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The effects of cold exposure on the physiological functions of animals have become an important part of the study of environmental physiology. As man migrates to colder climates, the knowledge of the mechanism of his responses and adjustments to the cold take on more significance. Investigations on different mammals during prolonged cold exposure show that a gradual change is undergone which apparently increases their resistance to the cold. The specific stressing property of cold is an increased heat loss. Much attention has been given this increased heat loss to determine its pathway.

This study was designed to investigate not only the extent of the increase in heat production of the golden hamster, but to determine some of the other changes that occur in this animal either as a cause or result of the increased resistance to prolonged cold exposure.

In addition, the length of time needed for the hamster to achieve complete acclimatization was recorded, and the extent of the changes in the animal after acclimatization was measured.

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EXPLANATION OF TERMS

Acclimatization. As used here the term refers to the changes that occurred in the animal as a result of being exposed to a cold environment. The changes which take place during acclimatization allow the animal to function adequately in the new environment.

The chief criterion employed to determine the extent of cold acclimatization in most animals is the change in resting metabolism as measured at or near the thermoneutral temperature (temperature at which resting metabolism is minimum). Hereafter, any reference to metabolism or oxygen consumption will be followed by the figures (30°C) or (6°C) which designate the approximate temperature at which these measurements were made.

A second method of determining the extent of cold acclimatization has been employed by Adolph and Richmond (2), using the body cooling and rewarming rates. They have demonstrated in hamsters and ground squirrels that acclimatized animals have a slower cooling rate than non-acclimatized animals. Fregly (20) has demonstrated the same phenomenon in rats.

Hibernation. Hibernation is a state exhibited by some mammals as a result of an adverse environmental condition, characterized by a reduction in physiological activities, i.e., a reduction in oxygen consumption, slowing of the heart and respiratory rates, and a fall in temperature to a few degrees above ambient and a decrease in serum magnesium (15).

Hibernation is of interest in these studies because the hamster is classified as a hibernator, although it is a relatively poor one (15,16,41). It appears to be closely related in some ways to non-hibernators (2,15), but possesses the property of entering the state of hibernation when necessary. The fact that the hamster is able to hibernate and also acclimatize to the cold (2,10) may provide information as to the accomplishment of natural hypothermia. It is also interesting to note that because the hamster responds similarly to non-hibernators on exposure to the cold, it might be considered a "missing link" between hibernators and non-hibernators.

The investigations presented herein are concerned with the hamster's ability to acclimatize to the cold, and are intended to compare these responses with those of other hibernating and non-hibernating mammals.

REVIEW OF LITERATURE

Numerous investigations have been made to determine the effects on animals of exposure to low temperatures. Most of these studies are concerned with the metabolic response in the form of heat production. The anatomical changes that occur under these conditions have been largely ignored. Most studies on cold acclimatization have been performed on the common laboratory animals such as the rat (1,7,12,20, 26,49), rabbit (5) and others (14,21,23,33,34,43). Fewer studies have been done on man (6,37), but some of the results of work on the small animals have been applied to man. The hamster has received very little attention in relation to its acclimatization to cold (2,35). Major emphasis placed on this animal has been on its ability to hibernate.

Cold-acclimatization in small mammals: oxygen consumption. The oxygen consumption (5°C) of small mammals exhibits an immediate increase upon cold exposure (5); an increase which is slower and of less magnitude occurs if the oxygen consumption is measured at 30°C in the same animal after the same length of cold exposure (7,24,32,50). The increase in oxygen consumption (5°C) is believed to be due primarily to shivering and an increase in muscular tone (5). Most investigators believe that the more gradual increase in resting metabolism (30°C) is not due entirely to an increased muscular tone, although there are some conflicting reports concerning this hypothesis (32). It is agreed by workers in this field that a large portion of the increased oxygen consumption (30°C) is due to adaptive changes in the physiology of the animal. The result of these adaptive changes is an increase in the basal metabolism of the tissues themselves. The increase is believed to be caused by a change in the endocrine functions initiated by the stress of cold exposure. A further discussion of the role the endocrines play in the process of acclimatization is found in the section on endocrine glands.

The primary function of an increased heat production in the acclimatized animal is to maintain its body temperature. The process of acclimatization is to accomplish this by the most efficient methods.

Body temperature. The body temperature of cold-acclimatized animals (5°C) is usually somewhat lower than non-acclimatized rats at 30°C. Yet the body temperature of the acclimatized animal is higher than that of the non-acclimatized animal when measured at the thermoneutral temperature (5). LeBlanc (37) suggests that the decrease in the body temperature is an example of an increased efficiency in the thermoregulatory powers. Its advantage to the animal

is that it would decrease the caloric cost necessary at these temperatures to maintain the normal body temperature at the new level.

Carlson (5) has made some interesting observations concerning the changes in heat production and the core temperature of the cold exposed rat. He noted that the heat production (5°C) increases on the first day, and remains very nearly at this high level during the remaining period of cold exposure, yet the core temperature (5°C) changes markedly. At the onset of cold exposure the core temperature (5°C) decreases 2 to 3 degrees, and following this, gradually increases until it is only slightly below the values obtained at 30°C. This leads one to believe that the heat-loss mechanism has improved so that only the necessary amount of heat is lost to maintain the core temperature. The essential factor of major concern in establishing a more efficient heat loss is the peripheral heat regulation, brought on by changes in peripheral circulation (13,26) and by changes in insulation (23,51).

Insulation. Insulation plays an important role in the ability of the animal to resist the effects of low environmental temperatures (23,32,-43,51). This phase of cold acclimatization is best exemplified in the arctic animals. Scholander (47) has studied many of these animals and demonstrated the importance of their insulation in the form of fur. The husky dog, for example, has very adequate insulation even in temperatures as low as −40°C; at this temperature there is no increase in the metabolic rate of these animals.

Sellers et al. (51) have recorded the oxygen consumption of clipped and unclipped cold-acclimatized rats to determine the importance of the fur of this animal. The oxygen consumption at 30°C was not influenced. Oxygen consumption (1.5°C) was 30% higher in the clipped animals.

Weight, food and water changes. A decrease in body weight has been observed in all small mammals studied during the first few days of cold exposure (2,15,25). After this initial weight loss two things may occur: (1) the weight may stabilize at a lower level, or (2) it may increase. After the weight increases as noted in some animals (2,50) it may return to the original level or in some instances continue to increase following the growth curve of similar animals at higher temperatures. This latter phenomenon has been observed in young rats.

As expected, there is an increase in food consumption of most animals exposed to the cold (7,39,48). The food consumption does not parallel the increase in oxygen consumption, therefore the decrease in weight noted above is observed. When the weight either stabilizes

or begins to increase in the cold-exposed animal, it is assumed that the animal has reached a stable state in which the caloric intake is equal to or greater than the caloric output.

Studies on water intake by the hamster during cold exposure have indicated an increase of approximately 65% (39).

Changes in endocrine function. Extensive studies have been made on the thyroid gland with reference to its size and activity in response to cold exposure (4,11,21,36,44,45,51). In the rat this gland shows an increase in activity as measured by: (1) weight increases of the organ itself, (2) histological changes, (3) removal of the organ, and (4) I^{131} uptake studies. Survival in the cold after thyroidectomy of non-acclimatized rats is short (5-12 days); acclimatized rats survive much longer (19-43 days) upon cold exposure if the thyroid gland is removed after acclimatization (51).

Radioactive iodine uptake studies imply, as a result of an increased uptake of iodine, that the thyroid is hyperactive, especially during the first month after cold exposure (4). It is agreed that the thyroxin requirement remains high for prolonged periods of cold exposure, but the role of this hormone has not been definitely established (32).

Heroux (28) has reported that there is a great increase in the activity of the adrenal cortex initially during the period of cold acclimatization, and after acclimatization has been completed the glands remain hypertrophied but return to their original activity. Heroux (27,29) observed that removal of the adrenal glands does not mean that acclimatization cannot occur; he was able to acclimatize adrenalectomized animals with salt therapy and gradual exposure to the cold environment.

