THE RELATED EFFECTS OF INANITION AND LOW ENVIRONMENTAL TEMPERATURE ON THE PATHOGENESIS OF TRYPANOSOMA DUTTONI INFECTION IN THE LABORATORY MOUSE

by

WALTER ALVIN SHEPPE, JR.

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS

in the Department

of

Zoology

We accept this thesis as conforming to the standard required from candidates for the degree of MASTER OF ARTS.

Members of the Department of

Zoology

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1951

ABSTRACT

Mice restricted to half of their daily food requirement lost weight and died. This effect was accelerated at low temperatures. Mice parasitized by Trypanosoma duttoni died sooner under these conditions than controls. Low temperatures did not affect weight gain or loss, but there is some indication that the parasite may have done so. Mice at 41°F showed transient edema of the extremities, but were not significantly harmed by 19 days' exposure to the cold. They showed enlarged adrenal glands within 3 days. The post mortem appearance of the starved animals included gastrointestinal hemorrhage and degeneration.

Those mice which died within two weeks usually still had stores of fat, but those which died later did not. It is suggested that the mice at low temperatures died earlier than controls because the higher metabolic rate at these temperatures caused exhaustion of carbohydrate stores before the animals could adjust to exclusive utilization of fat. The animals at room temperature had sufficient time to make this adjustment, and lived until they exhausted their fat stores. The effect of the parasite may have been through its utilization of host carbohydrate.

These results support the hypothesis that under stress conditions an animal may be adversely affected by a parasite which is ordinarily benign.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Parasitism is a widespread phenomenon. Probably every animal has living in or upon it other animals acting as parasites. The effects of these parasites on individual hosts have received considerable attention, but little is yet known about the effects of parasitism on a natural population. It is usually thought that most parasites are relatively harmless when the host is in good condition, but that under conditions which place a stress upon the host the parasites may become dangerous. This belief is based largely on observational data and has had little experimental verification.

The writer became interested in problems concerned with wildlife parasites and diseases, and intended to do field work on them in preparation for a thesis. It was pointed out by his advisors, though, that much basic information was needed before further field work could be very profitable. In the past, studies of wildlife parasites have been largely descriptive, and therefore of limited value. An experimental approach is now needed.

Before undertaking difficult field investigations, it seemed desirable to make preliminary laboratory experiments. These would show the effects of parasites on the host under known conditions, and provide the necessary controls for comparison. It was decided to conduct such an experiment, using a laboratory animal and one of its natural parasites.

This work consisted of exposing the parasitized animals and controls to stress conditions and observing the results. The stress conditions were planned to duplicate natural stresses as much as possible. The stress period for animals in the northern regions of the world is the winter, and for most mammals and birds this stress is in the form of low environmental temperature and inadequate food supply. These were the factors chosen for use here.

The effect of a parasite on wild animals must be measured in terms, not of individual comfort or condition, but of longevity and reproductive potential. That is, in terms of the things which directly influence population size. The projected series of experiments included tests using both of these criteria, but difficulties in obtaining suitable parasites postponed the experiments so that there was opportunity to study only the effect of the parasite on survival and weight of the host.

The parasite chosen for use here was Trypanosoma duttoni, a member of the T. lewisi group of trypanosomes occurring in Mus musculus. Because it is ordinarily non-pathogenic, any adverse effect which it might show in the present experiment may be expected to be due to the experimental conditions imposed. The host was the albino laboratory mouse.

METHODS

Experimental Design

The experiment was designed to provide several combinations of the three variables being studied--parasite, temperature, and diet. Each variable was represented by only two conditions, i.e. no attempt was made to measure the effect of several different degrees of intensity of each factor. Each animal was either parasitized or unparasitized, kept at room temperature or at low temperature, and fed ad libitum or restricted to one-half of the full ration.

This plan made eight groups of mice necessary. These groups were each subject to a different set of conditions, as shown in the following chart:

Temperature	Food Consumption	
	Ad Libitum	Restricted
Warm	1. Parasitized	5. Parasitized
	2. Unparasitized	6. Unparasitized
Cold	3. Parasitized	7. Parasitized
	4. Unparasitized	8. Unparasitized

This design provided one control group, without parasites and at room temperature on full diet, and one group subject to all three of the unfavorable conditions being tested. The other groups each were subject to one or two of these conditions, and were included as indicators of the individual effect of each condition and of the collective effect of each pair of conditions.

Actually, it was not possible to achieve the clear-cut distinction between favorable and unfavorable conditions mentioned above. The microclimates of the mice in a given room were probably nearly equal, but the parasitic infection and nutritional inadequacy varied considerably.

In referring to groups and to individual mice the following symbols will be used: warm room--W, cold room--C, full ration--F, half ration--H, parasitized--P, not parasitized--N. The individual mice are numbered from 1 to 15 in each group. Numbers 1-9 are females, 10-15 males. Thus, each group is represented by three letters, e.g. CHN, and an individual mouse by three letters and a number, e.g. WHP-14.

The Host

The host animal chosen was the albino mouse, because it is inexpensive to use and easy to work with, and its needs and reactions are better known than those of most animals. Most of the work on the effects of nutrition and temperature on animals has been done on the rat, but the cost of using rats was prohibitive. Much of the information obtained from rats is probably applicable to the mouse also, though the mouse is by no means just a small-scale copy of the rat.

The mice used were all from the stock of the Animal Nutrition Laboratory of the Faculty of Agriculture. This is a local inbred strain, and the mice used represented the twelth generation of brother-sister matings. Six males and nine females constituted each experimental group. The mice were

of diverse age and weight, and from a number of litters, so that no individual comparison is possible. An effort was made to equalize the weight distribution in each group. It was felt that the number of animals in each group (15) was great enough to largely eliminate significant genetic differences between the groups.

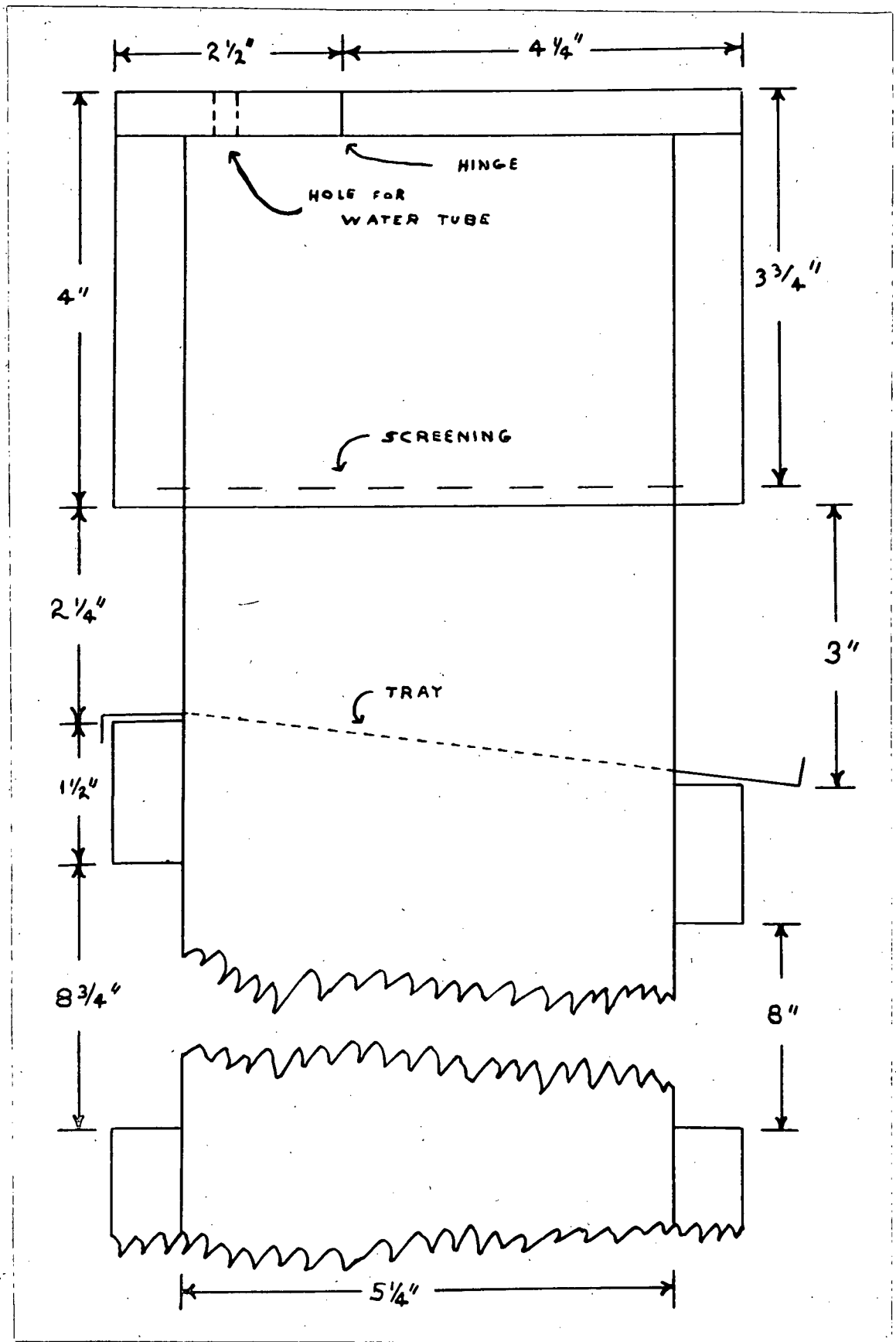
The natural mortality in this stock has been less than 1% per year, including animals destroyed because they had developed tumors. In the four-week period during which they were kept in the experimental cages before the initiation of the experimental conditions there were no natural deaths. It would seem that any deaths occurring during the experiment must probably be due to the experimental conditions, rather than to natural mortality.

The Cages

The mice were housed individually in two wooden racks. (Figure 1) The racks were built in four tiers, each of 15 individual compartments. Each tier held one of the eight groups of mice. The compartments had wooden front, back, and top, aluminum sides, and bottoms of $\frac{1}{4}$" wire screening. Access was from the top, which was hinged at the back. Three top sections served for each tier, each section covering five compartments. Continuous slits were cut in the top sections to provide ventilation.

The compartments were of approximately 10x13x9 cm. in size, giving 130 cm^2 of floor space and 1170 cm^3 volume. This

Figure 1. End view of one tier of cages. In the complete unit three other tiers occur below this. The front of the cages is on the right.


2 1/2"
4 1/4"
HINGE
HOLE FOR
WATER TUBE
4"
3 3/4"
SCREENING
2 1/4"
3"
TRAY
1 1/2"
8 3/4"
8"
5 1/4"

amount of space is large enough for ordinary purposes, but it is not large enough to keep the mice in best condition indefinitely, and may not be large enough to let them exercise as much as they wish in the cold.

Water was constantly provided from inverted half pint milk bottles. Each was provided with a short straight drinking tube inserted through a hole in the top of the cage.

The cages were kept in small private rooms, one maintained at a constant medium temperature, the other refrigerated and thermostatically controlled. Lighting in both rooms was irregular, but because of the wooden lids little light entered the cages.. Both rooms were mechanically ventilated.

The cages were designed by Dr. A. J. Wood, of the Faculty of Agriculture. They proved to be very convenient for work of this type, which involved considerable handling of mice and food, and the design can be recommended for use by other workers.

All but five of the mice had been in their cages for four weeks when infected with the trypanosomes. The remaining five were introduced at the time of infection. This four-week period provided abundant opportunity for them to become adjusted to the cages.

