A STUDY OF MAGNESIUM AND BODY FLUIDS OF THE
RAT DURING COLD ACCLIMATIZATION

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by

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The undersigned, appointed by the Dean of the Graduate Faculty, have
examined a thesis entitled

A STUDY OF MAGNESIUM AND BODY FLUIDS OF THE
RAT DURING COLD ACCLIMATIZATION

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a candidate for the degree of Master of Arts

and hereby certify that in their opinion it is worthy of acceptance.

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INTRODUCTION

Prolonged exposure to cold of most warm-blooded animals imparts various protective modifications in many of the physiological processes. These acquired, reversible responses may be termed expressions of acclimatization and discreetly used as measures thereof. Perhaps many of these modifications are of debatable value as primary causative instruments of the acclimatization process, but nevertheless all are salient in the determination of the existence of the acclimatized state.

An extensive study of hypothermia, or reduction of body temperature, has been made in the last two decades by numerous investigators; however, only in the last few years have the effects of prolonged cold exposure and acclimatization attracted attention. Manifestations occurring on short term cold exposure are usually regarded as due to hypothermia, while prolonged cold exposure usually leads to acclimatization.

Some of the changes in sodium, potassium, and chlorides due to hypothermia, prolonged cold exposure, and acclimatization have been investigated by Axelrod and Bass (1956) and Baker and Sellers (1957). However,

magnesium has been mostly studied with regard to hypothermia and hibernation and little attention has been allotted to the role of magnesium during acclimatization. Therefore this ion merits more detailed study.

Magnesium has been found to play an important role as an activator or coenzyme of many of the phosphatases and enolase (Scheer, 1953). Thus magnesium levels in liver, heart and skeletal muscle during acclimatization would be valuable in perhaps deducing some of these relationships. The recent report of Tapley and Cooper (1956) that the effectiveness of thyroxine as an uncoupler of oxidative phosphorylation is dependent on magnesium ion concentration and the work of Hegsted, Vitale, and McGrath (1956) stating that magnesium is the chief determinant of growth for rats at 13° C warranted investigation of magnesium levels in thyroid fed animals during acclimatization to cold. Because the concentration of magnesium in the serum depends on the amount of available fluid, it was thought that the fluid adjustments during acclimatization also deserved attention.

In assessing the numerous modifications imposed on the animal during the period of acclimatization, one must appreciate that no one or two of these responses are accountable for this complex state. Rather that a combination of alterations of the physiological patterns of

the animal, and the resultant adaptation of the compensatory homeostatic mechanisms, are responsible for the achievement of true acclimatization.

Since magnesium has been involved in temperature studies and hibernation, the present study is an attempt to ascertain the levels of magnesium in serum and various tissues of rats exposed to cold for prolonged periods and to possibly delineate a relationship between magnesium and acclimatization.

HISTORICAL REVIEW

A search through the literature has revealed a paucity of data relating magnesium to the acclimatization process. There are, however, a few reports which suggest that magnesium is intimately involved in acclimatization.

The observation of increased blood magnesium concentrations in the snail Helix pomatia during the winter by Lustig, Ernst, and Reuss (1937) and the increased serum magnesium concentration of hibernating hedgehogs by Soumalainen (1938) led investigators such as Steadman, Ariel, and Warren (1943) and Platner (1950) to study magnesium levels in cold exposed animals.

Steadman et al (1943) reported an average rise in serum magnesium of 24 per cent in rabbits whose body temperatures were reduced.* Platner (1950) showed a 159 per cent serum magnesium increase in goldfish exposed to 1° C for 60 hours and a 58 per cent serum increase in turtles exposed to 2° C for 11 days. On the virtue of a static Mg content of the red cells of the turtle and a decrease in skeletal muscle and skin Mg,

*Henceforth the word magnesium and the symbol Mg will be used interchangeably.

this author deduced that the increased magnesium content of the serum of the turtle was drawn from the skeletal muscles and skin. Following this report Platner and Hosko (1953) observed a linear relationship between the fall of rectal temperature and the rise of serum Mg in the turtle, hamster, rat, cat and dog. It is interesting to note that the prolonged exposure of turtles to 3° C resulted in a decline of serum Mg from the elevated level, approaching normal within 15-17 days of exposure in one third of the turtles. Although acclimatization of the turtles was not intended, it is apparent that the rise in serum Mg is perhaps a transient phenomenon because later this magnesium leveled off and declined with time. This is the first report suggesting that magnesium may be important in acclimatization.

The importance of the thyroid gland in resistance to cold exposure was reported by Leblond and Gross (1943) after observation of decreased survival of thyroidectomized rats in the cold. Supporting the significance of the thyroid in cold exposure was the report by Dempsey and Astwood (1943) of increased thyroid activity and increased production of the thyroid hormone in rats after 14 days of exposure to 1° C, and the report of Rand, Riggs and Talbot (1952) of increased utilization of the thyroid hormone by rats exposed to 1-7° C for 14 days. Evidence of the physiological prominence of the thyroid

gland in the acclimatization process was further supplied by the report of Sellers, Reichman, and Thomas (1951) of a modified form of artificial acclimatization with injections of cortisone and thyroxine. The survival rate of the clipped, artificially acclimatized rats was greater than clipped, unacclimatized rats, but less than clipped, normally acclimatized rats. These investigators have also observed survival of more than 18 months in 1.5°C and considerable fluctuations of survival of rats of the same age, same weight and same strain under the same environmental conditions, for no apparent reason.