Hsieh and Carlson (32) in a recent attempt to further evaluate the role of the adrenal medulla and the thyroid glands in acclimatization, curarized rats to eliminate the muscular tone contribution to the increased metabolic activity of acclimatized animals, and tested the contribution of each hormone to the increased metabolism. They concluded that both organs play an important role in acclimatization, with the adrenal medulla having the greatest role. They also feel that the calorigenic action of both thyroxin and adrenalin may depend upon the increased ability of the cold-acclimatized animal to utilize fat (25), as well as glucose (14) and protein.

Woods (55) compared the response of the domesticated rat and the wild Norway rat to cold and found that the adrenal glands of the domesticated rat increase in size, but there was no change in size of the adrenal glands in the wild rat. This would indicate that the

latter animal is constantly under stress to cope with nature and that the adrenals are operating at a maximum level of activity the major portion of the time. Since this animal is exposed to varying temperatures and other stress situations during the largest part of its life span, it is possible that it becomes adapted to the cold naturally and therefore the insulation provided by its fur, pilo-erection, superficial blood flow, and other factors would influence its response to cold exposure. Thus the thyroid, adrenal cortex, and the adrenal medulla (13) exhibit an increased activity during the initial phases of cold exposure, while acclimatization is taking place. After acclimatization is complete, these glands apparently function at a normal rate (8,27). The role of each of these glands is still obscure.

Cold exposure and fluid compartment shifts. Little is known about the body fluid changes in small animals during prolonged cold exposure. Deb and Hart (12) have made an extensive study of the blood and fluid volume changes in rats upon exposure to 30°C and 6°C. They found that total body water of the rats at 6°C was greater relative to body weight but was less in absolute terms. Blood and plasma volume was greater in the 6°C rats after an exposure of five weeks. Intracellular water was higher in the 30°C rats after three weeks, and extracellular water showed no change relative to total body water.

It has been suggested that the increased blood volume reported in the rat is due to the increased vascularity that occurs in the animal during cold acclimatization (26). This increased vascularity is a consequence of the increased metabolic capability of the animal after cold acclimatization has occurred (12).

The decrease in intracellular water is difficult to assess. There seem to be conflicting reports on what changes should be expected to occur in this fluid compartment. The suggested hypotheses are a result of both in vitro and in vivo studies. Adolph et al. (1), Robinson (46), and others suggest that the cellular water is dependent upon the metabolic integrity of the tissue and that an increase in metabolism causes a decrease in intracellular water. Wedgwood (54) related the fluid compartments to metabolism in human subjects and found an inverse relationship between metabolism and intracellular water when the total body water is held constant. In contrast to these reports, studies on hypothermia indicate an increased intracellular water during the first stages of reduction of body temperature (9). This is believed to be due to the increased muscular activity and metabolism occurring during this time.

Organ weight changes. Some changes in the weights of individual organs have been reported as a result of cold exposure. The liver increases in weight, and there is an increase in weight of both the rat and the mouse kidney (52). Sevenson (52) found an increase in heart weight in the rat but reported that no change occurred in the weight of the spleen when the value was expressed in terms of total body weight. When expressed as an absolute value, there appeared to be some decrease in spleen weight upon cold exposure. Little emphasis has been placed on this phase of acclimatization.

Comparisons of cold acclimatization in hibernators and non-hibernators. For the most part these comparisons are not easily made, since most hibernators will enter hibernation very soon after exposure to the cold (3,35,41,42), and few attempts have been made to determine if acclimatization to cold, as observed in the non-hibernators, takes place. The hamster, although a hibernator, differs in some respects from other hibernating mammals (15,41). One of the major differences observed in the hamster concerns the time it may remain in the cold before it enters a dormant state. It may remain in the cold (6°C) for long periods of time before it will enter hibernation and, in some instances, may never hibernate (38,41) under artificial conditions.

Other differences have been observed in the hamster that distinguish it from other hibernators (15). The fact that the hamster does not always hibernate upon exposure to the cold gives the investigator an opportunity to study its responses to the cold prior to hibernation, and compare them to non-hibernators.

In contrast to non-hibernators the activity of the endocrine glands of the hibernators seems to decrease (11,31,35); however, there is also evidence of increased activity of these glands in the hamster (36). Support for the former view has come from studies on the weight changes of these glands both in hibernators in the natural surroundings (31) and in the laboratory (11). The latter view was presented by Knigge (36) who suggested that these glands were hyperactive in the hamster during cold exposure. Holmes (31) studied the ascorbic acid content of the hamster adrenal glands during a period of cold exposure and found it to increase during the first hours, indicating a decrease in activity of this gland at this time. This increase in adrenal ascorbic acid was followed by a steady decrease during the next six or seven days until it had nearly reached control levels by the eighth day, indicating a normal output of the gland. Considering the increased oxygen consumption exhibited by the hamster during cold

exposure even at 30°C, it seems that the view of hyperactivity of the endocrine glands is more applicable than the hypoactivity hypothesis. It must be remembered that the cause of the increased oxygen consumption (30°C) in the hamster has not been determined.

Adolph and Richmond (2) have compared some of the responses of the cold-adapted hamster and ground squirrel to those of the cold-acclimatized rat and found that loss of cold adaptation as a result of exposure to warm temperatures takes much longer in the hibernators than in the rat; the oxygen consumption increases to the same extent in both groups of animals, rewarming from hypothermia does not change in the hibernators as a result of adaptation as it does in non-hibernators, and the pattern of rewarming differs (20).

Differences exist between hibernators and non-hibernators in their response to cold adaptation; there are differences present also within the hibernating group, particularly in the hamster.

PROCEDURES AND TECHNIQUES: EXPERIMENTS ON HAMSTERS

Maintenance of animals. All observations were made on male golden hamsters, ranging in age from 3 to 6 months and in weight from 71 to 114 grams. The animals were purchased from dealers in Wisconsin and California. They were maintained in individual wire cages (Bussey-type) and fed a dry food in pellet form (Rockland Rat Diet). Calibrated glass watering devices were used.

After a reasonable period of isolation has been allowed, half of the animals were placed in a cold room, maintained at 6°C ± 1°C in the same cages previously used. The remaining animals were used as control animals and maintained at 26°C ± 1°C. Daily records of food and water consumption were made during the entire experimental period. Most of the animals were weighed daily.

Measurement of oxygen consumption. The oxygen consumption was measured using a closed-circuit spirometric method (Fig. 1). The apparatus was operated by circulating the gases through the chamber and Baralime absorbent, with a small pump modified from an aquarium pump. Baralime was used also on the bottom of the animal chamber. This pump made it possible to operate only one chamber at a time and took up much less space than most more cumbersome types. The spirometer drop was recorded on a kymograph drum which traveled at a speed of one revolution per hour.

A large, heavy plastic box divided into five small compartments, each separate and sealed from one another, served as the metabolism chamber. The animals were placed in wire cages somewhat smaller than each plastic compartment, but not small enough to restrict movement. The top of the box was held in place with wing nuts with a heavy rubber gasket between the top and the chamber proper.

