED
JUN 3 1981

Report Presented July 1978

Responsible Investigator: G. Edgar/Folk, Jr.
Investigator: Mary A./Folk
Staff:

- Peter Ringens, M.D., Research Associate
- Barbara Grubb, Ph.D., Post-doctoral Fellow
- Karen Hagelstein, M.S., Student
- Martin Steiner, B.S., Student
- Jill Hunt, B.S., Medical Student
- Louis Arp, Technician

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(This report is organized in Parts, rather than Chapters.)

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1. Introduction

A. Objectives:

- a. To continue experiments on a hibernation-inducing factor obtained from arctic marmots.
- b. To do a study of photoperiodicity on brown lemmings, to test the function of their exceptionally large pineal gland by using four animal groups: continuous light control; continuous darkness control; and the same with pineal glands removed.
- c. To collect urine and blood from the polar bear, Irish, maintained at the Naval Arctic Research Laboratory, while he was under three dietary conditions: high fat, high protein, and fasted. This bear weighs 1,000 pounds.
- d. To determine whether the dominant wolf in the Behavior Pack at NARL is the breeder in the pack. The data are still being analyzed.

B. Procedure:

- a. The winter and spring of 1976 were spent preparing for summer field work at NARL. Two observers (E. Folk, M. Folk) went to NARL in March to make 24-hour observations of the wolf pack for two weeks.
- b. During June, July, and August of 1976 the experiment on brown lemmings was completed by E. Folk, M. Folk, P. Ringens, and an assistant, Louis Arp.
- c. During July, the polar bear, Irish, was studied, using the new urine collector funnel, essentially a chute upon which he stood. Twenty-four-hour urines were collected while the bear was under three dietary regimens. During 1977 these samples were analyzed in the Laboratory of Environmental Physiology: 30 determinations of each sample (voiding), often 20 samples per day. During 1977, Dr. Michael Philo was to repeat the dietary plan, taking blood samples, to correlate with the urine samples. The blood samples were not taken until spring 1978, and these results are now being combined with the urine analyses.
- d. The work of the year 1977 was involved with further experiments on the induction of hibernation by extracts of marmot blood. This material has been compared with hamster hibernating blood. The recipients were summer ground squirrels, which were not in "hibernating state". Both marmot and hamster hibernating blood were effective in producing hibernation.

The rest of 1977 was spent in preparing manuscripts for publication. Each one is considered separately in the following sections. The relation to earlier projects is explained. For example, the first manuscript was submitted by Dr. Joel Berberich from his thesis work on lemmings at NARL.

In many cases reprints have been provided to John Sater, Research Coordinator, AINA.

Part 2.

Title: Effects of cold exposure on urinary catecholamines in arctic lemmings.

Authors: J.J. Berberich, R.V. Andrews, and G.E. Folk, Jr.

Journal: Comparative Biochemistry and Physiology, 58C, pages 133-135, 1977.

Status: Published

Coordination with Program: Dr. Berberich did his thesis work at NARL on cold acclimation of lemmings. This paper on norepinephrine after cold exposure is a valuable contribution from his thesis data. The samples were collected at Point Barrow, then taken for analysis to Dr. Andrew's laboratory.

Part 3.

Title: Thermogeneiss of specific organs in cold acclimated rodents.

Authors: G.E. Folk, Jr. and B. Grubb

Journal: Federation Proceedings, Symposium Volume, 1978.

Status: As usual, the publication of this symposium is delayed. The title was "Thermogenesis."

Coordination with Program: This article was supposed to contain new information not published earlier. I included three principles which we showed at NARL: 1) the brown lemming becomes cold acclimated, instead of being acclimated all the time; 2) it shows a very large cold diuresis as part of cold acclimation; 3) it shows a conspicuous response to the norepinephrine test for cold acclimatization.

Part 4.

Title: Induction of summer hibernation in the 13-lined ground squirrel shown by comparative serum transfusions from arctic mammals.

Authors: W.A. Spurrier, G.E. Folk, Jr., and A.R. Dawe.

Journal: Cryobiology, vol. 13, pages 368-374, 1976.

Status: Published.

Coordination with Program: For several years we have collected serum from arctic marmots hibernating in our Laboratory of Environmental Physiology. These are difficult experiments. We are trying to find a better bioassay than simply injecting our extracts into ground squirrels in June and then waiting for them to go into hibernation in July. We are now purifying the extract we use by chromatography and electroporesis.

Part 5.

Title: Spontaneous and induced summer hibernation in 13-lined
ground squirrels.

Authors: Martin Steiner and G.E. Folk, Jr.

Journal: Cryobiology, 1978.