The report of Tipton, Leath, Tipton, and Nixon (1946) of increased activity of succinoxidase and cytochrome oxidase of liver tissue with thyroid feeding coupled with the findings of increased oxygen consumption and succinoxidase activity of liver tissue of rats exposed to 1.5°C for 27-70 days by You and Sellers (1951) strongly suggests an intimate connection between the thyroid hormone and tissue oxygen consumption during prolonged exposure to cold. The suspected relation of Mg to the thyroid hormone was substantiated by Tapley and Cooper (1956) reporting the efficiency of the uncoupling of oxidative phosphorylation by thyroxine in rat liver mitochondria dependent on the concentration of Mg in the test medium. Jenner and Kay (1931) reported magnesium responsible for the activation of the phosphatase enzymes

of the liver, kidney, bone, and intestinal mucosa. Thus an interrelation may be established between magnesium and the thyroid hormone during prolonged exposures to cold.

Hegsted et al (1956) reported that the magnesium requirement for growth at 12.8° C is 3 to 5 times as great as for comparable growth at 23.8° C. These investigators reported a 25 per cent decrease in efficiency of food utilization despite a 20 per cent increase in food intake in the cold. Vitale, Hegsted, Nakamura, and Connors (1956) subsequently reported an increase in magnesium requirement of rats administered thyroxine at room temperature in order to return the phosphorus to oxygen ratio of rat heart mitochondria preparations to almost normal levels and to attain a comparable weight gain of animals administered no thyroxine. It is interesting to note that these investigators obtained a 70 per cent decrease in serum Mg in rats on a normal diet by injecting thyroxine and a return to near normal levels of serum magnesium when the Mg content of the diet was increased 800 per cent.

The maintenance of high levels of oxygen consumption of rats after three months of exposure to 1.5° C in the face of sodium iodide injections which reduced the size and activity of the thyroid gland led Sellers and You (1950) to suggest that the increase in the

metabolic rate of acclimatized animals is not dependent on the hyperthyroid state, but on the presence of the thyroid hormone in the circulation. This hypothesis was supported by Starr and Roskelley (1940) in a report of increased cell height of thyroid epithelium of rats exposed to 15° C from 3 to 45 days, with a trend towards normal cell height after 56 days, and a report by Leblond, Gross, Peacock, and Evans (1944) demonstrating with radioactive iodine a definite thyroid stimulation after seven days of cold exposure, with a maximum at 26 days of exposure and a return to normal after 40 days of exposure.

It therefore seems possible that the increased activity of the thyroid gland is necessary to attain acclimatization but not necessary to maintain it, and that magnesium is intimately associated with the responses due to the increased thyroxine in the circulation.

Hematological and fluid adjustments during cold exposure have long been recognized and their importance in ionic imbalances acknowledged. The majority of the investigations have been done on short term cold exposure and only in the last few years has acclimatization occupied the attention of investigators in this field. Many of the reports are on humans, who are poor experimental subjects because of their inability to tolerate severe cold exposure for a prolonged period.

Bazett, Sunderman, Doupe, and Scott (1940), using human subjects exposed to 1°C and 32°C intermittently for 4 to 8 days, reported a static hematocrit and a decrease in blood and plasma volumes on six subjects. Conley and Nickerson (1945), using a similar experimental design, reported a static hematocrit, extracellular space and a decrease in plasma volume which returned to normal in three days. Elsner (1954) reported an unchanged plasma volume of men exposed to -45°F to $+34^{\circ}\text{F}$ for $3\frac{1}{2}$ weeks in Alaska. Brown, Bird, Boag, Delahaye, Green, Hatcher, and Page (1954) reported a decrease in blood and plasma volumes of Eskimos during the summer. The fact that blood and plasma volumes of the Eskimo are higher than the white man caused Brown et al (1954) to infer that a high blood volume is associated with acclimatization to cold. Garcia, Belknap, and Cook (1957) found no change in the hematocrit or blood volume of rats exposed to 3°C for 30 days. In view of the short term findings of reduced plasma and blood volumes and thiocyanate space of Rodbard, Saiki, and Malin (1950) there appears to be a discrepancy between short term cold exposure fluid movements and those of long term cold exposure.

This difference was further emphasized by Deb and Hart (1956) who reported an increase in blood volume relative to body weight at the five week level of rats

placed in 30° C for two weeks and then in 6° C for six weeks. Extracellular water had a tendency to increase relative to body weight, mostly between the third hour and one week determinations, increasing only very slightly between three and five weeks. Hemoconcentration was not a prominent feature of rats exposed to cold for several weeks.

Baker and Sellers (1957) exposed rats to 2° C for 45 days and reported an increased hematocrit for only the first few hours of exposure. Body water, plasma volume and blood volume were increased compared to controls of the same age and controls of the same weight. The increased body water of the acclimatized rats was interpreted as an indication of a greater proportion of actively metabolizing tissues, with the increased oxygen requirement acting as a stimulus for an increase in blood volume since the transient hemoconcentration exhibited during the very early stages of cold exposure had increased the total oxygen carrying capacity of the blood.