Since it seemed necessary to train these animals to the apparatus, they were placed in the small wire cages as often as possible before the initial oxygen consumption experiment was conducted; also, a few trial determinations were made with the animals in the plastic chambers. This procedure proved sufficient so that the animals were quiet during the experiments. The animals were placed in the chambers at least one-half hour before the beginning of the actual experiment to bring the system to the standard temperature (27°C). The temperature within each chamber was recorded both at the beginning and end of each determination and the barometric pressure was read once during the test. A compensating chamber was used to record any sudden changes which might occur in temperature or barometric pressure. This

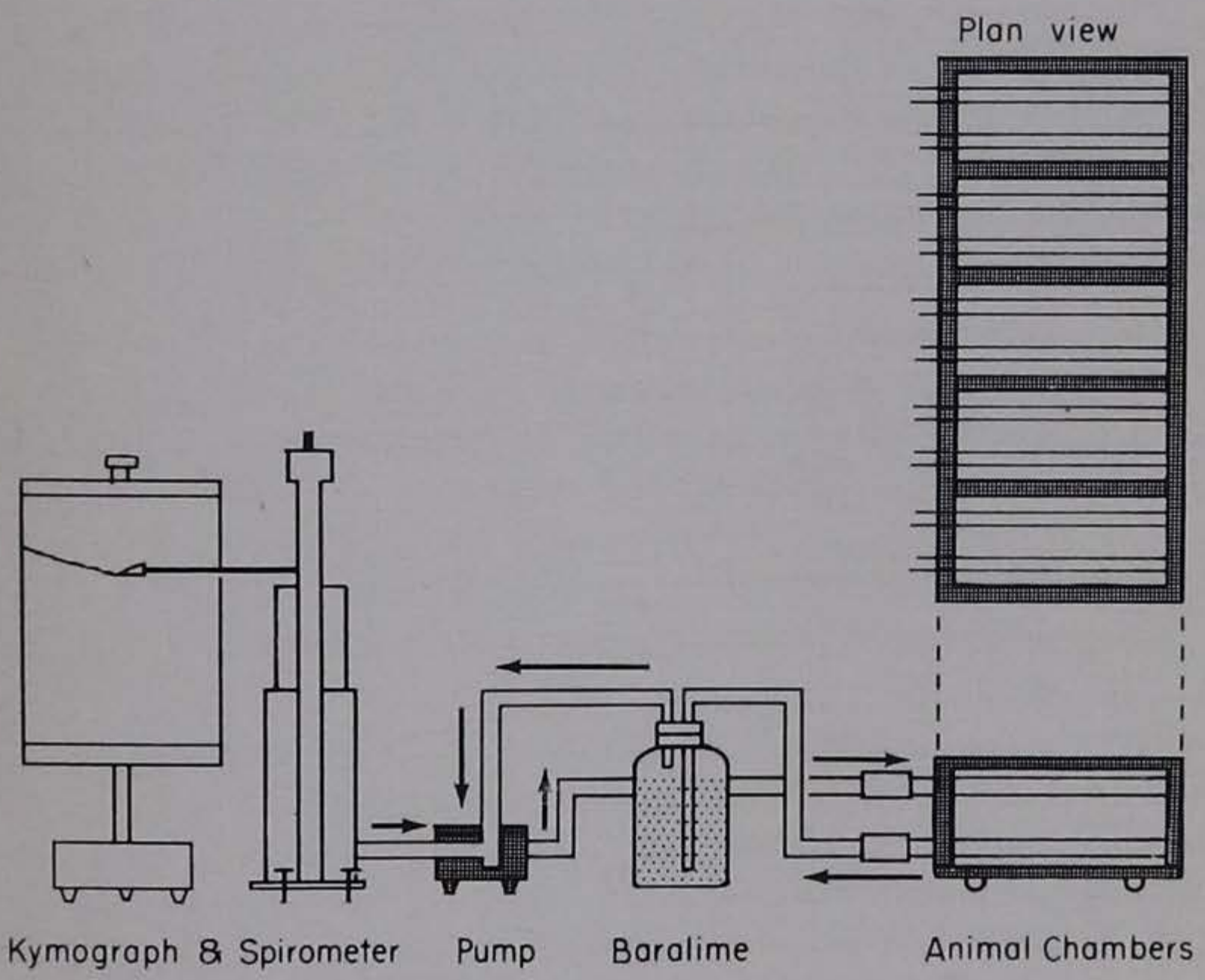

FIG. 1. Closed circuit apparatus for measuring oxygen consumption by the spirometric method.

consisted of a separate chamber connected in a manner similar to the others. Any changes occurring in these two factors were thus recorded on the kymograph record and corrections for these changes were made when reading the record. All runs were made for a period of one hour.

All oxygen consumption values were corrected to standard temperature and pressure, dry. By recording the drop of the spirometer on the paper, it was possible to ascertain if the animal had any gross movements during the determination. Any areas such as these were eliminated when analyzing the record. A typical record is shown in FIG. 2.

In order to standardize the condition of the animal, it was fasted for six hours prior to making a determination. The duration of fasting was established from Kayser's report (35) that at 6-7 hours of fasting the R.Q. is approximately 0.85 in the hamster. Fasting was begun from 6:00 to 8:00 a.m. since the animal's need for food would be at a minimum at this time, the hamster being a nocturnal animal (22,40). Also, this would enable oxygen consumption measurements to be made at 12:00 noon to 2:00 p.m., at a time when the animal should be at its most basal state (16).

Weight changes. Body weight changes were recorded daily in each animal. The body weight was recorded to the nearest gram on a dietetic scale. The organ weights, both wet and dry, were weighed on a torsion balance to the nearest 0.01 gram, and in some cases, to the nearest 0.005 gram (spleen). Dry weights were obtained by placing the organs in an oven at 80 to 100°C for 48 hours and weighing on removal from the oven.

Water and food consumption. Water intake was measured to the nearest 0.5 ml daily using calibrated water bottles. Food was weighed daily to the nearest 0.01 gram.

Surface area. All surface area measurements were made by skinning the animal carefully, placing the skin on squared paper and tracing around it.

Rectal temperature. Rectal temperatures were taken with a small thermistor designed for that purpose and recorded on a telethermometer. Recordings were made to the nearest 0.05°C. The thermistor was inserted approximately 1.5-2.0 cm into the rectum, and time was allowed for stabilization before making a reading. All body temperature was recorded at room temperature (26°C) immediately after the animal was removed from the oxygen consumption apparatus.

Body fluid compartment measurements. The animals were anesthetized with sodium pentobarbital (approximately 0.15 ml of a 5% solution) intraperitoneally. This dosage was usually sufficient to keep the animal in a state of anesthesia for the duration of the experiment. Following anesthesia the animals were placed on a dissecting board and both external jugular veins exposed. One vein was used for injecting solutions and the opposite one for withdrawal of samples. After completion of the sampling the animal was sacrificed, the organs weighed, and the carcass macerated in a Waring blender. The remains were placed in an oven at 80°C and allowed to dry. Daily weighings were made to determine the dry weight end point.

Extracellular fluid volume was measured by the single injection thiosulfate method of Frank et al. (18), modified for this small animal. Samples of blood were drawn at 10, 20, and 30 minutes after injection. Results were recorded as ml of thiosulfate space.

Plasma volumes were measured by the Evans Blue dye dilution technique modified for this animal, from the method of Frank et al. (19). This space was measured simultaneously with the thiosulfate space, the 30-minute reading being used for the calculation of plasma volume.

The hematocrit of each animal was measured with a van Allen type hematocrit tube which required 0.1 ml blood. These measurements were taken from the control sample of blood except when plasma volume measurements were taken, when an additional hematocrit sample was drawn at 30 minutes.

The sampling procedure used in the above determinations was as follows: A control sample (2.5 ml) was drawn from one of the jugular veins before any solution was injected. This sample was used for the hematocrit, blank, and standards for the determinations of Evans Blue and thiosulfate space. The animal was then injected with the sodium thiosulfate solution (10%) and Evans Blue. The volume of injected solution was 0.5 cc. At 10-minute intervals, 0.2 cc blood samples were drawn. The blood samples were centrifuged at 15,000 rpm for one minute and the plasma recovered for making a protein-free filtrate and the determination of Evans Blue. The protein-free plasma filtrate was used for titration of the thiosulfate samples; 0.05 ml samples were used and duplicate determinations made. Evans Blue was measured in a Coleman Jr. spectrophotometer at 620μ.

Experimental design. This complete study consisted of three separate experiments. The first was a study to determine the rate at

which the hamster can become adapted to the cold. The remaining experiments were designed to measure the changes which occurred in the acclimatized hamsters. Two groups of animals were used for these latter experiments since facilities and equipment limited the sample to a total of 24 animals.

Studies to determine the rate of cold acclimatization of the hamster. Twenty male hamsters approximately 6 months of age were used in this study. These animals were obtained from a dealer in Wisconsin. Five animals were used as controls and the remaining 15 were placed in the cold; measurements of food and water consumption, body weight, oxygen consumption and rectal temperatures were made. Each successive day in the cold, one animal was sacrificed and the organ weights, including the spleen, skin, heart, and kidneys, were recorded. The carcass was desiccated and the total body water determined in grams.