Experimental Temperatures

The two groups of mice--warm and cold--were housed at 68°F and 43°F respectively. The first temperature was intended as a more-or-less neutral control. All the mice had been

maintained at this temperature from birth until the beginning of the experiment. The low temperature level was a compromise between a desire to use the lowest possible temperature and the fear that the mice would be killed too rapidly if placed in very cold surroundings.

Bittner (1941) recommends a temperature of 72°F for ordinary purposes, and says that mice easily contract pneumonia. Ware et al. (1947) found that normal adult rats could maintain their body temperature at 4°C (39°F), but because the mouse is much smaller (about 1/20th the weight of the rat) it would be expected to be more susceptible to low environmental temperature.

The temperatures of both rooms varied considerably. The average in the warm room was 68.5°, in the cold room 43.3°. The ranges were 66-71° and 38-47° respectively. The cold-room temperature was observed to fall as much as 6° in half an hour.

The mice were housed individually, without bedding, on an open wire floor. This prevented their huddling together or building insulated nests to conserve heat and alter the microclimate of the environment. The temperature in the cages was one or two degrees above that outside. The cage temperatures of all four groups in both rooms were approximately equal.

The relative humidity in both rooms was measured with a Stewart Model Taylor Humiguide. This instrument is not suit-

able for accurate measurements, and gives readings considerably (perhaps 5-10%) below the actual humidity. During the period of heaviest dieoff the relative humidity in the cold room increased from 65% to 80%. Later it approached 100%. The humidity in the warm room averaged 56%, ranging from 50% to 62%.

This unequal humidity may have affected the results of the experiment. The most obvious effect of humidity is its interaction with temperature. The high humidity in the cold room would accentuate the effect of the low temperature, and the warm-room humidity is near the desired level of neutrality so that the present difference should serve to increase the temperature differential. It is entirely possible that humidity can have exerted some other, unknown, effect also.

The Ration

The diet of all the mice was qualitatively identical--Ration #10 of the Animal Nutrition Laboratory. Its composition is shown in Appendix 1. It is compressed into pellets about ½" in diameter and of variable length. These pellets were placed directly in the cage with the mouse, no effort being made to prevent wastage. A considerable proportion of the food was wasted by the careless eating habits of the mice, and all of the food data presented in a later section represent both actual food consumption and wastage.

It was expected that the mice on half-ration might utilize the food available more efficiently than those fed

ad libitum, but this was not the case. No measurements of wastage were made, but it seemed quite clear from observation of the amount of waste found in the pans below the cages that the restricted animals usually wasted at least as much food, proportionately, as did the unrestricted ones. There was considerable variation, between different individuals and for one individual at different times.

The mice in the full-ration groups were always supplied with an excess of food. The food quota for each half-ration mouse was determined individually by measuring its ad libitum utilization under the temperature conditions to which it would be exposed during the experiment. The voluntary utilization of the mice in the warm room was measured for several days shortly prior to the beginning of the experiment. The voluntary utilization of the cold-room mice was measured for the first two days at low temperatures. In each case the daily quota for the half-ration animals was set at one-half of the ad libitum utilization, though this was only approximate.

The method of measuring out the daily rations was not entirely satisfactory. Most of the rations were estimated, but frequently checked by weighing. The scales available were not accurate for the low weights concerned, and required considerable care in their use.

It may be that reduction of food volume itself, apart from reduction of nutrient intake, could be harmful to the

mouse. Carlson and Hoelzel (1948) found that added bulk increased the life span of rats. In the present work, no effort was made to equalize the bulk consumption of full-fed and half-fed mice.

Mice habitually practice coprophagy, and various workers have shown that they obtain certain vitamins in this way. One group of workers showed that rats could obtain part or all of their biotin and folic acid requirements from this source, but not their inositol, PABA, or niacin requirements (Barki et al. 1949). In the present experiment, most of the mouse droppings fell through the screen floor, beyond the reach of the animals, but some remained in the cages. Eventually perhaps a dozen or more droppings accumulated in many of the cages of full-ration mice, but not in the cages of half ration mice. It is possible that the half-ration mice were able to supplement their vitamin supply significantly in this way.

The nutritive requirements of the mouse are not as well known as those of the rat. The information available at the time was reviewed in 1944 by Morris, but he gives little data of immediate value in planning a balanced ration. Each strain of mouse probably has special requirements.

The ration used here has been in use for some time at the Animal Nutrition Laboratory, and has proved adequate for growth, maintenance, and breeding. Its biochemical makeup has not been assayed, but the computed protein content is 25%,

the fat content 6%. This protein content is within the limits recommended by Morris (20-30%), but the fat content is much below that shown by Deuel et al.(1947) to be optimal for the rat (20-40%).

It would seem at first sight to be unimportant whether the ration contains subminimal, adequate, or surplus amounts of each nutrient, since each group of mice receives the same ration. However, recent work has shown that the nutrient requirements of animals may in certain cases vary with the environmental temperature (Ershoff et al. 1949, Mills 1945).

The Parasite

The parasite used was Trypanosoma duttoni. It was supplied by Dr. P. V. Gustafson of the University of Washington, Seattle, from a culture originally obtained by the author from Dr. James Moulder, of the University of Chicago. An infected mouse was decapitated and its blood collected in a beaker of saline plus a few crystals of sodium citrate. The mice to be infected were injected intraperitoneally with 0.15cc of this very dilute blood mixture. The apparatus used was clean, but aseptic precautions were not taken, as this had been found unnecessary in previous work done by the author. All of the mice developed trypanosome infections from the original inoculations, and no associated bacterial infections were noted. The infection in the donor mouse was quite low. Blood for examining the trypanosomes was obtained by cutting off the tip of the host's tail.

A few of the mice became infested with mites (Myobia sp.) before the beginning of the experiment. Only one was seriously infested during the experiment. It developed sores, probably from scratching, appeared very sluggish, and utilized a great deal of food, most of which was wasted. It was still alive when the experiment ended. Olson and Dahms (1946) observed that hamsters, rats, and guinea pigs infested with Liponyssus bacoti lost weight and died, apparently of exsanguination. However it is apparent that in the present case the mites did not affect survival.

Sequence of Events

The proper sequence for initiating the several experimental conditions was a matter of some concern, but was decided by practical considerations. It was necessary to determine first that all the animals were infected, before applying the adverse environmental conditions. Then it was necessary to determine the food consumption of the mice under cold-room conditions, so that the half-ration could be calculated. The procedure, therefore, was this: (1) establish the infection, (2) ten days later test all animals for infection and lower the temperature, (3) measure food consumption for two days at the low temperature, and (4) reduce the ration. The experiment was considered to begin with the fourth step, and all of the following data are based on this.

It was necessary to end the experiments at 19 days. Mice in two of the groups were dying fairly rapidly at this

time, and the results would have been more complete and significant if it had been possible to continue observations for another week, but it seems quite probable that none of the conclusions drawn from the available data would have been changed. The two groups of animals which were of principal interest (CHP and CHN) had both died before the experiment was ended.

Records

Records of weight, food utilization, course of infection, survival time, and post mortem appearance were kept for each mouse. No post mortem examination was made of the mice killed at the end of the experiment. Water utilization was measured once.

RESULTS

Experimental Results

Survival. The number of days for which each mouse survived the experimental conditions is shown in Table I. The number of survivors in each group and the total survivors for each condition for each day are shown in Table II. The results of the analysis of variance are given in Appendix 3.

The first deaths occurred on the third day of the experiment, and the last observed deaths were on the eighteenth day. The experiment was ended on the nineteenth day. It is apparent from the raw data that each of the conditions employed affects survival, but each in different degree.

Table I. Number of days for which each mouse survived the experimental conditions. Blank spaces indicate that the animal was still alive after 19 days.

Group	Mouse															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	mean
WFN																
WHN	9		18			10										17.7
WFP																
WHP		16	16			15	6		18	9			16			16.5
CFN													3			
CHN	5	5	7	5	5	12	5	3	9	3	7	7	8	7	12	6.7
CFP																
CHP	3	3	3	5	12	3	3	6	7	3	3	7	5	3	3	4.6

Table II. Number of survivors on each day.

Group	Day of Experiment																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
WHN	15	15	15	15	15	15	15	15	13	13	13	13	13	13	13	13	13	12	12
WHP	15	15	15	15	15	14	14	14	13	13	13	13	13	13	12	9	9	8	8
CHN	15	15	13	13	8	8	4	3	2	2	2	0	0	0	0	0	0	0	0
CHP	15	15	6	6	4	3	1	1	1	1	1	0	0	0	0	0	0	0	0
W	60	60	60	60	60	59	59	59	56	56	56	56	56	56	55	52	52	50	50
C	60	60	48	48	41	40	34	33	32	32	32	29	29	29	29	29	29	29	29
F	60	60	59	59	59	59	59	59	59	59	59	59	59	59	59	59	59	59	59
H	60	60	49	49	42	40	34	33	29	29	29	26	26	26	25	22	22	20	20
N	60	60	57	57	52	52	48	47	44	44	44	42	42	42	42	42	42	41	41
P	60	60	52	52	49	47	45	45	44	44	44	43	43	43	42	39	39	38	38

Partial starvation was by far the most important factor in causing death. At the end of the experimental period 59 of 60 full-ration mice were still alive, while only 20 of 60 half-ration mice were alive. This effect would have been greater had the experiments been extended, because the half-ration animals were still dying, while none of the full-ration animals appeared moribund.

The only full-ration mouse which died during the course of the experiment was one of the cold-room males (CFN-13). It was dead on the third day, had no food in its cage, and had lost 8 grams weight since the previous weighing two weeks earlier. During this time the other males in this group all gained weight, averaging 2.4 grams. This was originally the second-largest mouse of the group. It is probable that some unrecognized factor was responsible for the death of this animal. This mouse is included in the following statistical analysis, except in a few cases were it was omitted to show significance in the difference between two sets of data.

The effect of low temperature was secondary to that of inanition, but was quite significant nevertheless. At the time when all 30 of the half-ration mice in the cold room had died, only 4 of the half-ration mice in the warm room were dead. By the end of the experiment one-third of the half-ration mice in the warm room were dead, and others were moribund. It is probable that all of these mice would have died had the observations been continued for a while longer. The

effect of low temperature would seem to be to hasten the death of animals which would soon have died of malnourishment even in the warm room. It is possible that the full-ration mice would have died earlier in the cold also, but this would probably have been a rather long-term effect.

The real purpose of the experiment was to determine whether the infection, ordinarily benign, would become malignant when the host was placed under adverse conditions. Since the full-ration animals did not die at all, it seems that low temperature alone is not sufficient to make the infection lethal, or rather that the combination of low temperature and infection is not lethal in adequately nourished animals.

The comparison of the parasitized and non-parasitized mice kept on half rations at low temperatures shows that the former died much more rapidly at first. On the third day nine of the 15 parasitized mice were dead, but only two of the non-parasitized mice. This difference diminished later, and the last mice of both groups died on the same day. Similar results were observed in the warm room, though the incompleteness of the data make it inadvisable to draw definite conclusions here. By the nineteenth day seven of the parasitized mice were dead, and only three of the non-parasitized mice.

The survival data were analyzed statistically to determine the significance of the results. The methods used are

discussed in Appendix 2. The results of the analysis of variance are given in Appendix 3. The analysis of variance for the most part confirms the conclusions drawn above from inspection of the crude data.

The calculated F value for temperature variance is 148. This is far greater than the tabulated F value (6.90 at p=.01) indicating that the probability of the survival difference between the two rooms being due to chance variation is extremely slight. The calculated F value for food variance is 223, indicating that food is even more important than temperature. The calculated F value for parasitism (1.23) is much lower than the tabulated value (3.94 at p=.05) and consequently we cannot say that the effect of parasitism on the animals as a whole is significant.