Status: In press.

Coordination with Program: Same as Part 4.

Part 6.

Title: Further evidence for hibernation in bears.

Authors: G.E. Folk, Jr., J.M. Hunt, and M.A. Folk.

Journal: Publication series of the International Union for Conservation of Nature, Symposium volume, 1977, No.42.

Status: In press.

Coordination with Program: We had recorded heart rates for as much as three weeks at a time, from three species of bears. During 1976, and 1977, we reanalyzed many crates of old records and selected the good EKG records. These are reported upon in the above paper, a symposium volume of the Society for Study of Biology of Bears.

Part 7.

Title: The role of adrenoceptors in norepinephrine stimulated
 $\dot{V}O_2$ in muscle.

Authors: B. Grubb and G.E. Folk, Jr.

Journal: European Journal of Pharmacology, vol. 43, pages 217-
223, 1977.

Status: Published.

Coordination with Program: Barbara Grubb joined our program as a NIH Postdoctoral Fellow for 1976 and 1977. Her research was to compare the thermogenesis of leg muscle of cold acclimated rats with cold acclimated lemmings. We maintained brown lemmings for her, provided by NARL. She built equipment to perfuse leg muscles, and published two fundamental, authoritative papers resulting from this development, on the white rat, and then turned her attention to lemmings. The paper on lemmings has been submitted to the Journal of Comparative Physiology.

Part 8.

Title: Ketamine-HCl anesthesia for the brown lemming (Lemmus trinucronatus).

Authors: P.J. Ringens, G.E. Folk, Jr., and C.B. Thayer.

Journal: Journal of Veterinarian Research.

Status: Returned by journal for changes. Is being rewritten.

Coordination with Program: This paper contains useful material concerning a new drug for lemming anesthesia. The principles worked out have been applied very successfully to our small arctic rodents such as tundra voles and red-backed voles.

Part 9.

Title: A comparison of body temperatures of least weasels and wolverines.

Authors: G.E. Folk, Jr., M.A. Folk, and D. Craighead.

Journal: Comparative Biochemistry and Physiology, vol. 58A, pages 229-234, 1977.

Status: Published.

Coordination with Program: This was a difficult experiment because we wanted to compare the body temperature of wolverines over four seasons. The technology of radio-telemetry is such that it has been very hard to obtain transmitters for the body cavity which last one year. We did succeed in obtaining summer and winter readings, as published above. The interest in this paper was astonishing. There were about 100 requests for reprints.

Part 10.

Title: The Pineal and Photoperiodism in Arctic Species.

Authors: G.E. Folk, Jr.

Journal: Chapter in The Pineal Gland and Reproduction, R.Reiter,
(ed.), Karger Publishers, Basel, Switzerland.

Status: Published, 1978.

Coordination with Program: This chapter in the book reviews the appropriate experiments related to arctic biology. These experiments are very scarce, but suggestions for future experiments are included.

Part 11.

Title: Cold acclimatization and the pineal gland of lemmings.

Authors: G.E. Folk, Jr., K.A. Hagelstein, and P. Ringens

Journal: Federation Proceedings, vol. 36, page 419, 1977.

Status: Published as an abstract.

Coordination with Program: This paper summarizes our major experiment in 1976 which concerned the effect of the pineal on lemming photoperiodism. The work was aimed at understanding cold acclimation and the fact that we have shown cold acclimation-without-cold, by using photoperiod alone. In this paper itself, there is little about cold acclimation and much about the large pineal gland of the lemming and its mode of control, in darkness, over the reproductive status of the lemming. A summary is found on the next page.

Part 11 (continued)

COLD ACCLIMATIZATION AND THE PINEAL GLAND OF LEMMINGS.

G. Edgar Folk, Jr., Karen A. Hagelstein and Peter J. Ringens.
Department of Physiology and Biophysics, The University of
Iowa, Iowa City, Iowa and the Department of Biochemistry,
University of Nijmegen, Nijmegen, The Netherlands.