EXPERIMENTS ON THE FULLY ACCLIMATIZED HAMSTER

Series A: Body water compartments and paired sample organ weight analysis. As a result of the first experiment, it was decided that in one week the hamster could be considered as being acclimatized to the cold. Therefore, this experiment was undertaken to determine the fluid compartment shifts and the magnitude of the other changes which are present in the cold-acclimatized hamster. For this study 24 male hamsters were used. These animals were obtained from a dealer in California. They were paired both by litter mates and weight. One member of each pair (12 animals) was placed in the cold room and maintained there for 12 days, during which time daily food and water consumption was recorded and body weight changes noted. On the 12th day the animals were removed from the cold room and measurements of weight, oxygen consumption, rectal temperature, extracellular fluid volume, plasma volume, organ weights, and total body water were made. Four animals died as a result of cold exposure during the course of the experiment.

Series B: Body water compartments and unpaired sample organ weight analysis. This experiment was conducted in order to obtain more data to add to the above experiment since the mortality rate was higher than expected in that series. Measurements of weight, oxygen consumption, rectal temperature, extracellular fluid volume, organ weights and total body water were taken. The same procedure was followed as above with the exception that dry weight measurements were made on the organs.

RESULTS

These experiments were conducted (1) to describe the rate of cold acclimatization in the golden hamster, and (2) to describe the magnitude of these changes after acclimatization has occurred.

Three major experiments were employed in obtaining these results. The first experiments to determine the rate of acclimatization included recording the organ weight changes, oxygen consumption, rectal temperature, food and water consumption, and total body water. With the exception of the control values, all of the data for each day of cold exposure were too small to warrant statistical analysis; therefore all results are presented in graphical form rather than in absolute terms.

The remaining experiments were conducted on the fully acclimatized hamster. The duration of cold exposure considered to be sufficient for complete acclimatization was 12 days. This time was determined from the former experiments. The same measurements were made as above, and in addition, the body fluid compartments were measured. Statistical analysis of the organ weights was divided into two series: the first was analyzed as paired samples, and the second series and all of the remaining data were analyzed as unpaired samples. The null hypothesis was rejected at the 5% level of significance.

Experiments to determine the rate of cold acclimatization in the hamster. The rate of cold acclimatization was determined by making the above daily measurements on one animal. The organs weighed in these experiments were the spleen, heart, skin and kidneys. Absolute values for each determination are presented in the sections which follow on fully acclimatized animals.

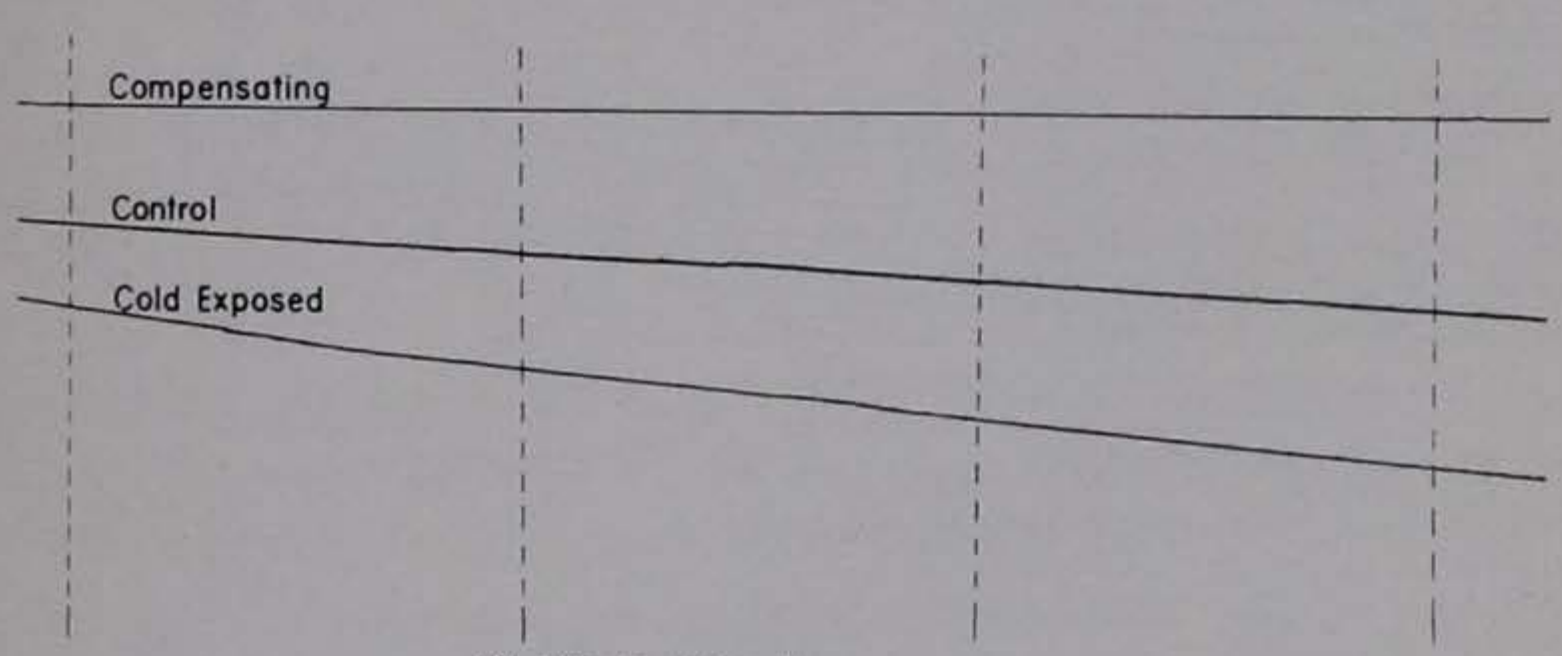

FIG. 2 An actual 30-minute record showing the spirometer drop for the cold-acclimatized and non-cold-acclimatized hamster.

Body weight. During the first four days of cold exposure there was a rapid drop in body weight which stabilized by about the sixth day. The total weight loss incurred during this time was approximately 20% that of the control animals (FIG. 3). This same pattern of weight loss was followed in a later series of experiments with a total mean weight loss of 18%.

Body temperature. The rectal temperature did not appear to change consistently in this group of cold exposed animals (FIG. 3).

Oxygen consumption. Oxygen consumption measured at 30°C increased progressively during the first three to four days of cold exposure. It then decreased slightly until by the 12th day it reached a level 30% above the control when expressed as total oxygen consumption. The increase by the sixth day is much greater (60%) when the oxygen consumption per gram per hour is used (FIG. 3).

Food consumption. Food consumption increased during the first six days of cold exposure. After this it stabilized at a maximum of approximately 100% (FIG. 4).

Water intake. Water intake decreased approximately 50% during the first two days of exposure to the cold and increased during the fourth and fifth days. During the sixth and seventh days it stabilized at some 125% above control values. These measurements were made on the later groups of animals and the same pattern was obtained, with the exception that the total increase was not as great (FIG. 4).

Total body water. The total body water when expressed relative to body weight seemed to increase during the first two days of cold exposure and then dropped slightly to approximately 10% above the control values. It remained at this level through the remaining period of cold exposure (FIG. 4).

Organ weights. There was a decrease in skin weight during the first five days of cold exposure, after which it remained at a lower level approximately 34% below control (absolute values). The decrease of body weight was approximately 20% (FIG. 5).

The weight of the heart when expressed in terms of body weight appeared to increase during the first three days of cold exposure. It remained at this level throughout the remainder of the cold exposure (FIG. 5).

The kidneys (presented separately) exhibited an increase in weight (relative to body weight) during the first few days of cold exposure (FIG. 5). Little, if any, change could be observed in the spleen weight in this study (FIG. 5).