Of more interest than the isolated effect of any one factor is the effect of the interaction of factors. The analysis of variance shows that the interaction of each factor with the other two is significant, i.e. these results would not be expected if each factor was independent and their combined effects were simply additive. Two-factor analysis shows that the interaction of food and parasite, at low temperature, is significant, but not the interaction of temperature and parasite.

Analysis of survival variance for the cold-room mice shows that the effect of parasitism is not significant, but that its interaction with food is. Repeating this analysis

with mouse CFN-13, an aberrant individual, omitted, shows significance of parasitism too. The "t" test shows that the difference between survival in groups CHP and CHN is almost, but not quite, significant at the 0.05 level of probability. Analysis of variance for the half-ration mice in both rooms shows definite significance of the infection on survival. The survival of the half-ration mice is plotted in Figure 2 for parasitized and unparasitized animals.

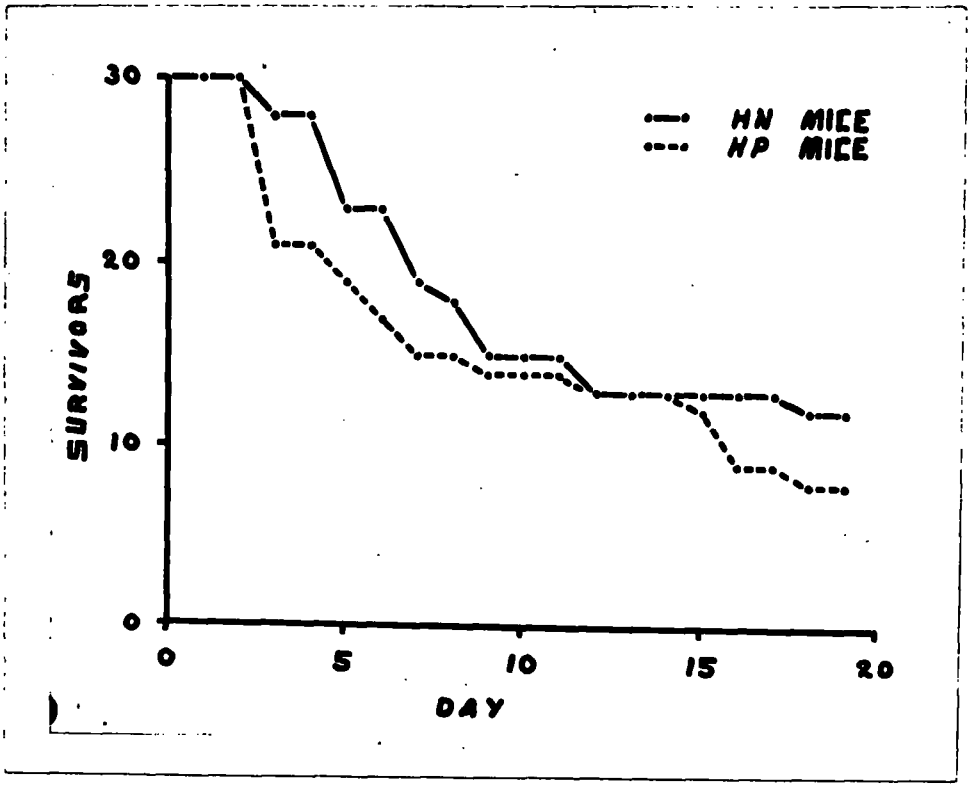


Figure 2. Survival of HN and HP mice.

Weight change. The mean weight changes of the mice in each group are given in Table III. During the experiment the half-ration mice lost weight and the full-ration mice gained weight, with few exceptions. The FN mice in both rooms made average gains of 3.67 grams; the FP mice averaged 1.87 grams. This similar difference at both temperatures appears significant. The "t" test was made for these differences at both temperatures. The warm-room difference is significant at p=.05, but the cold-room difference, because of greater variation within the groups is not.

Table III. Mean weight change, food consumption, and survival for each group.

Group	Weight (grams)					Food (grams/day)			Survival
	d_1	O	F	d_2	%d	Given	Calc	G/C	Days
WFN	≠ 5.67	25.6	28.3	≠ 3.67	≠14	6.47	--	--	19.00
WFP	≠ 5.07	24.6	26.5	≠ 1.87	≠ 7	6.07	--	--	19.00
WHN	≠ 4.07	23.7	18.3	- 4.73	-20	3.20	3.51	.91	17.67
WHP	≠ 4.21	25.4	19.7	- 4.00	-16	3.13	3.53	.87	16.53
CFN	≠3.93	24.1	27.7	≠ 3.67	≠15	11.18	--	--	17.93
CFP	≠ 4.71	25.0	26.8	≠ 1.87	≠ 7	11.30	--	--	19.00
CHN	≠ 4.60	24.7	19.9	- 4.80	-19	4.93	5.86	.84	6.67
CHP	≠ 4.15	25.1	19.1	- 6.00	-24	4.40	5.84	.75	4.60

d_1--weight change before experiment, d_2--weight change during experiment, O--original weight, F--final weight, %d--% change during experiment, G/C--given/calculated

Food consumption. The caloric requirement of mice at 70°F can be calculated from the formula $2 \times 70.5 \times (\text{body weight in kilograms})^{0.7}$. This was done for groups of three weights (Table IV) and the food requirement calculated from these values. The ration used contains 2.8 available calories per gram. This calculated requirement was then compared to the average utilization of all the mice in this weight group during the entire period of observation, except those being kept at low temperatures.

Table IV. Voluntary food utilization, by weight groups.

Mouse Weight	Calculated Requirement at 70°F		Utilized at 70°F		Utilized at 43°F	$\frac{\bar{x}\ 43}{\bar{x}\ 70}$
	Calories	Grams	$\bar{x}$	O/C	$\bar{x}$	
10-12	6.00	2.14	9.20	4.30	(14.81)	--
14-16	7.46	2.66	8.04	3.02	(12,94)	--
19-21	9.12	3.26	7.34	2.25	10.43	1.42
24-26	10.66	3.80	6.44	1.69	11.46	1.78
29-31	12.10	4.32	7.21	1.67	12.00	1.66
34-36	13.49	4.81	7.44	1.55	12.67	1.70
38-40	14.56	5.20	8.00	1.54	12.00	1.50
						$\bar{x} = 1.61$

O/C--observed utilization/calculated utilization

All figures except calories and ratios in grams.

Food utilization was greatest for the lowest weight group (10-12 grams), lowest for the middle group (24-26 grams), and rose again for the heavier groups. The ratio "observed utilization/calculated requirement" is very large, indicating considerable wastage. It declines steadily from 4.30 for the lightest group to 1.54 for the heaviest. The mice kept at 43°F used an average of 61% more food than those at 68°F.

Average daily food consumption by the full-ration mice during the experiment was: WFN--6.0gm, WFP--6.0gm, CFN--11.0 gm, CFP--11.5gm. This shows clearly that the infection had no effect on voluntary food consumption.

On the nineteenth day of the experiment all the surviving warm-room mice were fed ad libitum and their consumption during the next 24 hours was measured. The formerly half-ration mice used nearly twice as much food as the full-ration mice. Both groups of parasitized mice used slightly less food than their controls, but this could not justifiably be called a real difference, even if statistically significant, because the data are too few. The means were: WFN--6.5gm, WFP--5.3gm, WHN--10.3gm, WHP--10.0gm.

Water utilization. The amount of water used by the mice was measured for one four-day period late in the experiment, after the mice of the half-ration cold-room groups were dead. Only rough generalizations can be made from such data. In the warm room the half-ration mice used an average of 2.8cc per mouse per day, the full-ration mice used 5.6cc. The full-ration

mice in the cold room averaged 9.0cc per day. These differences were found to correlate with food consumption. The ratio "water utilization/food utilization" shows no great difference among the groups. The range is 0.77 to 0.97cc/gm food. Quimby (1948a) states that starved rats used less water than controls, but more in proportion to the food consumed. The present data clearly support the first statement, and tend to support the second.

Influence of sex. No clear-cut sex difference appears in any of the data. Certain slight survival differences in favor of males are probably due to weight rather than sex difference in the mice. It seems safe to say that there was no differential effect on the sexes.

Physical condition and behaviour. Most of the mice in each of the groups remained in good health until near death. As soon as their environmental temperature was lowered the cold-room mice ruffled their fur, as would be expected. The ruffling was quite uniform and orderly, not appearing at all unkempt.

Gilson (1950) reported edema of the feet and progressive gangrene of ears and tail in rats kept at 5°C. The present mice showed little evidence of this. The feet were, perhaps, slightly edematous, and the tail somewhat so. Five days after the temperature was lowered it was not possible to get sufficient blood from the tip of the tail of many of the cold-room mice to make a smear, but at 14 days there was no difficulty in doing this. The ears showed no involvement. The

tips of the tails of the parasitized mice in the cold room became dark for about ½ inch at the tip, which had been injured when blood samples were taken. This first appeared after several days, but disappeared within one week. Very occassionally a mouse was found with blood on its feet or tail, but this never persisted until the following day.

These results suggest that these mice, unlike Gilson's rats, were able to adapt themselves well to the low temperatures, possibly permanently. The experiment was too short, though, to justify such conclusions. The gangrene in Gilson's rats did not appear until between the tenth and thirtieth days.

No suitable apparatus was available for measuring the body temperature of the mice, but a few measurements with an ordinary clinical thermometer indicated no important difference between the cold-room and warm-room mice. A range of 35.0-39.0°C is normal, and all the observed readings fall within this range (Knorr 1926).

Most of the mice remained quite active. Though they showed an increased tendency to assume a hunched, shivering position in the cold, they were still alert when disturbed. Shortly before the period of their heaviest dieoff, the half-ration mice in the cold room became quite restless and made repeated attempts to climb from their cages.

Moribund mice sat hunched, and often could not be aroused by prodding. This was not a reliable indication of

approaching death, though. Several times an animal considered dying on one day appeared quite healthy later and lived until the end of the experiment, and many of the mice which died failed to appear moribund on the previous day. One mouse in the warm room was distinctly hypothermic on the day before its death. Most of the mice which died, especially the later ones, became quite thin before death.

When nearly dead the mice lay on their sides or sat on their backs in a corner of the cage, with their legs spread. They moved only with difficulty, apparently suffering paralysis of the hind legs. One mouse (a half-ration cold-room male) was observed while dying, on the third day. It was found lying on its side, and removed from the cage for observation. It made convulsive shivers, beginning at the head and continuing back to, but not including, the tail. The legs especially were involved.

When first observed the convulsions were spaced about five seconds apart, but later occurred much less frequently, though prodding elicited convulsive response at any time up until death. The later convulsions were confined to the head and neck alone. During the period of frequent convulsions, the animal urinated once, soiling its fur. From the first there was no pupillary reflex. Death was imperceptible. This mouse and two others died shortly after being disturbed for weighing.

The most striking feature seen at the post mortem examination was extensive gastric and intestinal hemorrhage. In most of the mice both stomach and intestine contained considerable blood, coffee-colored and containing thick sediment. This was most conspicuous in the cold-room mice, but definitely occurred also in most of those warm-room mice which died. This result would be expected in starved animals at low temperatures.

In most of the dead mice the intestine, rather than firm, round, white, thick-walled, was very flabby, distended, and transparent, showing the dark blood inside. This possibly was due to autolysis or bacterial decomposition, because the considerable variation in the extent of this condition may have been a result of different time lapses between death and examination. However this degeneration seemed to be correlated with the extent of the hemorrhage, and some of the mice examined shortly after death showed extensive degeneration of this type. Hemorrhage and degeneration were largely confined to the upper part of the small intestine, though in some cases they affected the entire intestine. (Figure 3)

The adrenals of all the cold-room mice were in a very active condition (described as "inflammed") at the time of death, but not those of the warm-room mice. The lungs of most of the mice appeared quite healthy, showing no evidence of pneumonia. Those in a few of the mice were hemorrhagic.