We demonstrated earlier that photoperiod can induce acclimatization without cold exposure in rodents; there is correlated functioning between the pineal gland and the hypothalamus during cold exposure. Furthermore, norepinephrine (the hormone of cold exposure) is associated with melatonin synthesis. The pineal glands of rats exposed to continuous darkness show increased synthesis of melatonin. Melatonin is antigonadal in rodents. To simulate cold acclimatization by photoperiod, we tested rodents in continuous light and continuous darkness. It was appropriate to do these studies on an arctic mammal that lives in extreme photoperiods (82 days of continuous light and of darkness), the brown lemming. These animals were tested in summer in artificial continuous light and darkness, some with pineal glands removed. The pineal glands were large (.66mg, 2.44mm/100g animal) compared to those of white rats (.41mg, .71mm/100g). After 30 days, the most conspicuous results were in the testes of the lemmings (expressed as grams/100grams of animal): continuous light normal lemmings $.88 \pm .07g$ compared to continuous darkness normal lemmings $.76 \pm .04g$. Thus preparation for cold exposure involved regression of testes. Continuous light stimulates testes but not through pineal activity since testes of continuous light lemmings without pineal weighed $.80 \pm .01g$, the same as continuous light (normal). Continuous darkness acts through the pineal (perhaps through melatonin) in this species, since testes of continuous darkness lemmings without pineals were $.84 \pm .11g$ (i.e. the same as continuous light, normal). Similar although unexpected results were obtained with brown adipose tissue; in continuous light the brown adipose tissue hypertrophied compared to lemmings in continuous darkness. (Supported by The Arctic Institute of North America with approval and financial support of the Office of Naval Research under contract number N00014-75-C-0635, subcontract ONR-455).

Reference: Fed. Proc. 36: 419.

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Part 12.

Title: Effects of photoperiod, cold acclimation and melatonin.

Authors: K. Hagelstein and G.E. Folk, Jr.

Journal: Federation Proceedings, vol. 37, page 249, 1977.

Status: Published as abstract.

Coordination with Program: This paper represents two years of work by K. Hagelstein, all preliminary to applying melatonin to lemmings in order to understand the role of the pineal in Arctic photoperiodism. The summary is on the next page. Her complete manuscript of 16 pages is now submitted to the Journal of Comparative Physiology.

Part 12 (continued)

EFFECTS OF PHOTOPERIOD, COLD ACCLIMATION AND MELATONIN.

Karen Hagelstein and G. Edgar Folk, Jr. Dept. of Physiology and Biophysics, and Environmental Engineering Program, The Univ. of Iowa, Iowa City, IA 52242.

We examined the effects of long (15L:9D) and short (9L:15D) daily photoperiods on white rat thermogenesis in warm conditions ($21 \pm 2^\circ\text{C}$ for 18 weeks) and again after 4 weeks of cold ($8^\circ \pm 2^\circ\text{C}$). Additionally, 4 mg/kg of melatonin was implanted in 18 of the long and short day rats prior to warm and cold exposure, to assess the possible mechanistic role of the pineal gland in the photoperiodic alteration of thermogenesis in preparation for cold. Combined short day and cold exposure elicited a number of responses: 1) a positive response at room temperature to NE (basal rate increased 23%); 2) growth of brown adipose tissue (BAT) (P .05); 3) an increase in the mean lowest daily rectal temperatures (P .01). Contrary to expectations, exogenous melatonin appeared to inhibit BAT growth in short day, cold acclimated rats. Melatonin depressed the mean daily lowest rectal temperature under warm conditions and raised the rectal temperatures of long day rats under cold conditions (P .01). Cold acclimation and short day, potentiated by melatonin, induced an increase (P .02) in mean pineal weight over long day, melatonin-treated, cold acclimated rat pineals. (Supported by AINA under ONR).

Reference: Fed. Proc. 37: 249.

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Part 13.

EXPENDITURES

<u>Travel to Point Barrow:</u>		\$3734
E. Folk	March 5, 1976	\$578
M. Folk	March 5, 1976	578
E. Folk	July 1, 1976	637
M. Folk	July 1, 1976	637
Peter Ringens	July 1, 1976	654
Louis Arp	July 1, 1976	650
<u>Travel to National Meetings:</u>		490
1. B. Grubb to FASEB, Anaheim		306
2. E. Folk to ASZ, New Orleans		184
<u>Salaries:</u>		3361
3. Peter Ringens		400
4. Mary DeVos (Animal Care in Lab.)		503
5. Jill Hunt (Technician)		179
6. Karen Hagelstein (Graduate Asst.)		476
7. Secretarial		1803
<u>Hardware:</u>		408
Parts and Electrical		130
Batteries and Radio Parts		170
Animal Cages		108
<u>Animal Care Charges in University Facility:</u>		938
For Marmots		291
For Lemmings		294
For Control White Rats		353
<u>Xerox:</u>		174
Reports and Publications		174
<u>Storeroom:</u>		192
Glassware		98
Chemicals		94
<u>Repairs:</u>		265
Refrigerator		86
Cold Box		72
Dictating Machine		17
Cold Room		55
Thermofax		35
<u>Office:</u>		564
Supplies		151
Postage		215
Telephone		198

Expenditures (continued)