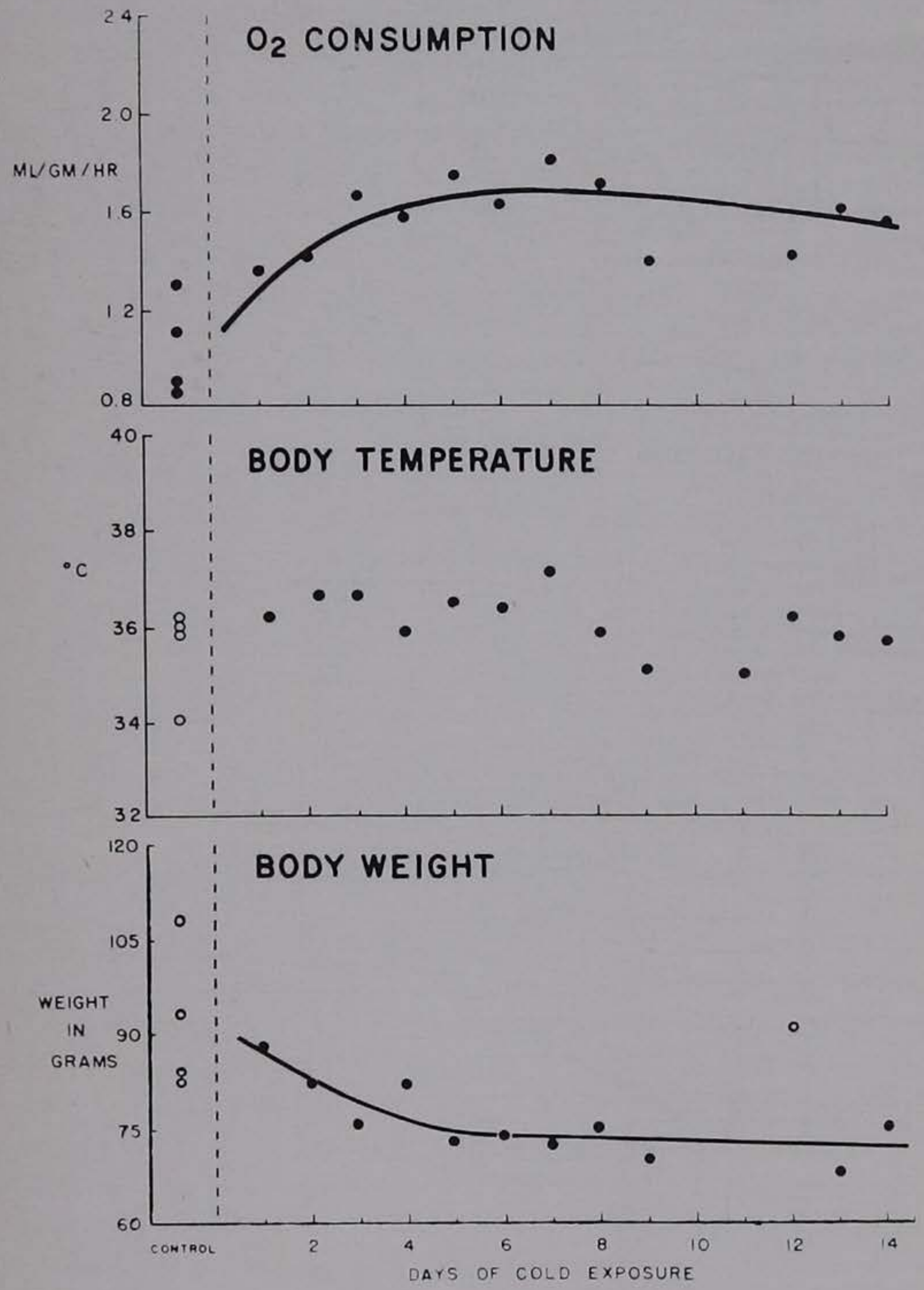

FIG. 3. Oxygen consumption, body temperature, and body weight during cold acclimatization (6°C) of the hamster.

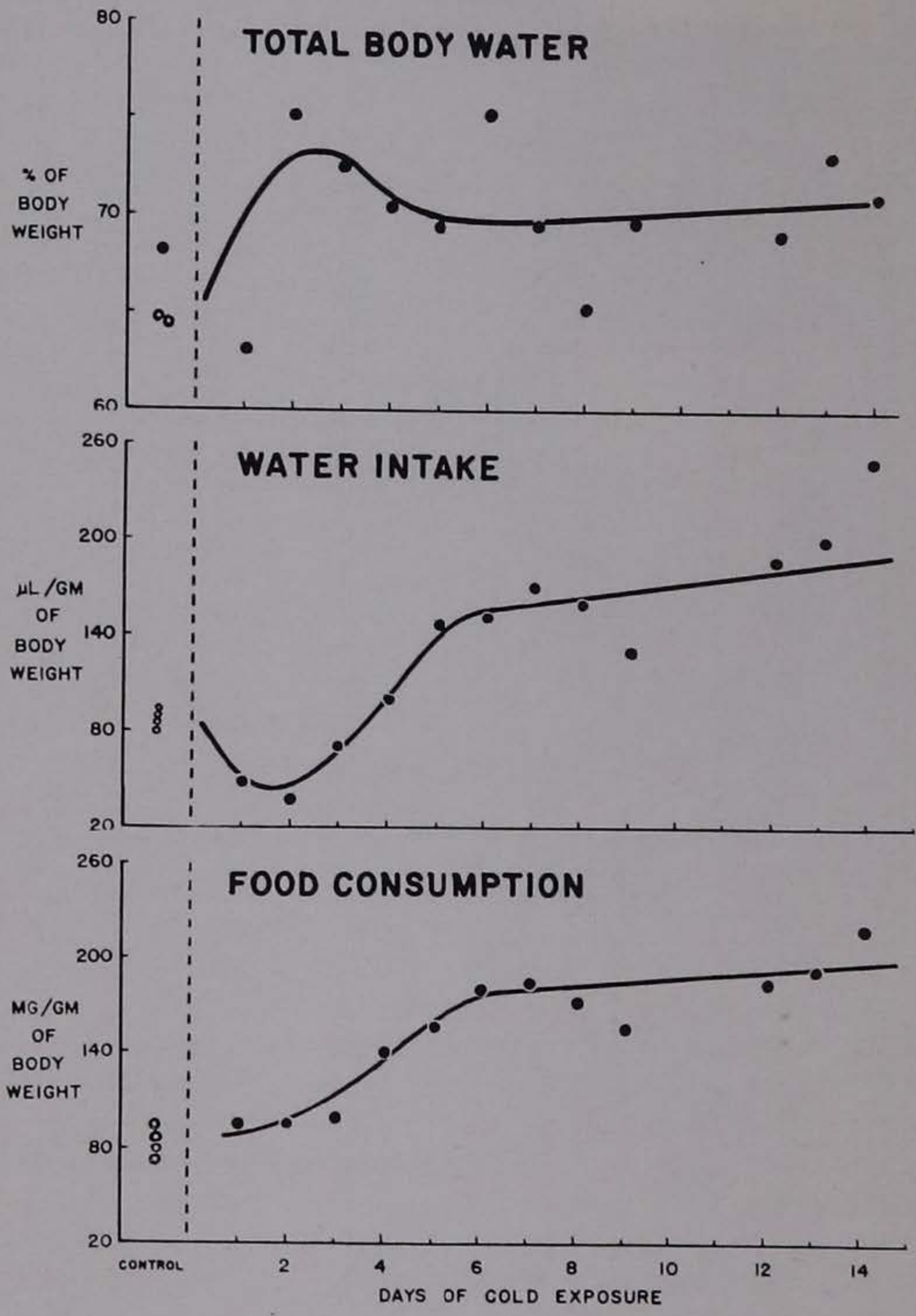

FIG. 4. Total body water, water intake, and food consumption of the hamster during acclimatization to the cold.