The earliest mice to die failed to exhaust their energy stores before dying. Most of them still had considerable body

fat, and their livers appeared normal. This continued to be true, on the whole, for about the first half of the experiment, though the extent of the body fat decreased. Most of the warm-room animals, which died toward the end of the period, had very little or no fat left and the last ones to die had very small livers, with darkened edges. This indicates that at low temperatures partially starved animals die before they can mobilize their energy reserves, but that at warm temperatures they exhaust their reserves before dying.

The blood glucose level of mice CHP-5 and CFP-1 was assayed on the day before they died. The blood (0.2cc) was removed by heart puhcture. The combined effects of the puncture and the loss of blood may have resulted in earlier deaths for these two mice. The blood glucose of CHP-5 was 108.8mgm%, that of CFP-1 was 98.0mgm%. This indicates that both starved and fully fed mice were able to maintain their blood glucose at a high level until near death.

The trypanosomes were very common in the blood of the mice after death. They were still quite numerous, but fewer, in one mouse 48 hours after death. This animal had been refrigerated all this time. Nowicki (1939) states that T. lewisi survives the death of the host only in the liver and other organs rich in nutritive substances, but this is not supported by the observations made here. The trypanosomes were observed in blood from the liver, heart, dorsal blood vessels, and brain, but not in spleen blood.

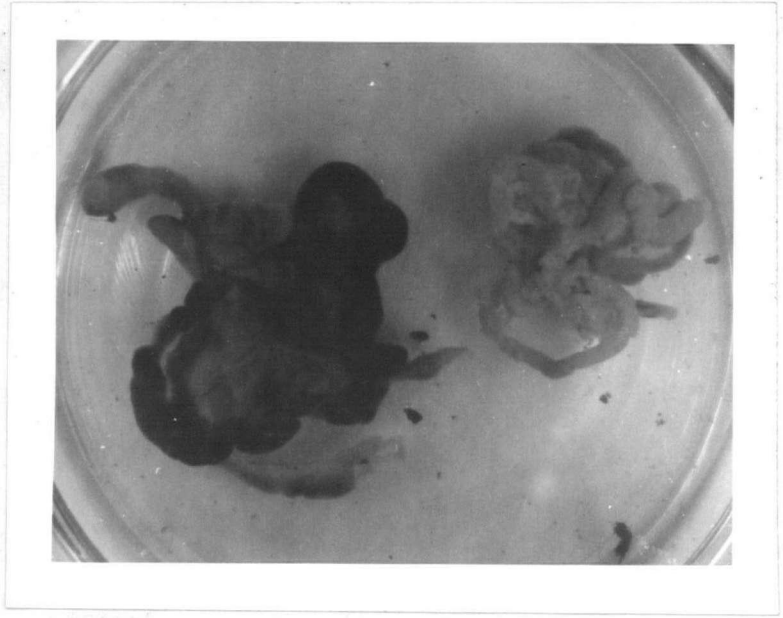


Figure 3. Post mortem appearance of mouse intestines. Left, from a CHP mouse which died on the third day of the experiment. Right, from a normal mouse killed for examination.

Influence of Unplanned Factors

The foregoing statistical analysis shows the very great probability that the results obtained in this experiment were not simply due to chance. It is very important to realize though that this does not permit the assumption that the observed results were due entirely to the experimental conditions. It is necessary first to show that the mice in each group were, for our purposes, identical or nearly so. It is also necessary to show that the experimental conditions were identical for each group concerned.

Initial similarity. It is impossible to assess adequately all the many characteristics of different animals in order to

demonstrate their identity. There are three methods commonly used to assure practical identity--(1) swamping out differences by the use of many replicates, (2) carefully selecting mice which are litter mates and of the same sex and weight, and (3) observing certain characteristics of the animals before initiating the experimental conditions.

Each of these methods has been utilized to some extent in the present work. (1) and (2) have been described under Methods. The most useful quantitative information for comparison of health and condition is growth data. The mice were weighed three times during a four-week period prior to the beginning of the experiment. The weight changes during this time are summarized in Table III.

Five of the eight groups averaged between 4 and 5 grams growth during this period. The extreme weight gains were 3.93 and 5.67 grams for the FN groups in the two rooms. The survival in these two groups was practically identical, so that any fundamental difference there may have been in the two groups was not reflected in the experimental results. A comparison which might have been significantly affected by pre-experimental differences in the mice was that between the CHP and CHN groups. The "t" test was made, and indicated that the difference between these two groups was not significant.

The food utilization was similar for all groups during this period. So far as available data indicate, the eight groups of mice were all comparable in this respect.

Equality of the ration. In planning the amount of food to be given the half-ration mice the consumption of each cold-room mouse was measured during the first two days at the low temperature and the mouse was later fed one-half of its average consumption during these two days. This did not prove to be entirely satisfactory, as the consumption of the CF mice increased during the course of the experiment, indicating that the food requirement increased. Also, there was a certain amount of variation in the day-to-day consumption of a given mouse, and the half-ration may have been calculated on the basis of a particularly high or low extreme. Voluntary consumption did not change significantly in the warm room during this period.

The requirement for a mouse of a given weight was calculated from the voluntary consumption of the full-ration groups (Table IV). A method of averaging grouped data was used, so the comparisons are only approximate. The ratio "food supplied/calculated food requirement" varies considerably. The range of group means is 0.75-0.91 (Table III).

The magnitude of this ratio shows an apparent correlation with the group survival, the lowest value (0.75) being for the CHP group, which died earliest, and the highest (0.91) for the WHN group, which showed the fewest deaths. The WHP group showed the greatest variation in ratio. The regression coefficient for this group is $b_{xy} = 3.07$. This was tested with the "t" test for regression significance (Johnson 1949,

p.48) and proved to be significant. The regression coefficient for the control group (CHP) was quite small (-0.24).

Degree of infection. The difficulties involved in making satisfactory trypanosome counts made it impossible to obtain counts from all of the smears which were available. Weekly smears were made from each mouse, and most of the mice were examined for live trypanosomes post mortem. The smears were stained with Wright's stain. Rough counts were made of sample smears, but no attempt was made to cover all of them.

Comparison of wet mount estimates made at the beginning of the experiment with those made after the animals were dead indicates that the infection increased greatly (five or more times) in the interval between the two estimates. This may have been entirely due to growth after the death of the host, however.

At the beginning of the experiment, at least, males had heavier infections than did females. The different groups had approximately equal parasite loads. Within each group there was variation of two or more to one. The available data do not show any correlation between degree of infection and survival.

To make any more definite statements would require reliable counts of all the smears and statistical analysis of the results. This is desirable, but not possible at this time.

DISCUSSION

A large body of information has been accumulated concerning the effects on animals of one or more of the factors used in the present work. Many of the results reported in the literature are not reliable, and the available information is quite incomplete. Furthermore, little or no effort has been made to reproduce in the laboratory all the major factors which would be expected to influence animals in the wild in order to determine the importance of each and the effect of their interaction.

Therefore there is no precedent to which the results of the present work may be compared. It is desirable however to consider briefly some of the principle findings which have a bearing on this work and to attempt to correlate the present findings with them.

Effects of Low Temperature

Temperature is a major regulating factor for all life. Homiothermic animals are not affected by temperature extremes as markedly as are poikilotherms, but nevertheless are profoundly influenced. Response to low temperature is of two types--(1) reduction of heat loss, largely by reducing the blood flow to the skin and extremities, and (2) increase in heat production by a raised metabolic level. By means of these two mechanisms the body temperature remains nearly constant at a wide range of environmental temperature.

The increased metabolism at low temperatures results in a greater caloric requirement, which means that the animal must consume more food. This increased energy requirement in the cold is especially serious for animals whose food supplies are very low during the winter.

The altered circulation which accompanies temperature changes is often responsible for considerable local damage. Frost bite and trench foot are some of the effects produced. The edema and erythema found by Gilson (1950) and the same phenomena observed in the present work resulted from such circulatory changes. There are varying degrees of severity of these pathological conditions. The gangrene of the tail and ears of Gilson's rats ended when the animals were moved to warm temperatures, but trench foot in man does not stop after being initiated, and it seems probable that the present mice recovered from these effects altogether while still in the cold.

Low temperature initiates a heightened metabolic level not only on a short-term basis but also over a long period of time. Man and other animals have been shown to have an annual metabolic cycle, the rate being higher in winter than in summer. (Benedict et al. 1929, Mills 1945). Schwabe (1938) found that exposure of rats to 7-12°C for extended periods of time increased the metabolic rate 11-16%, and the rate reached a maximum and sustained level at between 15 and 30 days. The body temperature was increased.

The response to change in environmental temperature is effected both by the nervous system and hormonally. Both types of regulation are complex and neither is thoroughly understood. Local and reflex nervous reactions occur, but the principal center of control is the hypothalamus, and all but the immediate reactions are mediated here.

Hormonal control is directed largely by the nervous system, through the pituitary. Both the thyroid and the adrenal cortex appear to be necessary for effective body temperature control. Their activity is regulated by the thyrotropic and adrenocorticotropic secretions of the pituitary. (Baillif 1937,1938; Gilson 1950; LeBlond 1943; Lesser et al. 1949; Ring 1936, 1938).

The over-all effect of climate on an animal has been extensively studied, especially for man. Mills (1939) maintains the importance of climate in the incidence of the metabolic diseases. He attempts to correlate these diseases with climate in the United States. Quin (1945) working with rats in South Africa, decided that the indirect effects of climate (nutrition and disease) are more important than the direct effects.

Gilson concludes that "certainly there is no evidence here of immediate acute damage to the rat by the cold air per se...the animals ultimately developed progressive gangrene of the tail, a sign that they were unable finally to cope with the environmental stress." (p.91)

The mice used in the present experiment reacted to the cold much as would be expected from the results of other laboratory and field studies. Fluffed fur, shivering, adrenal enlargement, and increased food consumption are characteristic reactions. The absence of deaths in the full-ration groups indicates that cold alone did not have a serious effect on the mice, but the more rapid mortality of the half-ration mice in the cold room shows that cold, in combination with other adverse conditions, can be effective in lowering survival. There was no consistent difference in weight change between the two rooms.

Effects of Inanition

A vast amount of work has been done on the requirements of various animals for specific nutrients, but little has been done on the effects of quantitative restriction of a balanced ration. Under such conditions of inanition the animal may be expected to suffer both from caloric restriction and from the inadequacy of specific nutrients. The nature of the deficiency responsible for any given effect will not be clear.

Chambers (1938) mentions the work of Morgulis (1923) on the changes in metabolism during inanition. The fasting period during complete inanition may be divided into four phases, each corresponding to a loss of about 1/8 of the normal body weight. "The first phase represents the transition from the well-fed to the fasting state and is marked by the rapid diminution of carbohydrate stores. During the second and third

phases, which are not distinctly separable, the body is deriving energy mainly from the oxidation of fat (80 to 90 per cent). When the reserve of fat becomes depleted the fourth phase is entered, which is generally characterized by the so-called 'premortal' rise in nitrogen excretion, denoting an excessive destruction of endogenous protein sometimes up to 100 per cent of the total energy requirement of the body." (p.257) The first phase, embodying a weight loss of 12.5 per cent, extends approximately for two days in the rat and guinea pig, four days in the rabbit, and 15 days in man.