The fluid adjustments during hypothermia, prolonged cold exposure and acclimatization are either accompanied by or preceded by ionic shifts across the cell membrane. Kuhl, Beck, Gerschberg, and Ralli (1954) reported a constant serum sodium, a decreased serum chloride, and an increased serum potassium 1 and 2 hours

after immersion of 55 human subjects in 9° C water for 8 minutes. Booker, Hayes, Da Costa, Jones, Hill, and Titus (1952) reported an increased potassium in blood, heart, and skeletal muscle in mice during cold stress, with the potassium decreasing to almost comparable levels with unstressed animals during the 1-2 hour recovery period. Baker and Sellers (1957) reported a significant loss of plasma chloride during the first 24 hours, and then a return to normal in rats exposed to 2° C for 45 days. A trend towards a temporary increase in plasma sodium was exhibited, but after 10 days a tendency to normal was observed. Plasma potassium increased significantly at the 1, 3, 16, and 192 hour periods during exposure, with the values falling towards normal after 12 days.

The report of Eichelberger and McLean (1942) that both potassium and magnesium were chiefly intracellular ions suggests an intimate relation between the two cations. Movement of these two cations during various physiological states has been studied extensively, and in many types of stress these two ions behave similarly. Hastings and Compere (1931) reported an increased serum potassium after removal of the adrenal cortex, and Bauman and Kurland (1926) reported an increase in serum magnesium after adrenalectomy.

In an attempt to relate magnesium to the adrenal, Glick, Frier, and Ochs (1957) analyzed microtome sections of the adrenal medulla and cortex but found no alterations in the quantitative distribution of Mg in the histological zones of the adrenal after administration of adrenalcorticotrophic hormone or hypophysectomy. However, Conway and Hingerty (1946) reported an increase in muscle and plasma magnesium in adrenalectomized rats, and Zwemer and Sullivan (1934) observed increased plasma magnesium in adrenalectomized cats while Harrop, Soffer, Ellsworth, and Trescher (1933) found similar increases in adrenalectomized dogs.

Recently Hingerty (1957) injected magnesium sulphate intraperitoneally into rats until the concentration of Mg in the muscle approximated that found in adrenalectomized rats. He showed a parallelism between the injected rats and the adrenalectomized rats by the appearance of increased phosphocreatine, slightly increased adenosine triphosphate, and decreased glucose 6-phosphate in both groups.

The necessity of the adrenals for survival in the cold was reported by Sellers, You, and Thomas (1951), who observed average survival time of 12 hours of rats transferred to 1.5° C 24 to 48 hours after adrenalectomy. The rapid hypertrophy of the adrenals upon exposure to

cold was observed by Selye (1937), who reported weight increases in the adrenals of rats exposed to -6°C for 48 hours. Katsh, Katsh, and Osher (1954) reported increases in adrenal weight after only 1 day of exposure of rats to 4-6 degrees C.

The failure of adrenal hypertrophy to be a reliable index of adrenal hyperactivity was reported by Heroux and Hart (1954b), who observed that a relatively low rate of administration of cortical hormones would maintain acclimatization in adrenalectomized, acclimatized rats.

The relationship between the ascorbic acid content of the adrenals and other tissues and the ability of an animal to survive and acclimatize to cold was established by Dugal and Thérien (1947).

Failure of control of body temperature of adrenalectomized rats over 16 days of age as reported by Holtkamp, Hill, Longwell, Rutledge and Buchanan (1949) and the report of a 75 per cent decrease in adrenal venous blood minute corticoid output in hypothermic dogs by Egdahl, Nelson, and Hume (1955) suggest an intimate association between adrenal cortical activity and body temperature control. This association is further supported by the report of Halberg and Spink (1955) of the maintenance of body temperature of adrenalectomized mice with injections of cortisone acetate and an endotoxin as compared to a

decreased rectal temperature with injections of endotoxin alone.

Katsh et al (1954), using the ascorbic acid response as an index of cortical activity, reported a reduction of more than 15 per cent of the ascorbic acid content of the adrenals of rats exposed to 2-4° C, reaching normal values about the third day. This finding suggests the occurrence of an adrenal insufficiency for the first few days in the cold.

As the result of work of Hastings and Compere and Bauman and Kurland cited previously it appears that both magnesium and potassium are linked to adrenal function. Serum concentrations of these two ions are increased by adrenal insufficiency. Since adrenal insufficiency is indicated at first upon exposure to cold, perhaps the activity of magnesium during the process of acclimatization is intimately related to adrenal function.

METHOD AND MATERIALS

Magnesium Determination Procedure

Kolthoff (1927) reported that the presence of the magnesium ion in alkaline solutions of titan yellow resulted in a red color, and that the amount of magnesium present could be determined colorimetrically. The method utilized in this study was based on the method of Garner (1946), Heagy (1948), Orange and Rhein (1951), with some of the modifications of Platner and Hosko (1953).