The gross changes in the hamster during cold acclimatization occur during the first seven days. Body weight, oxygen consumption, total body water, food and water consumption stabilize during the fifth to the seventh day, while the organ weights seem to make their major changes during the first two or three days. Therefore, cold acclimatization of the golden hamster, as determined by the above changes, is complete by the end of the first week. The ensuing experiments on the fully acclimatized hamster were carried out on the twelfth day after onset of cold exposure to assure that all of the gross changes were complete.

EXPERIMENTS ON THE FULLY ACCLIMATIZED HAMSTER

All data collected from the two experiments, with the exception of the organ weights, were grouped and analyzed together. The organ weights were analyzed under two series: Series A includes a paired sample analysis of the wet weights and Series B consists of an unpaired sample and analysis of both the wet and dry weights of the organs.

Body weight. The body weight changes of these animals followed the same pattern observed in the first study; there was a loss in weight the first six or seven days, followed by stabilization at this lower level during the remainder of the cold period. The mean weight of the control animals was 87.5 grams and the cold-exposed animals at termination of the experiment (twelve days) weighed 70.9 grams. This represented a 19% difference in weight in the two groups of animals (TABLE I).

Food and water consumption. Food and water consumption also followed the same pattern as in the first experiment. The food consumption for the control group was 77.5 mg/gm body wt/day, and for the cold-exposed group on the twelfth day it was 154 mg/gm body wt/day. A 99% increase in food consumption occurred in twelve days of cold exposure. Water consumption decreased the first two days of exposure from 99.4 μl/gm body wt/day to 48 μl/gm body wt/day, approximately a 50% decrease. This was followed by a rapid increase, approximately 13% above control by the seventh day, after which it remained constant for the period of exposure. On the twelfth day of exposure the water consumption was 128 μl/gm body wt/day (TABLE I).

Body temperature. There appeared to be no important difference in body temperature of the control animals (36.17°C) as compared with that of the cold-exposed animals (36.39°C) at the termination of these experiments.

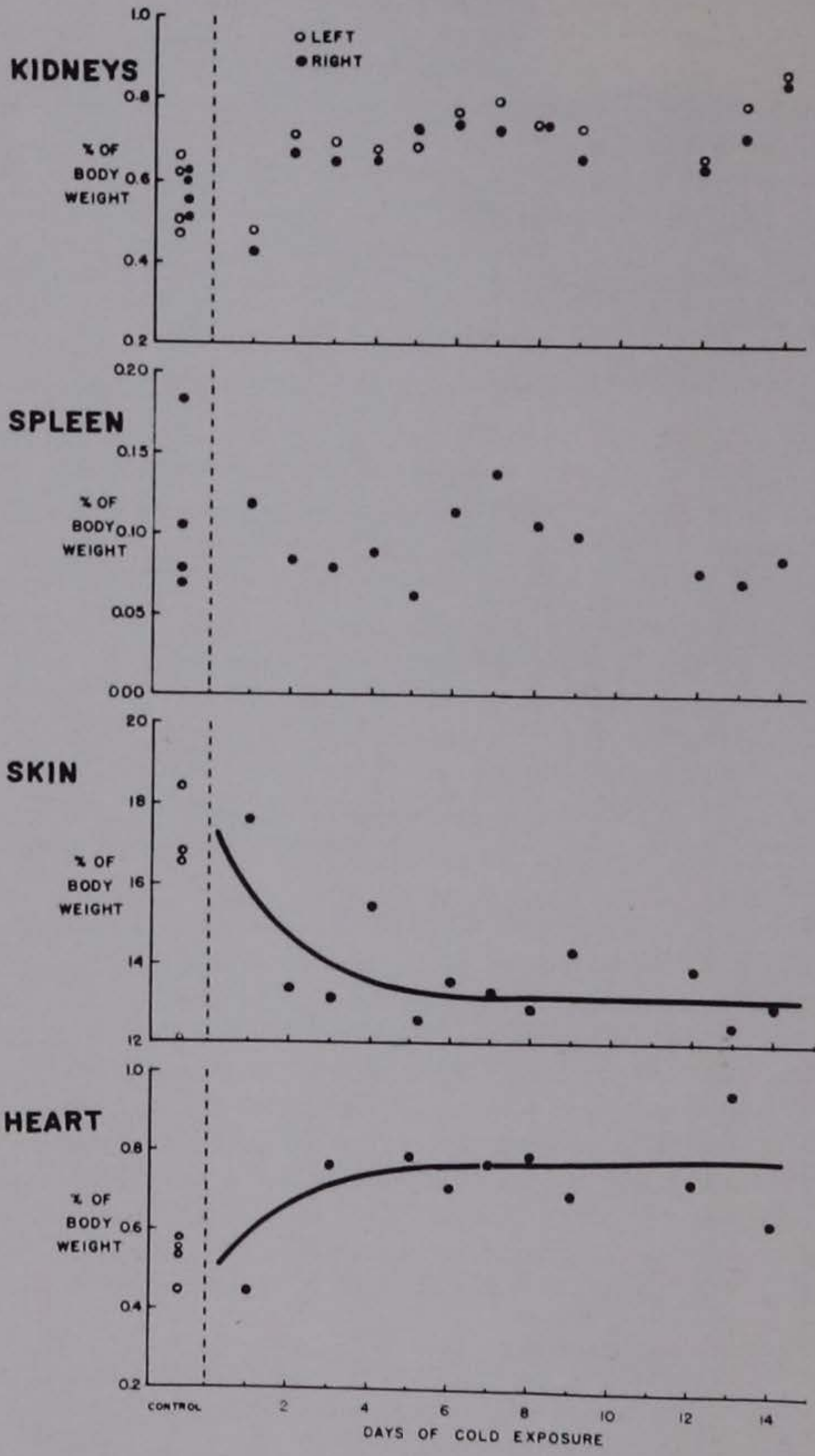

FIG. 5. Organ weights during cold acclimatization of the hamster.

Oxygen consumption. The total oxygen consumption (ml/hour) was greater in the cold-acclimatized hamsters (approximately 43%). The mean value for the controls was 92.06 ml/hour, and the cold acclimatized hamster was 131.7 ml/hour (TABLE I). The increase in oxygen consumption was 72% when expressed in ml/gm body wt/hour. The mean value for controls was 1.06 ml oxygen/gm/hr while for the cold-exposed animals it was 1.82 ml oxygen/gm/hr.

Body fluid compartments: Total body water. The total body water of the fully acclimatized hamster was lower than that of the non-acclimatized hamster in absolute values (49.42 grams in the acclimatized animal and 57.09 in the control TABLE I). Due to the change in body weight of the cold-exposed animal, the total body water relative to body weight is greater than in the control animals, i.e., 71% in the cold-exposed and 66% in the controls.

Evans Blue fluid volume (plasma volume) and blood volume. The Evans Blue volume appeared to be larger in the cold-acclimatized animal (3.14 ml) as compared with the non-acclimatized animal (2.91 ml). No statistical difference ($p = 0.10$) was evident, due to the range

TABLE I

Mean Values for Changes in the Hamster After Exposure to the Cold for Twelve Days

	Control (26°C)		Cold-Acclimatized (6°C)	
	Mean	Number of determinations	Mean	Number of determinations
Body weight (gms)	87.5	18	70.9	16
0_2 consumption (ml/hr)	92.6	16	131.7	12
Rectal temperature (°C)	36.19	17	36.39	15
Total body water (grams)	57.09	17	49.42	15
Thiosulfate volume (ml)	21.57	11	20.75	10
Intracellular vol. (ml)	32.74	10	29.26	9
Plasma volume (ml)	2.91	6	3.14	6
Hematocrit (%)	43.8	14	42.2	12
Food consumption (mg/gm body wt/day)	77.5	10	154.0	10
Water intake (µl/gm/day)	86.6	10	128.0	10

in values. Relative to body weight the Evans Blue volume of the cold-acclimatized animals was approximately 4.44 ml/100 gm body weight and in the non-acclimatized it was approximately 3.33 ml/100 gm body weight.