Chronic inanition of young rats resulted in a 10 per cent reduction in metabolic rate after 30 days and a 20 per cent reduction after 90 days. This drop may have been caused by a deficiency of thiamin in the diet, the effect being mediated through the pituitary and thyroid glands. The respiratory quotient averaged 0.75 in fasting rats and 0.94 in non-fasting rats, indicating a shift from carbohydrate to fat metabolism in the fasting animals. (Quimby et al. 1948)

During inanition all or almost all of the organs of rats lose weight, but some suffer much more loss than others. The head and skeleton lose little weight, so increase in relative weight. The integument, musculature, and viscera change little in relative weight, and the remainder decreases in relative weight, chiefly due to loss of fat. The adrenals, thyroid, and central nervous system maintain their original weight, while the spleen, liver, and alimentary canal decrease in

relative weight. (Jackson 1915) Apparently the adrenals of different animals may be affected by starvation in different ways. In guinea pigs they undergo true hypertrophy, but in rats they show only an erratic false hypertrophy, with increased water content but no increase in solids. (D'Angelo et al. 1948)

These statements are supported by the rather meager data of this type collected during this experiment. The first effect observed was intestinal degeneration, followed by depletion of body fat stores, this becoming complete only after two weeks or more on half rations. Only the last mice to die showed decrease in liver size. No adrenal enlargement was noted, but the examination was too superficial and the examiner too untrained for this to be considered evidence.

Inanition has been found to increase the voluntary activity of rats. Rats on a diet which produced 42% weight loss showed seven times the activity of rats which were fed ad libitum and gained 121% of their weight. (Krzywicki and Costa 1949) There was no opportunity during the present work to measure activity, but for the most part the half-ration mice were more quiet than their controls. The unusual activity of certain groups shortly before death supports the reports of great pre-mortal activity.

This probably may be interpreted as an attempt to leave a barren area in search of food. This is an obvious reaction, and quite common in many animals, including rodents. Some

animals however, especially ungulates, are so controlled by territorial behaviour patterns that they will not leave their established routes of travel to get food which is close by.

Relation Between Temperature and Nutrition

Regulation of food intake is an important means of regulating body temperature through metabolism. Brobeck (1948) studied the food intake of rats adapted to 82-84°F when they were exposed to temperatures of 65-76° and 92-95°. At the lower temperatures the intake was increased and the rats gained weight normally and avoided hypothermia. At the higher temperatures the intake was decreased and the rats lost weight and became hyperthermic. This same general effect has often been observed. Better nutrition produces earlier development of temperature control. (Hill 1947)

Seven-month female rats survived on the average 16½ days of fasting at 26°C, losing 49% of their body weight, and 11 days at 16°C, losing 44% of their body weight. "The length of survival of these fasting rats refutes the supposition that small animals succumb quickly to fasting." (Horst et al. 1930, p.199)

The results reported here emphasize the importance of low temperature in increasing the food requirement of mice. The cold-room mice fed ad libitum utilized 60% more food than those at room temperature. However, since the half-ration mice at both temperatures supposedly received the same proportion of their actual requirements, the longer survival of the

warm-room mice apparently cannot be explained on a calorie intake basis alone. It is suggested that the low temperature increased the energy requirements so greatly that the mice exhausted their carbohydrate stores and died before they could adjust to the exclusive use of fats for energy.

Various workers have shown the importance of fat in the diet. Deuel et al. (1947) tested in several ways the influence of varying dietary fat levels on rats, and found that in every case the performance of the animals was best when their diets contained liberal amounts of fat. Optimum growth was obtained on diets of 20-40% fat.

Rats previously fed a high-fat diet survive longer during fasting than those fed a high-protein diet. The former are adapted to burning mainly fat for energy, saving their carbohydrate and protein. The latter cannot do this, quickly exhaust their carbohydrate reserves, and then utilize their body protein for energy. (Roberts and Samuels 1949)

The ration used for the mice in the present work contained little fat (6%) compared to that recommended by these workers. It is possible that had they been previously fed a high-fat diet the cold-room mice would have been able to utilize all of their body fat before succumbing. Rats at low temperatures choose a diet high in fats (LeBlond et al. 1944), and Treichler and coworkers (1946) found that the prefered winter diet of ruffed grouse was characterized by high energy content.

Recent work has indicated that not only is the caloric requirement influenced by environmental temperature, but also certain specific food substances may be needed in greater or lesser amounts at extreme temperatures. Ershoff et al. (1949) state that "available data indicate...that requirements for essential nutrients may be significantly increased by exposure to cold and other conditions of 'stress'." (p.288)

Mills (1945) has found that the requirements for certain vitamins is greatly increased in the heat, but his various statements on the subject are not entirely consistent. It seems certain that a higher concentration of certain vitamins in the food is necessary in the heat, because of reduced voluntary food consumption under such conditions. Lesser et al. (1949) found that hyperplasia of thyroid epithelium of cold-exposed rats could be prevented by increasing the iodine content of the diet.

It was not possible to determine whether the mice used here developed any vitamin or mineral deficiencies. It is probable that increased metabolism in the cold would, over a period of time, make increased intake of the vitamins involved in metabolism necessary.

Effects of Parasitism

Wild animals almost universally have protozoan, helminth, and arthropod infestations, but they usually are thought to be more or less benign. Griffiths (1944) states that trapped rats are generally strong, vigorous, and free of signs of dietary

deficiency, often in spite of heavy parasite loads. Experimental work has indicated that most parasites are not serious if the host is in good condition.

Van Cleave (1937) suggests that wildlife may be adversely affected by parasites in several ways--mechanical injury, competition for food, toxin production, sterility, or predisposal to disease. Sprent (1949) could find no conclusive evidence that Ascaris produces toxins, but did establish that sensitized hosts show an anaphylactic reaction. Chandler (1943) suggests that the pathogenic effects of Hymenolepis diminuta in rats may be a result of absorption of vitamins by the worms.

Strongyloides papillosus in calves produces diarrhea, anorexia, loss of condition, retarded growth (33-79% less than in controls), and sometimes death. (Vegors and Porter 1950) Rats infected with Cysticercus fasciolaris become severely anemic, but seldom die of the infestation. (Miller and Dawley 1928) Pigs with heavy helminth loads grow more slowly and require more food per unit growth than controls with light loads. (Andrews and Jones 1948) Hawkins (1946) found no marked pathology associated with Moniezia expansa infections of sheep, but Hansen et al. (1950) found consistent and significant retardation of weight gain in infested lambs.

Russian workers have studied the influence of parasitism on fat storage in various mammals. Arctic foxes apparently store more fat when they have few helminths. (Perelshin 1943)

Marmots with heavy ascarid infections sometimes do not store sufficient fat for survival during hibernation. (Dubinin and Leshkovitch 1945)

The degree and pathogenicity of parasitism is often influenced by external conditions, especially weather conditions. Boughton (1933) found a definite correlation between meteorological conditions and the percentage of snowshoe rabbits infected by parasites. Erickson (1944) states that temperature, precipitation, and type of soil influence the percentage and degree of parasitic infection of snowshoe hares but gives no supporting data. Eichler (1942), working with swallows and martin, concluded that coincidence of parasitism and wet weather may have grave consequences for the broods, while either factor alone need not be important.

Parasites and infectious disease have often been suggested as a cause of the periodic heavy mortalities of certain animal species. Cox (1936) associates heavy tick infestations with declines in numbers of hares, but cites only casual observational evidence. Erickson (1944) states that both shock disease and heavy helminth infections are correlated with hare declines, and believes that the two factors may be related. Dubinin and Leshkovitch (1945) found that the marmot population density of the coming year could be predicted on the basis of a complete parasitological census in the fall. However, it is now generally believed that parasites are of only secondary importance as a cause of these declines.

Figure 4 shows the course of a typical Trypanosoma duttoni infection in the mouse. The parasites increase in number rather slowly until a reproduction-inhibiting antibody largely stops their division. After this the trypanosomes already in the blood are slowly destroyed by one or more trypanocidal antibodies produced by the host. The infection never reaches a high level, and apparently is harmless to the host. (Taliaferro and Pavlinova 1936)

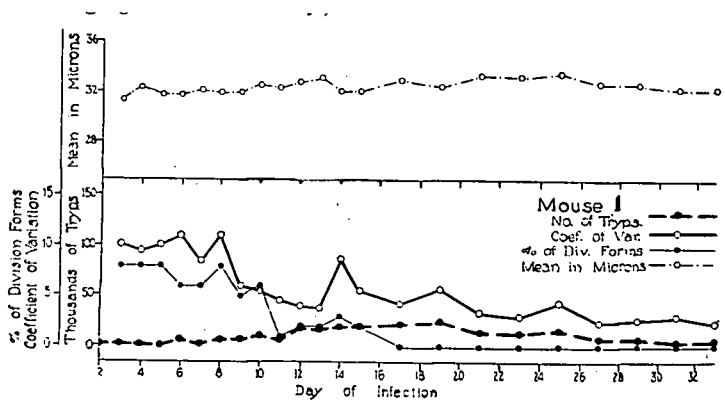


FIG. 3. Course of infection of *T. duttoni* in normal mouse 1. Note the sluggish but prolonged reproductive activity with its eventual cessation (Coefficient of variation and percentage of dividing forms) and the concomitant paucity in numbers (No. of trypanosomes).

Figure 4. Course of T. duttoni infection in mouse. From Taliaferro and Pavlinova 1936, p.34)

The effects of parasitism in this experiment were necessarily determined largely by the characteristic nature of the host-parasite relationship. Since T. duttoni is ordinarily a benign parasite it would not be expected to have any adverse

effect on the host unless influenced to do so by other factors. The absence of death in the full-ration parasitized groups shows that T. duttoni infection alone is not lethal to mice. The statistically significant difference in weight gain between the WFN and WFP mice merits further investigation.

Relation Between Parasitism and Nutrition

A recent symposium on the influence of nutrition on experimental infection (Schneider et al. 1949) has summarized most of the work which has been done in this field. Faulty techniques and inadequate knowledge of the nutritional factors concerned have invalidated much of this work, but nevertheless a considerable body of knowledge has been built up. Most of it is concerned with specific nutritional deficiencies, usually protein or vitamins, rather than with inanition.

Studies on general malnutrition have shown that it always or almost always lowers the host resistance to infection. The common picture is reduced resistance to the original infection, slow development of immunity, and low resistance to reinfection. Parasite loads are heavier and more seriously pathogenic. Infections which would ordinarily be benign may become lethal. Reproduction is hindered. This has all been demonstrated for man and laboratory animals, but never for wild populations. Ecologists often seem to assume that the effect of parasites under conditions of malnutrition is due to competition for food (Einarsen 1946), but this idea, while

probably sound in part, has not been adequately established.

Certain diseases, notably hookworm infection and amebic dysentery, are now recognized as a result of the interaction of two factors--malnutrition and the causative organism. Mice underweight as a result of undernourishment from birth are more susceptible to infection with Trichinella spiralis. (Hendricks 1950) Ackert and Wisseman (1946), working with two intestinal helminths in fowl, concluded that "if all of the ingredients required by growing chickens are supplied, the young fowls can tolerate considerable numbers of tapeworms without serious effects." (p.728)

Caloric deficiency, even if all specific nutrients are supplied, increases the susceptibility of rats and mice to Salmonella typhi-murium infection (Guggenheim and Buechler 1947) and of sheep to trichostrongylid infection. (Whitlock 1949a)

Protein deficiency increases the susceptibility of rats and mice to Salmonella (Guggenheim and Buechler 1947) and of rats to typhus (Fitzpatrick 1948); is a predisposing factor to trichostrongylidosis of sheep (Whitlock 1949a); and interferes with the development of immunity against trichinosis in rats (Taliaferro 1949), Hymenolepis in mice (Larsh 1950), and Nippostrongylus in rats (Donaldson and Otto 1946). Lambs which are protein-deficient do not survive Haemonchus infections as well as controls (Weir et al. 1948), and hamsters

infected with Leishmania show early emaciation and death on a protein-deficient diet. (Ritterson and Stauber 1949) However, other workers have reported no such effect of protein deficiency. (Becker and Morehouse 1936, Metcoff 1949)

The importance of various minerals and vitamins for resistance to a wide variety of parasitic infections has been indicated by the work of Weir, Whitlock, Wright, Kartmann, Lawler, McCoy, Frye and Meleney, Fitzpatrick, Gyorgy, Watt, and many others. Here too the results are not always the same. In some cases no effect is observed and in some the host's resistance may even be increased by a nutritional deficiency. It is absolutely necessary to consider the role of each nutrient in each host-parasite relationship, rather than speaking in terms such as "general rundown condition".