Reagents:

- (1) Magnesium stock solution - 10.131 gm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.5 ml chloroform made up with water to one liter. 1 ml. = 1mg. Mg.
- (2) Magnesium working solution - 2 ml. of Mg stock solution made up to 100 ml. 1 ml. = .02 mg. Mg.
- (3) Trichloroacetic acid - 10 per cent. .614 Normal.
- (4) Sodium Hydroxide - 2 Normal. 80 gm. per liter.
- (5) Titan Yellow solution - 375 mgs. of titan yellow made up to 500 ml. with .1% Elvanol (polyvinyl alcohol - Grade 71-74. E. I. DuPont De Nemours and Co.) Heated until

dissolved and filtered. Stored in a refrigerator at about 5° C.

- (6) Elvanol solution - 1 gm. Elvanol made up to one liter. Heated until dissolved and filtered. Stored in a refrigerator at about 5° C.
- (7) Glass distilled water was used for all reagents.

Serum Magnesium Determination

To 0.5 ml serum in a centrifuge tube 1.0 ml. of trichloroacetic acid was added and then centrifuged at about 3000 rpm for 10 minutes. After removal of 1 ml. of the supernatant to a 12X75 mm cuvette containing 1.5 ml. of glass distilled water, 1 ml. of titan yellow solution in Elvanol and 1.5 ml. of sodium hydroxide solution were added and mixed thoroughly by passing air through a small capillary pipette. The mixture was allowed to stand for twenty minutes to stabilize the color lake and then read in a Coleman Junior Spectrophotometer, Model 6 A, at a wavelength of 560 mu.

A calibration curve was established for each set of samples. To prepare the calibration series magnesium working solution was placed in five cuvettes in the following volumes: 0.0 ml. (blank), 1.0 ml., 1.5 ml., 2.0 ml.; this represented 0.00, 0.02, 0.03, and 0.04 mg. of Mg respectively. To each tube was added 0.5 ml. tri-

chloroacetic acid, 1 ml. of titan yellow solution in Elvanol, and 1.5 ml. of sodium hydroxide solution. Glass distilled water was added where necessary to bring final volume to 5 ml. and the solution was then mixed as previously described. Optical density readings for the standard curve were plotted on linear graph paper. Using this method, Beer's law holds over the range of 0.0 mg. to 0.06 mg. magnesium. New titan yellow in Elvanol solution was prepared every ten days.

Tissue Magnesium Determination

Tissue magnesium determinations were carried out by placing a weighed piece of tissue in an electrically powered Ten Broeck tissue grinder, adding 5 ml. of 10 per cent trichloroacetic acid and grinding until the tissue was thoroughly homogenated. The homogenate was transferred to a centrifuge tube. The grinder was rinsed with 5 ml. of trichloroacetic acid, which was added to the homogenate. Centrifugation was carried out for 10 minutes at about 3000 rpm, and one ml. was taken from the supernatant and analyzed as previously described. The method of tissue magnesium determination used herein was similar to that of Stoner (1950), in which the tissue (muscle) was ground with quartz and 10 per cent trichloroacetic acid, filtered after 30 minutes, and allowed to stand overnight at 4° C. An aliquot was then taken

and the amount of Mg determined.

Hematocrit Determination

Hematocrits were determined by centrifuging a sealed, heparinized capillary tube filled with fresh blood in a High Speed Micro-Hematocrit Centrifuge for ten minutes and reading on a Graphic Reader provided with the instrument.

Determination of Per Cent of Water in Skeletal Muscle

The total water content of skeletal muscle was determined by weighing a freshly excised piece of thigh muscle and placing it in a crucible previously dried at 105° C for 24 hours and weighed. The tissue was then dried at 105° C for 24 hours to a constant weight and reweighed.

Plasma Volume, Blood Volume and Thiocyanate Space Determinations

The method used for the determination of sodium thiocyanate space as a measure of extracellular fluids was that of Laviètes, Bourdillon and Klinghoffer (1936). The use of T-1824 (Evans Blue) in measuring plasma and blood volumes was derived from Metcalf and Favour (1944). The mixture of T-1824 and sodium thiocyanate for simultaneous determination was in accordance with the pro-

cedure described by Wang and Hegsted (1948), and the method of dye injection was taken directly from D'Amour and Blood (1948).

Reagents:

- (1) 225 mg. of T-1824 mixed with a 5 per cent solution of sodium thiocyanate. Hereafter referred to as dye solution.
- (2) 5 per cent solution of ferric nitrate.
- (3) 20 per cent solution of trichloroacetic acid.
- (4) Glass distilled water was used for all reagents.

Plasma Volume and Blood Volume Determinations

After injection of 0.4 ml. of the dye solution through a carotid cannula the syringe was refluxed several times with blood to insure mixture of all the dye solution. The blood was withdrawn after 6 minutes with a mineral oil coated syringe by direct heart stab. After the blood had clotted centrifugation was performed at about 3000 rpm for 20 minutes. Glass distilled water was used to dilute 0.2 ml. of the serum twenty times the original volume. One half of the diluted serum was read in the Coleman Junior Spectrophotometer, Model 6 A, at 620 mu and the concentration of T-1824 determined from a previously constructed standard curve. A water

blank was used. The remainder of the serum was used for magnesium determination, while the rest of the diluted serum solution was used for determination of thiocyanate space.