The hematocrit values were no different in these two animals (43.8 in the controls and 42.2 in the cold-acclimatized). Therefore, the blood volume relationship should be nearly the same as for the plasma volume. The control blood volumes (5.83 ml/100 gm body wt) obtained here are lower than those obtained by Suihla (53) which averaged 7.14 ml/100 gm body wt.

Thiosulfate fluid volume (extracellular volume). The thiosulfate volume was slightly lower in the cold-acclimatized animals, i.e., 21.57 ml controls and 20.74 cold-acclimatized animals (TABLE I). Relative to body weight the acclimatized hamsters had a larger volume (25.2% controls and 28.7% acclimatized).

Intracellular fluid volume. The intracellular fluid absolute volume (total body water minus the thiosulfate volume) was greater in the control animals (32.74 ml) than the acclimatized hamsters (29.26 ml). Relative to body weight there is no important difference between the two groups (38.40% control and 39.80% acclimatized (TABLE I).

Organ weight changes. The analysis of the changes in organ weights in these two experiments was carried out separately. The first group (Series A) was treated on a paired sample basis of the wet weights only. The wet and dry weights of the second group (Series B) were analyzed as unpaired samples.

Skin. The skin wet weights of the acclimatized animals were lower in both groups. The control weight in Series A was 12.21 and in Series B, 10.81 grams, and the acclimatized weights were 11.26 in Series A and 8.59 grams in Series B (TABLES II, III). The dry skin weight was also significantly lower in the acclimatized hamsters ($p < 0.01$). The dry weight of the controls was 5.28 and of the acclimatized was 3.75 grams (TABLE III).

Heart. The heart weights of the Series A acclimatized animals (0.51 grams) were slightly larger than in the controls (0.42 grams) $p<0.05$ (TABLE II). However, the heart weights of Series B were not significantly different ($p > 0.05$). Wet weight of the controls was 0.881 and the acclimatized wet weight was 0.87 gram. The dry weight of the controls was 0.36 and of the acclimatized 0.374 gram. More care was taken to remove all the residual blood from the heart chambers of the Series B hearts than in Series A; therefore, the increase in wet weight of Series A may be more apparent than real.

Kidneys. The weights of both kidneys were combined for these analyses. The wet weights of both series of experiments were greater in the cold-acclimatized animals. In Series A the mean weight of the kidneys of the control animals was 0.88 grams and of the acclimatized 1 gram ($p < 0.01$, TABLE II). In Series B the wet weights were 0.938 grams in the controls and 1.057, acclimatized ($p = 0.01$, TABLE III).

There was no significant difference ($p > 0.05$) in the dry weights of the kidneys of the control and acclimatized animals. The control weight was 0.211 grams and the acclimatized weight was 0.23 grams (TABLE III).

Spleen. There was no significant change ($p > 0.05$) in the spleen weight in the Series A animals (control was 0.086 and acclimatized 0.065 grams). Both wet and dry weights of the Series B spleens were less in the acclimatized hamsters ($p < 0.05$ in both cases, TABLE III). The control weight was 0.077 grams and the acclimatized 0.047 grams; the dry weights were 0.028 control and 0.011 grams acclimatized. More samples need to be taken to definitely establish the difference in Series B.

DISCUSSION

Significance of the adaptive changes in the hamster during cold exposure. The gross adaptive changes apparent in the hamster, a hibernator, during acclimatization to the cold are complete after the seventh day of cold exposure. These changes are characterized by an

TABLE II

Wet Organ Weight Changes in Hamsters Exposed to the Cold (6°C) for Twelve Days

(Weights in grams: 't' score on paired sample basis.)

	Weight	Number of determinations	't' score	'p'
Skin				
Control	12.21	9	5.36	0.01
Cold exposed	11.26	9		
Kidneys				
Control	0.88	9	3.69	0.01
Cold exposed	1.00	9		
Heart				
Control	0.42	9	2.75	0.05
Cold exposed	0.51	9		
Spleen				
Control	0.086	9	1.90	0.20
Cold exposed	0.065	9		

increase in oxygen consumption (30°C), decrease in body weight, an increase in food and water consumption, certain organ weight changes, and an increase in total body water relative to body weight.

Metabolic changes. The increased oxygen consumption (30°C) of the cold-acclimatized, small, non-hibernating mammals is also present in the acclimatized hamster (35). Studies on the rat indicate that this increased standard metabolism is largely due to an increased chemical heat production[2] (30,32), and little is due to physical heat production[2]. The source of the increased oxygen consumption in the hamster has not been studied as such. A few studies have been undertaken concerning the changes in endocrine function in the hamster, but they tend to confuse the issue even more.

Two views concerning the hormonal changes of the cold-exposed hamster are in the published record; the first describes a decreased output of hormone from the pituitary, thyroid glands and the adrenal

TABLE III

Wet and Dry Organ Weight Changes in Hamsters Exposed to the Cold (6°C) for Twelve Days

(Weights in grams; 't' score on unpaired sample basis)

	Wet Weight		Dry Weight	
Skin	Control	Cold Exposed	Control	Cold Exposed
Mean	10.81	8.59	5.28	3.75
Std. Dev.	0.75	1.08	0.43	0.26
No. deter.	8	6	8	6
't' score		4.28		8.08
'p'		0.01		0.001
Kidneys				
Mean	0.938	1.057	0.211	0.230
Std. Dev.	0.079	0.588	0.025	0.014
No. deter.	8	6	8	6
't' score		3.19		1.58
'p'		0.01		———
Heart				
Mean	0.881	0.870	0.360	0.374
Std. Dev.	0.015	0.006	0.032	0.025
No. deter.	8	6	8	6
't' score		0.18		0.89
'p'		———		———
Spleen				
Mean	0.077	0.047	0.028	0.011
Std. Dev.	0.012	0.012	0.010	0.006
No. deter.	8	6	8	6
't' score		2.42		2.31
'p'		0.05		0.05

cortex (11,31), and the second presents the opposite picture, that there is an increase in hormonal output of these glands (36). If the first hypothesis is correct, the increased oxygen consumption of the cold-acclimatized hamster would be primarily due to physical factors. The second hypothesis indicates that chemical heat production plays an important part.

That physical heat production plays a major role in the increased oxygen consumption of the cold-acclimatized hamster seems to be supported by observations made during the course of these studies but not discussed in earlier sections of this paper. It was noted that the body temperature of the anesthetized cold-exposed animal decreased approximately 5°C at 26°C. Assuming that the anesthetic causes a depression of muscular activity and an increased blood volume to the skin (45), a decreased heat production and an increased heat loss may occur. If chemical heat production were high in this animal, body temperature would be expected to remain higher. At the present time it is impossible to determine which of these two factors plays the more significant role.

The hamster increases its heat production in the cold to the extent that there is no reduction in body temperature at cold temperatures (11,36,38). These studies indicate that there is no difference in rectal temperature of the acclimatized hamster and the control when measured at room temperatures. This may signify that this animal has an efficient heat loss mechanism (11,38), since the oxygen consumption of the acclimatized animal is higher at these temperatures.

The weight loss incurred in the hamster during cold acclimatization may be attributed to a decrease in fat, water, and some protein (25) in order of their greatest contribution to the weight loss. The stabilization of the weight at a lower level can be attributed to the increased food consumption that has reached a point where the caloric intake is equal to the output. The decrease in water consumption during the first few days of cold exposure probably indicates that the metabolic and stored water is sufficient to provide a portion of the water necessary (50%). Lyman (40) has suggested that the hamster in its natural state probably does not have access to water during the winter and must depend upon stored and metabolic water, but if water is available it will drink.

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[2]Chemical heat production refers to that under the influence of endocrine function, and physical heat production refers to that influenced by shivering, muscular tone and circulation.

Kidneys. The increased weight of the kidneys in the cold-acclimatized hamster is due to an increased water content, as demonstrated by the dry weights of the control and cold-acclimatized animals. The location of this increased water content is not presently clear; however, due to the increased urine output at this time. The possibility exists that the number of active nephrons has increased or the volume of blood has increased.

Skin and spleen weights. The decrease in both wet and dry weights of the skin is probably due to loss in fat content. The decrease in spleen weight noted in part of these experiments cannot be explained. The same change in spleen weights has been observed in a large number of autopsies performed previously in this laboratory.