<u>Freight:</u>		\$ 285
To and From Barrow	285	
<u>Photography:</u>		245
For Publications	245	
<u>Surgical Supplies:</u>		250
Microinstruments	67	
Drugs, Sutures, Supplies	183	
<u>Reprints and Literature:</u>		353
Reprints	295	
Government Documents, Monographs	58	
	GRAND TOTAL	<u>\$11,234</u>

Part 14, Explanation of Budget Items

Salaries: There was no salary charge for the technical assistant, Louis Arp. He received no compensation but his airplane travel from The University of Iowa was paid (\$650). Peter Ringens has been on this project for three years and has contributed numerous publications. He received recompense of \$400. Results of this work are found in paper number 100 and number 112; and in preliminary report number 89 and number 96. Mary DeVos took care of lemmings and at various times, especially during the hibernation season, several marmots. For this she was paid \$503. Jill Hunt analyzed records of heart rate and EKG obtained by radio-telemetry, while she was a medical student. Her pay was \$179. The results of this work is found in paper number 111, and preliminary reports 90, 91, and 97. Karen Hagelstein carried out the work which determined how we could administer melatonin to lemmings and rats. For this she was paid \$476. Her work is recorded in preliminary report 89, and 99, and her work is discussed in detail in the body of this report. Mrs. Louise Janes was recompensed for her work as a general coordinator, and for editing and typing reports, duplicating reprints, correspondence, and manuscripts (\$1,803).

Travel: M. Folk and E. Folk traveled twice to Point Barrow. The time spent there in March was for the study of wolves and wolverines. The travel of Barbara Grubb was to the Federated Association for the Study of Experimental Biology, at Anaheim, California. The travel cost was \$306 and she delivered a paper (#105) and a preliminary report (#85). The travel for E. Folk to a meeting was the American Society of Zoologists in New Orleans. There he delivered preliminary report #86 which resulted in the published paper, #110.

Animal Care Charges: Although during experiments we maintained a large series of animals in the Laboratory of Environmental Physiology, at various times of the year they had to be kept in the Animal Care Facility of the University. This involved primarily marmots, ground squirrels for the bioassay of hibernation factor, and white rats. The charges over essentially a two-year period amounted to \$938.

We consider that all other items on the Budget are self-explanatory.

PAPERS

100. 1977 Ringens, P., G.E. Folk, Jr., and J.J. Berberich. Cold acclimation in the tundra vole. *Acta Theriologica* 21:67-74.
111. 1977 Folk, G.E., Jr., J.M. Hunt, and M.A. Folk. Further evidence for hibernation in bears. Pub. series of Int. Union for Conservation of Nature, Morges, Switzerland, No. 42, 463 pp., C.J. Martinka (Ed.).
112. 1977 Ringens, P.J., G.E. Folk, Jr., and C.B. Thayer. Ketamine-HCl anesthesia for the brown lemming (Lemmus trimucronatus) (submitted).

PRELIMINARY REPORTS

89. 1977 Folk, G.E., Jr., K.A. Hagelstein, and P. Ringens. Cold acclimatization and the pineal gland of lemmings. *Fed. Proc.* 36:419.
90. 1977 Folk, G.E., Jr., J. Hunt and M.A. Folk. EKG by radiotelemetry of hibernating bears. *Fed. Proc.* 36:543.
91. 1977 Folk, G.E., Jr., J. Hunt and M.A. Folk. Hibernation of Bears: Further Evidence and Bioenergetics. *Proc. of 4th Int. Conf. on Bear Research, Feb., Univ. of Montana, IV*:21.
92. 1976 Folk, G.E., Jr. Symposium on Photoperiodism, Chairman's Report (7th Congress, Univ. of Maryland) *Int. J. Biometeorol.* 20:211.
93. 1977 Folk, G.E., Jr., M.A. Folk, and A.B. Moos. Obtaining body temperatures of small mammals. *Abstracts Iowa Acad. Sci.* 89:53-54.
94. 1977 Steiner, M., G.E. Folk, Jr., and A.B. Moos. Induction of summer hibernation in 13-lined ground squirrels. *Proc. Iowa Acad. Sci.* 89:54-55.
95. 1977 Folk, G.E., Jr. Hypothermia in Biology and Medicine (Book Review). *The Physiology Teacher* 6:8.
96. 1977 Folk, G.E., Jr. and P.J. Ringens. Cold acclimatization and the pineal gland. *Proc. Internat. Congress Physiological Sciences, Paris.*
97. 1977 Folk, G.E., Jr., J.M. Hunt and M.A. Folk. The EKG of mammalian hibernators and non-hibernators. *College of Medicine Research Day.*

(These are the few pertinent excerpts from recent publications lists.)