The plasma volume was determined by dividing the mg. of dye injected by the concentration of dye in the sample. The blood volume was determined by dividing the product of the plasma volume times 100 by the percentage of plasma (from the hematocrit).

Thiocyanate Space Determination

From the preceeding diluted serum solution 1 ml. was taken and added to 1 ml. of 20 per cent trichloroacetic acid, allowed to stand for 10 minutes and then centrifuged at approximately 3000 rpm for 10 minutes. With a pipette, 1 ml. of supernatant was removed and added to 1 ml. of ferric nitrate solution and read immediately in the Coleman Junior Spectrophotometer, at 460 mu. A blank with water was similarly treated. The concentration of thiocyanate was determined from a previously constructed standard curve. The thiocyanate space was determined by dividing the mg. of thiocyanate injected by the concentration of thiocyanate in the sample.

Experimental Procedure and Materials

Experimental animals used were Sprague-Dawley

(Holtzman, Wisconsin) rats of mixed sex. The males were 16 days older and approximately 150 grams heavier. Control animals were studied prior to experimental animals, and both were treated in the same manner in regard to individual determinations. Forty-seven rats were placed in a cold room at 10° C and 5 days later forty-six rats were added.

The temperature of the cold room was lowered approximately 1° C each day until it reached 3.5° C. The experimental animals were fed the same diet as the control animals, namely Purina Laboratory Chow and tap water ad libitum. Experimental animals were placed in individual cages on wire bottoms. The cage racks were periodically moved about the cold room to compensate for any possible temperature differentials within the cold room itself. The temperature and humidity of the cold room were monitored daily for about an hour with a Leeds and Northrup 12 channel Type G "Speedomax".

The experimental rats were weighed and their deep (5cm.) rectal temperature taken with a direct reading thermocouple type clinical thermometer (Micron Instrument Corp. "Rapid-Temp") before being placed in the cold. Deep rectal temperatures were subsequently taken at 5, 16, 23, 29, 41, 65, and 71 hours and on the 4th, 11th, 21st, and 40th day of exposure. Ground Purina Laboratory Chow

containing 0.1 per cent thyroid extract (U.S.P., Eli Lilly Co.) was fed to 14 rats which remained in the cold from 42 to 45 days. Due to mechanical failure the temperature of the cold room was elevated to 14° C for approximately eight hours, 35 days after the first group had been placed in the cold.

Approximately 16 rats were removed from the cold on the 10th, 20th, 30th, and 40th day of exposure for the various analyses. The rats were anesthetized with an intra-peritoneal injection of pentobarbital (32 mg/kg) after being weighed, and removed to an adjacent room at a temperature of about 25° C. The abdominal cavity and the thoracic cage were opened. Approximately 5 ml. of blood was taken directly from the heart with a mineral oil coated syringe (to prevent hemolysis). Two hematocrit tubes were immediately filled and the remainder of the blood set aside for 2 hours to clot. After clotting the blood was centrifuged at circa 3000 rpm for 20 minutes, the serum removed and placed in the cold until the determinations were made. After the blood was withdrawn a piece of liver from the right lobe, the entire heart, a gastrocnemius muscle, and some thigh muscle were ablated and weighed. All were then placed in individual beakers and put in the cold until determinations were made except the thigh muscle, which was immediately placed in the oven for total water determination. If the animal was

to be used for blood volume and thiocyanate space determinations in addition to the magnesium determinations, carotid cannulation and dye solution injection were performed prior to the abdominal incision. Tissue determinations were made within 36 hours and serum determinations within 48 hours.

RESULTS

The data contained in Tables I through X and Figures I, II and III show the observations on control animals and animals exposed to $3.5^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$ for 10, 20, 30 and 40 days. Tables I through IX also show the Mean, Standard Deviation, and Standard Error of the mean of the various determinations. Table X shows the statistical levels of significance of the values of the various determinations at the four points of observation as compared to the respective values of the control animals.

Figure I illustrates the variations in liver, heart, skeletal muscle and serum magnesium, and deep rectal temperature, room temperature, and relative humidity. The values of the control animals are placed at 0 (zero) days. Figure II illustrates the variations in blood and plasma volumes, thiocyanate space, per cent of water in skeletal muscle, hematocrit, room temperature and relative humidity. For a comparison of these values to body temperature the reader should refer to Figure I for the body temperature values.

Figure III illustrates the weight changes in the male and female rats.