The usual major defense against invasive organisms is the development of antibodies against specific proteins of the invader. The antibodies are modified serum globulin, and their production is hindered by anything which hinders production of serum globulin. This is largely dependent on dietary protein. (Cannon 1942, McNaught et al. 1936) Here we have a mechanism of wide application and of considerable practical importance by which diet may influence resistance.

Intestinal helminths have been found to be dependent on the food ingested by the host for their carbohydrate supply on a day-to-day basis. Chickens eliminate their Ascaridia, Raillietina, and Hymenolepis after short periods of inanition.

(Reid 1945, Chandler 1943) Ascaridia lose up to 89% of their glycogen after 48 hourse of host inanition.

Parasites are as dependent on vitamins as are their hosts. The source of a vitamin depends on the particular parasite and vitamin involved. Some parasites synthesize certain of their own vitamins. Others, especially arthropods, utilize those synthesized in their guts by the microflora and fauna. Intestinal forms take some from the lumen and others from the mucosa. Many parasites must get some or all of their vitamins directly from the host. (Beck and Chandler 1950)

At least two vitamins seem to be important for the resistance of the rat to T. lewisi. In pantothenate-deficient rats the reproductive phase of the parasite is prolonged and there is greater parasitemia. Anemia, leucopenia, neutrophilia basiphilia, reduced growth rate, and, in extreme cases, death result. (Becker et al. 1947) Infections in biotin-deficient rats show longer periods of reproduction, delay in initial trypanolytic crisis, and delay in terminating the infection. Such rats apparently are not protected by receiving immune serum. Biotin appears to be necessary for the activation of the immune bodies in this infection. (Caldwell and Gyorgy 1943, 1947)

It seems certain that the death of the mice during the present experiment was largely due to caloric insufficiency. It is doubtful if any intense avitaminosis could develop in three days, though this may have occurred later in the period.

Relation Between Parasitism and Temperature

Environmental temperature has been found to influence profoundly the host-parasite relationship in many cases. Mills (1945) cites work showing that resistance of laboratory animals to several diseases is lowered by high temperatures. This seems to be associated with lowered phagocytic activity under such conditions. Other work has shown that the immediate cause of the lowered phagocytic activity is inadequate intake of certain vitamins, which is caused by reduced total food intake. McDowell (1923) found that rats exposed to high temperatures were more resistant to pneumococcic infection than those at medium temperatures.

Exposure of rats to low temperatures increased the mortality from polyarthritis (caused by pleuro-pneumonia-like organisms), but decreased the incidence and severity. High temperatures had little effect. (Tripi and coworkers 1949) Kolodny (1940) working with Trypanosoma cruzi in rats found that high environmental temperature (90-95°F) slightly increased resistance, while low temperature (40-45°F) generally resulted in heavy blood stream invasion, and light doses caused death. Hegner (1937) has found that increasing the temperature and humidity of a bird's environment brings about modifications favorable to malaria parasites.

It is well known that a sudden drop in temperature lowers resistance to infection, especially pneumococcus infection. Pounden et al. (1947) found that diarrhea in calves

was much more common after sudden drops in temperature or after irregular feeding.

Disease sometimes reduces the ability of an animal to control its temperature. Ware and coworkers (1947) observed that rats with fleas could not maintain their body temperatures in a cold environment, while rats without fleas could. They believe that flea infestation may be a pathological condition of sufficient severity to decrease the resistance of rats to change in body temperature. Another group of rats, with "snuffles", showed the same effect.

The infection employed in the present experiment seems to have had no effect on the survival of the full-ration mice at either temperature, and the analysis of variance indicates that there was no interaction between temperature and infection.

Related Effects of all the Experimental Conditions

Inanition was the primary cause of death in these mice. Cold and parasitism hastened this effect, but did not initiate it. The infection was clearly the least important of the three factors, but it seems safe to say that it did exert a definite influence on the mice which were partially starved.

The mechanism of this effect is not clear. It probably was due either to the exaggeration by the experimental conditions of some natural but ordinarily insignificant effect of the infection, or to changes in the nature of the infection

brought about by these conditions.

It is suggested that utilization of host carbohydrate may have been the influential factor. Trypanosomes have a high rate of carbohydrate utilization--from 50 to 100% of their dry weight per hour (Von Brand 1950). This rate was measured in the African pathogenic species, but may well be similar in the non-pathogenic species. Hypoglycemia has been found to be associated with fatal infections of the pathogenic trypanosomes, but apparently it is only a secondary factor. (Scheff and Thatcher 1949) The blood analyses made for these mice indicate that they had a high glucose level to at least within 24 hours of death. Nevertheless, it seems quite possible that the infection of mice deficient in carbohydrate by a parasite which uses large amounts of it would be disadvantageous to the mice.

The results of this experiment must be interpreted with caution. Only one parasite was used, and only one grade of temperature and food stress. Under other conditions the results would undoubtedly have been different. Also the various imperfections of this experiment leave the results open to some doubt. This work may be considered indicative, but no general theory of parasitism could justifiably be based on this data.

The Laboratory Mouse as a Representative Mammal

The use of laboratory animals for studies such as this has numerous advantages over the use of captive wild animals. Laboratory animals are easy to house and handle; their spatial, dietary, temperature, and psychological requirements are fairly well known; and they are in their natural environment in the laboratory.

On the other hand there are serious disadvantages in their use. The purpose of the study is to gain information in the laboratory which may be applicable to animals in the wild, but in the process of domestication the laboratory animals have lost some of the mechanisms which enable the free-living animals to adjust to the conditions in question.

Donaldson (1924) summarizes data comparing the albino rat to its wild ancestor, the Norway rat. Albinos are heavier but have lighter skeleton, adrenals, and gonads. The thyroid weight is similar in the two forms; the albino hypophysis is heavier. The brain and spinal cord are lighter in the albino, due to lower water content.

Nichols (1950) made a comparative study of the adrenal glands in the two forms. He states that "the wild Norway rat in the natural state has an adrenal gland about twice the size of the adrenal gland of the domestic rat of comparable weight. The adrenal gland of the wild Norway rat has cholesterol content of approximately 10 per cent, which is about twice that of the domestic rat. Hence the wild Norway rat has about

4 times as much adrenal cholesterol per unit of body weight as does the domestic rat...

"It is interesting to consider the differences in behaviour of the 2 types of rats and the differences between their adrenal glands. The wild rat has a 'faster pace of living' than does the domestic rat, being subjected to extremes of climate, uncertain food supply, natural enemies, etc.; indeed he owes his very existence to a more alert and aggressive attitude than is characteristic of the sedentary domestic rat. It is logical to assume that this requires more cortical hormone. The large gland of the wild animal is apparently under both genetic and environmental control." (pp.7-9)

The mouse, because of its small size and consequent high surface-volume ratio, is more susceptible to low temperatures than most animals. Horvath (1948) found that mice survived for 0.4 hours at -35°C. Rats and rabbits of comparable age survived for 0.75-2.0 and 3.5-6.5 hours respectively.

Application to Field Conditions

The work of Boughton, Erickson, and Eichler mentioned above demonstrates, in so far as it is applicable, that climatic and other environmental factors really do affect the parasitic infections of animals in the wild. Erickson says that soil type also is important. This may be through its effect on the survival of eggs and larvae of the parasites or its effect on the chemical composition of the plants growing

on it, poor soil resulting in malnourished wildlife. The influence of soil quality on wildlife productivity has been shown by Albrecht (1944) and others.

Einarsen (1946) points out the importance of the protein content of deer browse. The protein content of the important browse plants is shown to be significantly greater when the plants receive abundant sunshine. Deer living on the edges of areas which have recently been logged or burned have two advantages--the browse plants are both more abundant and more nutritious than those under a closed forest canopy. This is especially true after a burn, which enriches the soil with minerals.

The importance of this was shown by a comparison of deer populations living under both types of conditions. Those from recently cleared areas were more abundant, healthier, and larger than those from climax areas. Periodic heavy losses occur in the latter. He states that when the protein content of browse "falls below 5 per cent, a deer crisis is at hand... parasites were not a factor on burned over land. When proteins fell to a critical level (on a climax area), deer losses were increased by parasitic infestations as they took nourishment greatly needed by deer whose digestion was overtaxed in handling an increased bulk of fibrous browse." (p.311) He gives no evidence to support this last statement, so it cannot be considered well established.

A study of the effect of forest succession on the nutrition of moose has shown these changes in quantity and quality of food in some detail. "In general terms it can be concluded that the youngest stages in forest succession are, in this habitat, providing the most nutritious vegetation, but for certain nutrients access to older stands bearing well grown coniferous trees is desireable. It is also clearly evident that a flora containing several different palatable browse species is most important in the constitution of a satisfactory winter range." (Cowan et al. 1950, p.270)

Both of the above papers show that the nutritive quality of browse is lower in winter than in summer, on the whole. The stresses presented by winter conditions are reduced food volume, lower food quality, increased energy requirement, and possibly direct effects of cold. The first three effects all have one result--malnutrition. Increased metabolism requires not only fat and carbohydrate for energy but also additional protein for tissue maintenance, and protein has been shown to be especially low in the winter.

The relationship between parasites and nutrition is demonstrated by field data in a report by Van Volkenburg and Nicholson (1943) on the relationship between parasitism and malnutrition in deer. Parasites are apparently unimportant on ranges with sufficient browse, but shortage of browse, and the resultant grass eating, result in malnutrition and in the acquisition of parasites which occur on grass but not on

browse. Heavier infestation results.

The ability of natural populations to live at very low temperatures was studied by Laurie (1946). He found colonies of house mice in cold storage plants, living all their lives at temperatures of -21 to -10°C. The temperatures of their nests were higher than those outside, but the mice were exposed to the full effect of the cold when searching for food. These mice were much larger than those from other environments, indicating adaptation according to Bergmann's rule. The reproductive rate was relatively high.

Criticism of the Experiment and Suggestions for Further Work

The work reported here must be considered as merely a preliminary exploration of the field. It must be repeated and extended, using improved techniques. It was, of course, impossible to foresee all of the problems which arose in time to treat them correctly.

Two major mistakes were made--insufficient records were taken, and the method of feeding was not satisfactory. It is doubtful if either of these faults invalidates the results, but they do make them less trustworthy.

Weight, food consumption, and parasite load should be determined more often than was done here. Each mouse should be weighed after death. All the surviving mice should be examined internally at the end of the experiment. It would be

helpful to include in each group a few extra mice for examination during the experiment.

The food should be given in a way which would eliminate wastage, so that the exact consumption could be determined. This is a very necessary improvement. The method of calculating half-rations proved to be unsatisfactory. Few of the half-ration mice actually received half of the food eaten by the full-ration mice, and the fraction of a full ration which each half-ration mouse received was different for different mice. A better procedure would be to base the half-rations on the consumption of the full-ration mice during the previous day. This method presents difficulties in determining the food requirement for each weight class, and involves much more work. Food measurements should be more accurate than was possible here.