Body water compartments. It is difficult to assess the changes that take place in the body water compartments in the acclimatized hamster because of the changes in body weight with cold exposure. Deb and Hart (12) are the only other investigators who have reported studies such as these on rodents. Studies on the shifts in body water have been reported on larger animals during hypothermia (9), and there have been in vitro studies on the water shifts in different tissues (2,46). This type of experiment involves altering the temperature of the tissues and therefore is not applicable to the changes occurring where the tissues do not change their temperatures, as in the intact animal.

The decrease in total body water observed in these studies apparently is due to a decrease in the intracellular fluid, the only fluid compartment in which a decrease occurred. The absolute volumes of the thiosulfate space did not appear to change appreciably. The mean value for the Evans Blue volume was higher in the cold-acclimatized animals, but due to the large range in values for both groups of animals, no statistical difference could be obtained. Relative to body weight, the plasma and blood volume appear to have increased considerably (3.33 ml/100 gm body wt to 4.44 ml/100 gm body wt). This may imply that there is an increase in vascularization in the cold-acclimatized hamster as has been observed in the rat (26).

The decrease in the absolute volume of the intracellular fluid is probably best explained as being due to the increased oxygen consumption. This hypothesis emerged from the reports of Robinson (46) who believes that the intracellular fluid is influenced by the metabolic activity of the cell, i.e., as the metabolism increases, the cellular water decreases as a result of active transport of water out of the cell. Adolph and Richmond (2) also reported that the cellular water

increases with anoxia and decreases with the addition of oxygen. The consequence of a decreased cellular water would probably be a decrease in the size of the cells. This is reported to occur in the muscle fibers of the rat (26). No reports have been forthcoming as to any change in size of cells or muscle fibers in the hamster.

Acclimatization to the cold, during which major changes occur in the hamster, requires an exposure period of at least seven days. The changes of importance considered in this study include decreases in body weight and total body water, and increases in oxygen consumption, food and water consumption, and kidney weight.

SUMMARY

1. Acclimatization to cold (6°C) of golden hamsters occurs in a period of seven days of exposure.

2. Fully cold-acclimatized hamsters exhibit a large increase in oxygen consumption when measured under standard conditions at 30°C.

3. A persistent decrease in body weight and increase in food and water consumption is characteristic of fully cold-acclimatized hamsters.

4. Cold-acclimatization in hamsters results in increased kidney weights, and decreased spleen and skin weights. The heart does not appear to change during cold exposure.

5. There was a decrease in total body water which appeared to be due to a decrease in the intracellular fluid during cold exposure. When expressed in terms of body weight, plasma and blood volume increased with cold exposure.

BIBLIOGRAPHY

1. Adolph, E. F. and J. Richmond. Am. J. Physiol. 187: 437. 1957.
2. Adolph, E. F. and J. Richmond. J. Appl. Physiol. 9: 53. 1956.
3. Bartholemew, G. A. and T. S. Code. J. Mamm. 38: 60. 1957.
4. Brown-Grant, K. J. Physiol. 131: 52. 1956.
5. Carlson, L. D. Cold Injury. New York: Josiah Macy, Jr. Foundation. 1952.
6. Carlson, L. D. J. Appl. Physiol. 5: 672. 1953.
7. Cottle, W. H. and L. D. Carlson. Proc. Soc. Exp. Biol. and Med. 92: 845. 1956.
8. Dahlstron, H. Acta Physiol. scandinav. Supp. 71, 21: 1. 1950.
9. D'Amato, H. E. Am. J. Physiol. 178: 143. 1954.
10. Dawson, W. R. J. Mamm. 36: 543. 1955.
11. Deane, H. W. and C. P. Lyman. Endocrinology 55: 300. 1954.
12. Deb, C. and J. S. Hart. Canad. J. Biochem. and Physiol. 34: 959. 1957.
13. Des Marais, A. and L. P. Dugal. Canad. J. Med. Sci. 29: 90. 1951.
14. Depocos, F., G. K. MaCleod and J. S. Hart. Revue Canadienne de Biologie 16: 83. 1957.
15. Farrand, R. L., G. E. Folk, Jr. and M. L. Riedesel. Proc. Iowa Acad. Sci. 63: 724. 1956.
16. Folk, G. E. Jr. and R. L. Farrand. Brit. J. Animal Tech. Assoc. 6: (5). 1956.
17. Folk, G. E. Jr. and R. R. Schellinger. Anat. Rec. 120: 3. 1954.
18. Frank, H. A. and M. H. Carr. J. Lab. and Clin. Med. 45: 973. 1955.
19. Frank, H. A. and M. H. Carr. J. Lab. and Clin. Med. 45: 977. 1955.
20. Fregly, M. J. Am. J. Physiol. 173: 393. 1953.
21. Freinfel, N. and D. Lewis. J. Physiol. 135: 288. 1957
22. Gault, M. R. Fed. Proc. 16: No. 1, 44. 1957.
23. Hart, J. S. Canad. J. Zool. 30: 83. 1952.
24. Hart, J. S. Canad. J. Zool. 31: 99. 1953.
25. Hart, J. S. and O. Heroux. Canad. J. Biochem. and Physiol. 34: 414. 1956.
26. Heroux, O. and J. St. Pierre. Am. J. Physiol. 188: 163. 1957.
27. Heroux, O. and J. S. Hart. Am. J. Physiol. 178: 449. 1954.
28. Heroux, O. and J. S. Hart. Am. J. Physiol. 178: 453. 1954.
29. Heroux, O. Am. J. Physiol. 181: 75. 1955.
30. Heroux, O. J. Appl. Physiol. 9: 399. 1956.
31. Holmes, W. N. Endocrinology 57: 409. 1955.
32. Hsieh, A. C. L. and L. D. Carlson. Am. J. Physiol. 188: 40. 1957.
33. Irving, L. J. Appl. Physiol. 9: 414. 1956.
34. Irving, L. Fed. Proc. 10: 543. 1951.
35. Kayser, C. L'Année Biologique 29: 109. 1953.
36. Knigge, K. M. Anat. Rec. 127: 75. 1957.
37. LeBlanc, J. J. Appl. Physiol. 9: 395. 1956.
38. Lyman, C. P. J. Exp. Zool. 109: 55. 1948.
39. Lyman, C. P. and P. O. Chatfield. J. Exp. Zool. 114: 491. 1950.
40. Lyman, C. P. J. Mamm. 35: 545. 1954.
41. Lyman, C. P. and P. O. Chatfield. Physiol. Rev. 35: 403. 1955.
42. Mullally, D. P. J. Mamm. 34: 65. 1953.
43. Morrison, P. R. and W. J. Tietz. J. Mamm. 38: 78. 1957.
44. Rand, C. G., D. S. Riggs and N. B. Talbot. Endocrinology 51: 562. 1952.
45. Rieke, W. O. and N. B. Everett. Am. J. Physiol. 188: 403. 1957.

46. Robinson, J. R. Proc. Roy. Soc. London s.B. 137: 378. 1950.
47. Scholander, P. F., V. Walters, R. Hock and L. Irving. Biol. Bull. 99: 225. 1950.
48. Sealander, J. A. J. Mamm. 32: 206. 1952.
49. Sealander, J. A. Biol. Bull. 104: 87. 1953.
50. Sellers, E. A., S. S. Yow and N. Thomas. Am. J. Physiol. 165: 481. 1951.
51. Sellers, E. A., A. Reichman, N. Thomas and S. S. Yow. Am. J. Physiol. 167: 651. 1951.
52. Stevenson, J. F. Cold Injury. New York: Josiah Macy, Jr. Foundation. 1952.
53. Suihla, A. and H. Bowman. J. Mamm. 36: 135. 1955.
54. Wedgwood, R. J., D. E. Bass, J. A. Klimas, C. R. Kleeman and M. Quinn. J. Appl. Physiol. 6: 317. 1953.
55. Woods, J. W. J. Physiol. 135: 384. 1957.

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