TABLE I
Serum Magnesium Concentration in Control
Rats and Rats Exposed to Cold

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%
5	2.60	12	3.14	29	2.60	15	2.40	24	3.50
6	2.40	23	3.60	9	3.06	3	2.40	36	3.80
7	2.50	28	3.20	16	3.10	38	2.20	41	1.84
8	2.42	8	2.96	34	3.50	26	3.12	17	2.30
9	1.80	32	3.00	37	2.70	35	2.40	45	1.26
13	2.00	11	2.50	42	3.48	14	2.08	40	2.14
14	1.74	4	3.68	10	3.00	13	3.50	47	3.24
15	2.44	33	2.96	7	2.26	44	3.36	20	2.14
16	2.60	53	3.54	76	2.20	5	2.08	94	2.20
17	1.80	64	2.90	77	2.84	79	2.90	62	2.64
18	1.40	82	3.24	60	2.70	95	1.60	68	1.58
19	2.20	89	2.44	55	2.10	50	2.20	51	2.64
20	2.30	70	2.28	92	2.40	57	2.70	69	2.64
21	1.80	87	4.48	93	2.80	72	2.36	66	1.76
23	2.00	75	2.48			81	2.44		
24	1.93	56	3.20			58	1.72		
25	2.10								
26	2.20								
27	2.80								
28	1.94								
29	2.34								
30	2.10								
31	2.10								
33	2.20								
34	2.40								
35	1.60								
37	2.18								
40	1.75								
42	2.80								
43	2.40								
44	3.20								
45	2.56								
46	2.10								
47	3.52								
48	2.72								
49	2.10								
Mean	2.25	3.10		2.75		2.50		2.39	
S.D.	±.42	±.52		±.41		±.52		±.68	
S.E.m.	±.068	±.13		±.11		±.13		±.18	

TABLE II
Liver Magnesium Concentration in Control
Rats and Rats Exposed to Cold

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%
1	18.0	12	22.4	29	23.0	15	23.1	24	20.9
2	20.5	23	23.8	9	20.0	3	25.3	36	25.1
3	21.8	28	19.2	16	20.7	38	21.3	41	21.7
4	19.5	8	21.5	34	23.2	26	22.9	17	21.8
5	18.6	32	25.2	37	22.8	35	18.8	45	20.2
6	18.6	11	25.6	42	20.7	14	19.9	40	19.5
7	24.0	4	18.3	10	19.9	13	17.7	47	18.0
8	22.3	33	26.2	7	22.7	44	19.0	20	16.3
9	20.3	53	22.0	27	21.2	5	20.5	94	20.0
10	22.4	64	19.8	76	20.2	79	26.9	62	18.4
11	20.8	82	20.2	77	21.4	95	24.1	68	19.4
12	19.1	89	21.4	60	17.1	50	19.3	51	19.7
13	20.3	70	22.6	55	22.2	57	21.4	69	19.0
14	20.3	87	21.0	92	19.7	72	22.4	66	19.7
15	21.1	74	23.1	93	20.8	52	20.4	63	19.6
16	21.1	75	23.9	65	19.1	81	27.2		
17	27.8	56	21.7	80	20.4	58	25.9		
18	24.0								
19	22.9								
20	25.3								
21	26.3								
22	23.3								
23	20.5								
24	25.2								
25	25.1								
26	24.5								
27	20.2								
28	22.0								
36	24.7								
37	22.7								
38	21.2								
39	19.4								
Mean	22.0		22.2		21.0		22.1		20.0
S.D.	± 2.42		± 2.17		± 1.76		± 2.56		± 1.92
S.E.m.	± .43		± .54		± .44		± .71		± .51

TABLE III

Heart Magnesium Concentration in Control

Rats and Rats Exposed to Cold

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%
1	14.0	12	19.9	29	17.5	15	17.5	24	17.5
2	16.9	23	19.6	9	16.0	3	18.6	36	19.0
3	16.8	8	20.1	16	17.3	38	15.2	41	16.0
4	16.4	32	16.0	34	14.5	26	16.1	17	17.5
5	14.1	11	19.6	37	19.6	35	16.5	45	17.9
6	15.4	4	21.0	42	17.2	14	18.0	40	16.3
7	17.5	33	20.4	10	17.4	13	16.4	47	14.5
8	18.7	53	19.3	7	15.8	44	16.2	20	16.9
9	16.2	64	18.4	27	16.7	5	18.4	94	17.7
10	17.8	82	17.1	76	16.1	79	22.6	62	16.0
11	14.7	89	19.4	77	14.5	95	20.8	68	17.4
12	14.5	70	17.0	60	16.7	50	17.2	51	16.2
13	15.0	87	15.9	55	15.4	57	16.6	69	19.5
14	15.1	74	17.1	92	13.9	72	15.4	66	18.2
15	17.0	75	13.8	93	17.1	52	17.2	63	18.0
16	15.3	56	20.2	65	17.3	81	24.4		
17	16.9			80	14.8	58	19.9		
18	16.9								
19	17.1								
20	25.8								
21	22.0								
22	23.0								
23	18.5								
24	17.0								
25	15.5								
26	14.4								
27	12.3								
28	17.3								
36	15.9								
37	16.0								
38	15.3								
39	17.4								
Mean	16.8		18.4		16.3		18.1		17.2
S.D.	± 2.64		± 1.97		± 1.39		± 2.47		± 1.24
S.E.m.	$\pm .47$		$\pm .51$		$\pm .35$		$\pm .61$		$\pm .33$

TABLE IV
Skeletal Muscle Magnesium Concentration in
Control Rats and Rats Exposed to Cold