The experimental conditions used here were not suitable for such a short-term experiment, since there was no mortality in half of the groups. This makes all calculations based on survival misleading, since mice which probably would have lived much longer must be considered to have died as a result of the experimental conditions by the end of the experimental period. This can be corrected by extending the experiment, which is very important, and by changing the experimental conditions to give a better gradation of results. The reduced ration could be increased from one-half to two-thirds in order to give the other conditions an opportunity to exert their

effects. The cold-room temperature could be lowered to freezing or below. A more serious parasite could be used. Every combination of conditions, though, must be considered an independent and valid experimental design.

This work can be extended in a number of ways. One is to include controlled breeding, as was originally planned for the present experiment. An ambitious extension of this would be to establish large laboratory populations under the experimental conditions and observe them over a long period of time, studying acclimatization, individual and population size, longevity, and birth rate. Other hosts, including native mammals, can be used, and a wide variety of parasites.

Any such project will be relatively sterile unless the main interest--field conditions--is kept constantly in mind. There must be a constant interchange of ideas between laboratory and field, so that the laboratory findings may be tested in the field, and field observations used as the basis for planning laboratory experiments.

This experiment and report were designed as a problem in experimental ecology, with interest centered on the gross effects of the experimental conditions on survival and condition. Before any real understanding of the problem can be reached the physiological mechanisms involved must be elucidated. It is therefore highly desireable that in the future physiological studies be included in any experiments of this type.

SUMMARY

1. Laboratory mice were infected with Trypanosoma duttoni and subjected to stress conditions--low environmental temperature and inadequate food consumption. Various combinations of these stress factors for infected and uninfected mice were used, involving a total of eight groups, containing 15 mice each.

2. The half-ration mice early began to die off. Over half of them had died after 19 days, and the remainder probably would have died shortly. None of the full-ration mice died during the course of the experiment.

3. The half-ration mice kept at low environmental temperature (43°F) died sooner than those at room temperature (68°F). By the end of the experiment there had been 10 deaths among the room-temperature mice and 31 among the cold ones. The full-ration mice adapted themselves well to the low temperature.

4. The infected mice died more rapidly than did the uninfected ones. This difference is slight, but is statistically significant. The parasite is ordinarily benign.

5. The full-ration mice gained weight; the half-ration mice lost weight. Weight change shows no consistent correlation with the other factors.

6. There was no correlation between sex and survival or weight change.

7. The mice which died showed gastro-intestinal hemorrhage and degeneration. The fat stores were not exhausted in the earliest mice to die, but were in those which died toward the end of the experimental period.

8. The pertinent literature is reviewed and the application of this information to field conditions is discussed.

ACKNOWLEDGEMENTS

Much of the credit for this work belongs to the numerous persons who contributed so greatly in advice and personal assistance. The idea and principal details were suggested at a meeting with Drs. W. A. Clemens, J. R. Adams, and I. McT. Cowan. Dr. Clemens helped obtain financial support, and showed constant interest in the work. Dr. Adams, my advisor, was helpful in many ways, especially during the rather trying period before the experiment could be begun. Dr. Cowan helped obtain certain difficult materials. Mr. G. J. Spencer identified the mites. Dr. P. A. Larkin helped plan the statistical analysis.

The work was done at the Animal Nutrition Laboratory of the Faculty of Agriculture. Dr. A. J. Wood, the director, gave much valuable advice, both on the maintenance of the mice and in interpreting the results. He and his staff did much of the actual operation of the experiment. Without Dr. Wood's constant support the experiment could not have been carried out. Mr. W. H. Markham of this laboratory made the blood sugar determinations.

The parasites used were supplied by Dr. P. V. Gustafson of the University of Washington and Dr. J. W. Moulder of the University of Chicago. Dr. Constantinides of the Faculty of Medicine made a post mortem examination of several mice.

The project was supported by a grant from the University Research Fund.

The author wishes to express his deepest appreciation to all the above persons and to those others who also contributed ideas, encouragement, and advice.

APPENDICES

Appendix 1. Composition of Ration #10 of the Animal Nutrition Laboratory.

wheat flakes	450	parts
wheat bran	45	"
fish meal (70%)	50	"
wheat germ meal	75	"
powdered skim milk	50	"
dried yeast	10	"
apple pomace	30	"
beet pulp	30	"
oats, ground	40	"
meat scrap	75	"
soya meal	75	"
liver meal	50	"
bone meal	10	"
carragrass	5	"
salt	5	"
	1000	"

Appendix 2. Statistical methods employed.

All of the statistical methods employed here are widely used, and are described in Snedecor (1946), but a brief description of the methods used is given for convenience and to permit comparison.

Standard deviation. (σ)

$$\sigma = \sqrt{\frac{\Sigma x^2 - (\Sigma x)^2/N}{N-1}}$$

The "t" test.

$$t = \frac{\bar{x}_a - \bar{x}_b}{\sqrt{\left(\frac{\sigma a}{\sqrt{Na}}\right)^2 + \left(\frac{\sigma b}{\sqrt{Nb}}\right)^2}}$$

Analysis of variance. The purpose of analysis of variance is to determine which of two or more experimental conditions is responsible for observed variation in results. The variation due to each condition is isolated and compared to the variation among animals treated in the same way, to determine the significance of the inter-group variation. The probability of the results being affected by the interaction of the experimental factors is also indicated.

The present analysis of variance was based on the method described by Snedecor (pp.275-277) for analysis with more than one item in the subclasses. The computations involved need not be mentioned here. The results are given in Appendix 3.

"F" values are arbitrary units used in all analysis of variance. The calculated "F" is compared with the proper tabled "F", and if larger indicates that the variance is statistically significant. The degree of significance is dependent on the probability (p) level used in selecting the tabled "F". For most biological purposes the p=.05 level is used. This means that the chances are 5 in 100 that the observed results might have occurred as a result of chance alone. For further refinement the p=.01 level is used, indicating that chance alone would be expected to produce such results once in 100 tries.

Appendix 3. Analysis of survival variance.

Source of Variance	Calc. F	Tab. F p=.05	Signi-ficant	Tab. F p=.01	Signi-ficant
1. Temperature vs. food and parasitism.					
Temperature	148	--	--	6.90	Yes
Food and parasite	76	--	--	3.98	Yes
Interaction	41	--	--	3.98	Yes
2. Food vs. temperature and parasitism.					
Food	223	--	--	6.90	Yes
Temperature and parasite	50	--	--	3.98	Yes
Interaction	43	--	--	3.98	Yes
3. Parasitism vs. temperature and food.					
Parasite	1.23	3.94	No	--	--
Temperature and food	12.46	--	--	3.98	Yes
Interaction	154	--	--	3.98	Yes
4. Parasitism vs. temperature for half-ration mice					
Parasite	7.68	3.92	Yes	6.84	Yes
Temperature	398	--	--	6.84	Yes
Interaction	.808	3.92	No	--	--

Appendix 3. Continued

Source of Variance	Calc. F	Tab. F p=.05	Signi-ficant	Tab. F p=.01	Signi-ficant
5. Parasitism vs. food for cold-room mice.					
Parasite	1.06	3.92	No	--	--
Food	657	--	--	6.84	Yes
Interaction	9.57	--	--	6.84	Yes
6. Parasitism vs. food for cold-room mice, omitting CFN-13.					
Parasite	9.41	--	--	6.84	Yes
Food	1576	--	--	6.84	Yes
Interaction	9.41	--	--	6.84	Yes

LITERATURE CITED

Ackert, J. E. and Charles L. Wisseman, Jr. 1946. Tolerance of fowls for moderate infections of intestinal helminths. Am.J.Trop.Med. 26: 721-728.

Albrecht, William A. 1944. Soil fertility and wildlife--cause and effect. Trans. Ninth N. A. Wildlife Conf. pp.19-29.

Andrews, John S. and D. J. Jones. 1948. Effect of worm parasites on the growth of and feed utilization by pigs. J.Parasit. 34(6-2): 13-14.

Baillif, Ralph N. 1937. Cytological changes in the rat thyroid following exposure to heat and cold, and their relationship to the physiology. Am.J.Anat. 61: 1-19.

-----. 1938. Microscopic changes in the hypophysis of the albino rat following exposure to cold, and their relationship to the physiology of secretion. Am.J.Anat. 62: 475-495.

Barki, Victor H., P. H. Derse, R. A. Collins, E. B. Hart, and C. A. Elvehjem. 1949. The influence of coprophagy on the biotin and folic acid requirements of the rat. J.Nutr. 37: 443-456.

Beck, J. Walter and Asa C. Chandler. 1950. Experiments on the nutrition and host relations of Hymenolepis diminuta in white rats, with special reference to

vitamins and hormones. J.Parasit. 36(6-2): 44.

Becker, Elery R. and Neal F. Morehouse. 1936. The effect of diet on the coccidian infection of the rat. J.Parasit. 22: 60-67.

-----, Jane Taylor, and Caroline Fuhrmeister. 1947. The effect of pantothenate deficiency on Trypanosoma lewisi infection in the rat. Iowa.St.Coll.J.Sci. 21: 237-243. (Biol.Abs. 20882, 1947).

Benedict, Francis G. and Grace MacLeod. 1929. The heat production of the albino rat. II. Influence of environmental temperature, age, and sex; comparison with basal metabolism of man. J.Nutr. 1:367-398.

Bittner, John J. 1941. Care and recording, in Snell, G. D. Biology of the laboratory mouse. Philadelphia: Blakiston. 497pp.

Boughton, R. V. 1932. The influence of helminth parasitism on the abundance of the snowshoe rabbit in western Canada. Can.J.Res. 7: 524-547.

Brobeck, John R. 1948. Food intake as a mechanism of temperature regulation. Yale J.Biol.and Med. 20: 545-552. (Biol.Abs. 20867, 1949).

Caldwell, Frederick E. and Paul Gyorgy. 1943. Effect of biotin deficiency on duration of infection with Trypanosoma lewisi in the rat. Proc.Soc.Exp.Biol. and Med. 53: 116-119.

---- and ----. 1947. The influence of biotin deficiency on the course of infection with Trypanosoma lewisi in the albino rat. J.Inf.Dis. 81: 197-208.

Cannon, Paul R. 1942. Antibodies and the protein-reserves. J.Immun. 44: 107-114.

Carlson, A. J. and Frederick Hoelzel. 1948. Prolongation of the life span of rats by bulk-formers in the diet. J.Nutr. 36: 27-40.

Chambers, William H. 1938. Undernutrition and carbohydrate metabolism. Physiol.Rev. 18: 248-296.

Chandler, Asa C. 1943. Studies on the nutrition of tapeworms. Am.J.Hyg. 37: 121-130.

Cowan, I. McT., W. S. Hoar, and J. Hatter. 1950. The effect of forest succession upon the quantity and upon the nutritive values of woody plants used as food by moose. Can.J.Res.,D 28:249-271.

Cox, W. T. 1936. Snowshoe rabbit migration, tick infestation, and weather cycles. J.Mam. 17: 216-221.

D'Angelo, S. A., A. S. Gordon, and H. A. Charipper. 1948. A differential response of the rodent adrenal gland in acute starvation. Proc.Soc.Exp.Biol.and Med. 68: 527-529.

Deuel, H. J. Jr., E. R. Meserve, Evelyn Straub, Cornelia Hendrick, and B. T. Scheer. 1947. The effect of fat level of the diet on general nutrition. J.Nutr. 33: 569-582, 582-592, 641-648.

Donaldson, Alan W. and G. F. Otto. 1946. Effects of protein-deficient diets on immunity to a nematode (Nippo strongylus muris) infection. Am.J.Hyg. 44: 384-399.