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%	Rat	mg%
1	23.6	12	27.5	29	26.3	15	21.5	24	26.3
2	22.5	23	27.1	9	22.9	3	24.2	36	27.8
3	24.4	28	27.0	16	24.6	38	25.9	41	27.4
4	26.0	8	26.1	34	23.2	26	26.8	17	24.6
5	22.8	32	31.5	37	25.7	35	24.7	45	27.8
6	22.9	11	29.5	42	26.8	14	27.1	40	21.6
7	25.0	4	26.4	10	23.1	13	24.3	47	22.6
8	24.7	33	31.1	7	26.5	44	23.4	20	21.1
9	27.1	53	24.4	27	24.9	5	23.0	94	22.8
10	23.0	64	23.6	76	24.1	79	31.0	62	23.7
11	24.5	82	25.8	77	23.7	95	28.8	68	24.7
12	23.6	89	26.9	60	21.1	50	27.2	51	23.0
13	22.4	70	26.3	55	30.1	57	21.7	69	24.9
14	23.3	87	30.0	92	26.0	72	24.8	66	24.0
15	23.2	74	27.2	93	26.4	52	24.5	63	24.4
16	24.5	75	29.5	65	22.6	81	29.3		
17	26.2	56	29.7	80	23.0	58	26.9		
18	24.0								
19	23.2								
20	27.1								
21	26.4								
22	25.1								
23	25.5								
24	26.2								
25	24.0								
26	26.9								
27	24.3								
28	25.5								
36	31.0								
37	24.0								
38	24.6								
39	24.8								
Mean	24.8		27.6		24.8		25.6		24.4
S.D.	±1.75		±2.12		±2.09		±2.56		±2.05
S.E.m.	±.31		±.53		±.52		±.64		±.54

TABLE V

Water Content of Skeletal Muscle in Control

Rats and Rats Exposed to Cold

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	%	Rat	%	Rat	%	Rat	%	Rat	%
25	75.3	28	75.6	16	75.0	15	75.4	24	76.2
26	75.1	8	74.9	34	74.5	3	74.6	36	74.2
27	76.2	32	69.7	37	74.6	38	73.7	41	77.2
29	75.6	74	74.9	76	74.7	79	73.8	94	75.9
30	76.0	64	77.0	77	68.8	95	65.9	62	75.0
31	76.0	82	75.9	55	68.9	50	75.6	68	76.5
36	77.7								
38	75.9								
39	77.1								
43	76.2								
44	76.0								
45	76.1								
50	76.3								
51	76.2								
55	76.1								
56	75.9								
Mean	76.1		74.7		72.8		73.2		75.8
S.D.	±.59		±2.33		±2.76		±3.33		±.98
S.E.m.	±.15		±1.04		±1.23		±1.49		±.44

TABLE VI
Thiocyanate Space as per cent of Body Weight
in Control Rats and Rats Exposed to Cold

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	%	Rat	%	Rat	%	Rat	%	Rat	%
43	22.9	105	24.5	80	24.8	44	23.8	47	19.8
44	21.4	106	28.6	103	23.7	5	20.9	20	21.6
45	18.2	99	26.0	98	25.6	81	21.8	66	17.6
47	17.1	102	23.7	100	23.3	58	23.6	63	22.9
48	18.0			104	21.3				
49	19.6			97	27.2				
50	17.4								
52	22.3								
54	21.8								
55	21.0								
56	19.1								
Mean	19.9		25.7		24.3		22.5		20.4
S.D.	±1.98		±1.87		±1.86		±1.11		±2.04
S.E.m.	±.62		±1.08		±.83		±.64		±1.18

TABLE VII
Blood Volume as per cent of Body Weight
on Control and Cold Exposed Rats

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	%	Rat	%	Rat	%	Rat	%	Rat	%
43	6.9	105	8.9	80	9.2	44	9.1	47	10.3
44	6.3	106	12.1	103	9.5	5	9.6	20	10.1
45	7.1	99	9.6	98	11.0	81	9.5	66	7.2
47	5.8	102	9.3	100	9.3	58	9.4	63	10.4
48	6.9			104	9.8				
49	7.6			97	11.2				
50	7.9								
52	8.6								
54	8.8								
55	7.8								
56	6.8								
Mean	7.3	10.1		10.0		9.4		9.5	
S.D.	±.89	±1.24		±.81		±.19		±1.31	
S.E.m.	±.28	±.71		±.36		±.11		±.75	

TABLE VIII
Plasma Volume as per cent of Body Weight
on Control and Cold Exposed Rats

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	%	Rat	%	Rat	%	Rat	%	Rat	%
43	4.2	105	5.0	80	5.1	44	4.9	47	5.7
44	3.7	106	5.9	103	4.7	5	5.5	20	5.8
45	4.1	99	5.4	98	5.8	81	4.8	66	4.1
47	2.9	102	4.6	100	4.5	58	4.9	63	5.7
48	3.4			104	5.4				
49	3.5			97	5.7				
50	4.4								
52	4.7								
54	4.7								
55	4.1								
56	3.7								
Mean	4.0		5.2		5.2		5.0		5.3
S.D.	±.30		±.47		±.47		±.26		±.70
S.E.m.	±.09		±.27		±.21		±.15		±.41