Donaldson, Henry H. 1924. The rat--Data and reference tables. 2nd ed. Mem.Wistar Inst.Anat.and Biol.No.6. 469pp.

Dubinin, V. B. and L. I. Leshkovitch. 1945. On the fattening of the marmot (Marmota sibirica Radde) and their infestation by ascarids before entering hibernation. Zool.J.Moscow 24: 373-378. (Biol.Abs. 30716, 1949).

Eichler, W. 1942. Untersuchungen zur epidemiologie der aussenparasiten. IV. Nest, witterung und parasiten-befall bei schwalben und einigen anderen wirten. Anz.Schadlingsk 18: 4-10. (Biol.Abs. 7533, 1950).

Einarsen, Arthur S. 1946. Crude protein determination as an applied management technique. Trans. Eleventh N. A. Wildlife Conf. pp.309-312.

Erickson, Arnold B. 1944. Helminth infections in relation to population fluctuations in snowshoe hares. J.Wildlife Man. 8: 134-153.

Ershoff, B. H., J. N. Pagones, and H. J. Deuel Jr. 1949. Comparative nutritive value of butter and vegetable fats under conditions of low environmental temperature. Proc.Soc.Exp.Biol.and Med. 70: 287-290.

Fitzpatrick, F. K. 1948. Susceptibility to typhus of rats on deficient diets. Am.J.Pub.Health 38: 676-681.

Frye, W. H. and H. E. Meleney. 1937. Vitamin A-deficient diet in rats in relation to infection with E. muris. J.Parasit. 23: 228-229.

Gilson, Saul B. 1950. Studies on adaptation to cold air in the rat. Am.J.Physiol. 161: 87-91.

Griffiths, W. J. Jr. 1944. Absence of audiogenic seizures in wild Norway and Alexandrine rats. Science 99:62-63.

Guggenheim, K. and Edith Buechler. 1947. Nutritional deficiency and resistance to infection. The effect of caloric and protein deficiency on the susceptibility of rats and mice to infection with Salmonella typhi murium. J.Hyg. 45: 103-109.

Gyorgy, Paul. 1938. Pediculosis in rats kept on a riboflavin-deficient diet. Proc.Soc.Exp.Biol. and Med. 38: 383-385.

Hansen, M. F., A. C. Todd, and G. W. Kelley. 1950. Effects of a pure infection of the tapeworm Moniezia expansa on lambs. J.Parasit. 36(6-2): 45.

Hawkins, Philip A. 1946. Studies of sheep parasites. VII. Moniezia expansa infections. J.Parasit. 32(6-2): 14.

Hegner, Robert. 1937. Parasite reactions to host modifications. J.Parasit. 23: 1-12.

Hendricks, James R. 1950. The effect of body weight on the natural resistance of mice to Trichinella spiralis. J.Parasit. 36(6-2): 39-40.

Hill, Robert M. 1947. The control of body-temperatures in white rats. Am.J.Physiol. 149: 650-656.

Horst, Kathryn, L. B. Mendel, and F. G. Benedict. 1930. The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. J.Nutr. 3: 177-200.

Horvath, S. M., G. E. Folk, F. N. Craig, and W. Fleischmann. 1948. Survival time of various warm-blooded animals in extreme cold. Science 107: 171-172.

Jackson, C. M. 1915. Effects of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. Am.J.Anat. 18: 75-116.

Johnson, L. P. V. 1950. An introduction to applied biometrics. Minneapolis: Burgess. 165pp.

Kartman, Leo. 1943. New developments in the study of ectoparasite resistance. J.Econ.Entom. 36: 372-375.

Knorr, M. 1926. Die weisse maus als versuchstier. Centr. Bakt., 1 Abt., Orig. 99: 576-584.

Kolodny, Maxwell H. 1940. The effect of environmental temperature upon experimental trypanosomiasis (T. cruzi) of rats. Am.J.Hyg. 31(C): 21-23.

Krzywicki, Harry and Esther da Costa. 1949. Effect of dietary restriction and rehabilitation upon the spontaneous activity of rats. Fed.Proc. 8: 388.

Larsh, John E. Jr. 1950. The effects of a protein-deficient diet on resistance of mice to Hymenolepis infection.

J.Parasit. 36(6-2): 45-46.

Laurie, E. M. O. 1946. The reproduction of the house mouse (Mus musculus) living in different environments. Proc.Roy.Soc.,B 133: 248-281.

Lawler, H. J. 1941. The relation of vitamin A to immunity to Strongyloides infection. Am.J.Hyg. 34(D): 65-72.

LeBlond, C.-P., L.-P. Dugal, and M. Therien. 1944. Les aliments choisis par le rat blanc au froid et a la chaleur. Rev.Can.Biol. 3: 127-129.

---- and J. Gross. 1943. Effect of thyroidectomy on resistance to low environmental temperature. Endocrinology 33: 155-160.

Lesser, A. J., R. J. Winzler, and J. B. Michaelson. 1949. Effect of iodide on thyroid glands of rats kept at low temperatures. Proc.Soc.Exp.Biol.and Med. 70: 571-573.

McCoy, O. R. 1934. The effect of vitamin A deficiency on the resistance of rats to infection with Trichinella spiralis. Am.J. Hyg. 20: 169-180.

McDowell, Claire. 1923. The effect of different temperatures and humidities on the resistance of rats to a pneumococcus infection. Am.J.Hyg. 3: 521-546.

McNaught, J. B., V. C. Scott, F. M. Woods, and G. H. Whipple. 1936. Blood plasma protein regeneration controlled by diet. J.Exp.Med. 63: 277-301.

Metcoff, Jack. 1949. The influence of protein nutrition on experimental infection. Am.J.Pub.Health 39: 862-865.

Miller, Harry M. and C. W. Dawley. 1928. An experimental study of some effects of Cysticercus fasciolaris Rud. on the white rat. J.Parasit. 15: 87-103.

Mills, C. A. 1939. Climate and metabolic stress. Am.J.Hyg. 29(A): 147-164.

----. 1945. Influence of environmental temperatures on warm-blooded animals. Ann.N.Y.Acad.Sci. 46: 97-113.

Morgulis, S. 1923. Fasting and undernutrition. New York: Dutton.

Morris, H. P. 1944. Review of the nutritive requirements of normal mice for growth, maintenance, reproduction, and lactation. J.Nat.Cancer Inst. 5: 115-141.

Nichols, John. 1950. Effects of captivity on adrenal gland of wild Norway rat. Am.J.Physiol. 162: 5-9.

Nowicki, E. 1939. Postmortales infektionsfahiges uberdaurn von Schizotrypanum cruzi, Trypanosoma congolense, und Trypanosoma equinum in versuchstierorganen. Zentralbl. Bakt. I. Abt. Orig. 143: 386-392. (Biol.Abs. 3845,1940)

Olson, T. A. and R. G. Dahms. 1946. Observations on the tropical rat mite, Liponyssus bacoti, as an ectoparasite of laboratory animals and suggestions for its control. J.Parasit. 32: 56-60.

Perelshin, S. D. 1943. Winter nutrition of the polar fox in the Yamal district. Zool.Zhur. 22: 299-313.

Pounden, W. D., J. W. Hibbs, and W. E. Krauss. 1947. Weather and management influence calf health. Ohio Farm and Home Res. 32: 203-206. (Biol.Abs. 8194, 1949).

Quimby, Freeman H. 1948. Food and water economy of the young rat during chronic starvation and recovery. J.Nutr. 36: 177-186.

----, N. E. Philips, and I. V. White. 1948. Chronic inanition, recovery, and metabolic rate of young rats. Am.J.Physiol. 154: 188-192.

Quin, J. I. 1945. Comparative effects of climate as studied on white rats in various South African localities. S.Afr.J.Sci. 41: 304-309.

Reid, W. M. 1945. The relationship between glycogen depletion in the nematode Ascaridia galli (Schrank) and elimination of the parasite by the host. Am.J.Hyg. 41: 150-155.

Ring, G. C. 1936. An attempt to stimulate the thyroid gland in rats by exposure to cold. Am.J.Physiol. 116: 129.

----. 1938. Metabolism and body temperature of normal and adrenalectomized rats during exposure to cold. Am.J.Physiol. 122: 435-445.

Ritterson, Albert L. and Leslie A. Stauber. 1949. Protein intake and leishmaniasis.in the hamster. Proc.Soc. Exp.Biol.and Med. 70: 47-50.

Roberts, Sidney and Leo T. Samuels. 1949. Influence of previous diet on metabolism during fasting. Am.J.Physiol. 158: 57-62.

Scheff, G. J. and J. S. Thatcher. 1949. The role of potassium as cause of death in experimental trypanosomiasis. J.Parasit. 35: 35-40.

Schneider, H. A. 1949. Influence of nutrition in experimental infection. Bact.Rev. 13: 99-134.

Schwabe, E. L., F. E. Emery, and R. Griffith Jr. 1938. The effect of prolonged exposure to low temperature on the basal metabolism of the rat. J.Nutr. 15: 199-210.

Snedecor, George W. 1946. Statistical methods applied to experiments in agriculture and biology. Ames: Iowa St.Coll.Press. 485pp.

Sprent, J. F. A. 1949. On the toxic and allergic manifestations produced by the tissues and fluids of Ascaris. I. Effect of different tissues. J.Inf.Dis. 84: 221-229.

Taliaferro, W. H. and Yelena Pavlinova. 1936. The course of infection of Trypanosoma duttoni in normal and in splenectomized and blockaded mice. J.Parasit. 22: 29-41.

----, R. L. Woolridge, and E. P. Benditt. 1949. The effect of protein depletion on acquired immunity in trichinosis. Science 109: 443.

Treichler, Ray, R. W. Stow, and A. L. Nelson. 1946. Nutrient content of some winter foods of ruffed grouse. J.Wildlife Man. 10: 12-17.

Tripi, H. B., G. M. Gardner, and W. C. Kuzell. 1949. Effects of temperature and ultraviolet light on experimental polyarthritis of rats. Proc.Soc.Exp.Biol.Med. 70: 45-47.

Van Cleave, H. J. 1937. Worm parasites in their relation to wildlife investigations. J.Wildlife Man. 1: 21-27.

Van Volkenberg, H. L., and A. J. Nicholson. 1943. Parasitism and malnutrition of deer in Texas. J.Wildlife Man. 7: 220-223.

Vegors, H. H., and Dale A. Porter. 1950. Studies on the life history and pathogenicity of the intestinal nematode Strongyloides papillosus in calves. J.Parasit. 36(6-2): 33.

Von Brand, Theodor. 1950. The carbohydrate metabolism of parasites. J.Parasit. 36: 178-192.

Ware, A. G., R. M. Hill, and F. H. Schultz. 1947. The effect of interference with respiration on the control of body-temperature in white rats and New Zealand rabbits. Am.J.Physiol. 149:657-666.

Weir, W. C., T. L. Bahler, A. L. Pope, P. H. Philips, C. A. Herrick, and G. Bohstedt. 1948. The effect of hemopoietic dietary factors on the resistance of lambs to

parasitism with the stomach worm, Haemonchus contortus. J.Animal Sci. 7: 466-474. (Biol.Abs. 9043, 1949)

Whitlock, John H. 1949. Thyroid abnormalities as a possible factor in trichostrongylidosis. J.Parasit. 35(6-2): 12.

----. 1949a. The relationship of nutrition to the development of the trichostrongylidoses. Cornell Vet. 39: 146-182. (Biol.Abs. 30415, 1949).

Wright, Willard H. 1935. The relation of vitamin deficiency to ascariasis in the dog. J.Parasit. 21: 433.