TABLE IX
Per Cent Hematocrit in Control Rats and Rats
Exposed to Cold

Control		Experimental							
		10 Day Exposure		20 Day Exposure		30 Day Exposure		40 Day Exposure	
Rat	%	Rat	%	Rat	%	Rat	%	Rat	%
17	42	12	43	29	47	15	36	24	41
18	37	28	43	9	44	35	48	36	36
19	37	8	44	16	45	38	45	41	44
20	48	53	47	34	45	26	45	17	44
21	46	64	44	42	46	35	46	45	40
22	50	82	45	10	48	14	46	40	46
25	43	89	41	7	47	13	52	47	45
26	48	70	48	76	45	44	46	20	43
27	53	87	44	77	49	5	43	94	46
28	49	74	41	60	49	79	40	62	45
30	42	75	41	55	42	95	46	68	47
31	40	56	32	92	49	50	49	51	42
35	39	105	44	93	44	57	44	69	46
39	43	106	51	65	48	72	46	66	43
43	39	99	44	80	45	52	48	63	45
44	42	102	50			81	49		
45	42					58	47		
46	37								
53	43								
47	50								
48	50								
49	54								
Mean	44.3	43.9		46.2		45.6		43.5	
S.D.	± 5.14	± 4.21		± 2.07		± 3.53		± 2.73	
S.E.m.	± 1.12	± 1.08		± .55		± .88		± .73	

TABLE X

Statistical Levels of Significance of Rats Exposed to Cold as
Compared to Control Rats

	Control		Experimental											
			10 Day Exposure		20 Day Exposure			30 Day Exposure			40 Day Exposure			
	Mean	Mean	t	P	Mean	t	P	Mean	t	P	Mean	t	P	
Hematocrit (%)	44.3	43.9	.91	>.05	46.2	1.52	>.05	45.6	.91	>.05	43.5	.59	>.05	
Liver Mg (mg%)	22.0	22.2	.29	>.05	21.0	1.93	>.05	22.1	.12	>.05	20.0	2.35	<.05	
Heart Mg (mg%)	16.8	18.4	2.32	<.05	16.3	.86	>.05	18.1	1.81	>.05	17.2	.69	>.05	
Skeletal Muscle Mg (mg%)	24.8	27.6	4.51	<.01	24.8	0.0	1.0	25.6	1.12	>.05	24.4	.63	>.05	
Serum Mg (mg%)	2.25	3.10	5.78	<.01	2.75	3.84	<.01	2.50	1.66	>.05	2.39	.73	>.05	
% H ₂ O in Skeletal Muscle	76.1	74.7	1.33	>.05	72.8	2.66	<.05	73.2	1.93	>.05	75.8	.18	>.05	
Blood (% of Body Volume Weight)	7.3	10.1	3.57	<.01	10.0	5.70	<.01	9.4	6.85	<.01	9.5	2.66	<.05	
Plasma (% of Body Volume Weight)	4.0	5.2	4.43	<.01	5.2	5.70	<.01	5.0	5.64	<.01	5.3	3.12	<.01	
Thiocyanate (% of Space (Body Weight))	19.9	25.7	4.64	<.01	24.3	4.31	<.01	22.5	2.89	<.05	20.4	.36	>.05

Fig. 2. Body Fluid Alterations, Cold Room Temperature and Relative Humidity During Cold Exposure.

Fig. 3. Weight Changes in Male and Female Rats During Acclimatization to Cold.

The determinations made on rats fed 0.1 per cent thyroid extract in their ground feed are contained in Table XI. A hematocrit mean was not considered representative as two of the animals had lost considerable blood from the tail. Table XII shows the statistical levels of significance of the various determinations (excluding hematocrit) as compared to rats exposed to cold for 40 days with no thyroid extract.

All statistical treatment was in accordance with standard procedures (Edwards, 1955).

TABLE XI

Data on Rats Fed Thyroid during 40 Days Exposure to Cold

Rat	Hemato- crit in %	mg % Magnesium				% of Body Weight			%H ₂ O in Muscle
		Liver	Muscle	Heart	Serum	Blood Volume	Plasma Volume	SCN Space	
43	---	21.6	24.6	15.8	--	---	---	--	75.2
19	48	23.1	23.1	17.7	1.2	9.3	4.8	24.7	--
22	40	22.5	27.5	17.0	2.4	8.9	5.3	29.1	--
30	45	20.3	23.9	14.5	3.2	7.4	4.1	--	70.7
25	52	19.5	22.8	16.5	3.6	9.5	4.5	24.5	--
90	47	24.4	23.1	18.4	4.2	---	---	--	--
85	42	22.1	26.7	22.6	2.4	--	---	--	75.8
78	42	23.9	27.8	20.2	2.2	--	--	--	--
86*	28	25.7	29.3	21.0	1.6	---	---	--	--
96*	30	27.7	28.4	16.5	1.8	--	--	--	74.2
Mean	--	23.1	25.4	18.0	2.51	8.8	4.7	26.1	74.0
S.D.	--	±2.34	±2.03	±2.4	±.92	±1.14	±.45	±2.12	±1.98
S.E.m.	--	±.78	±.67	±.80	±.32	±.47	±.26	±1.50	±1.14

*These animals were bleeding from their tails.

TABLE XII

Statistical Levels of Significance of 40 Day Cold
Exposed Thyroid fed Rats as Compared to 40 Day Cold
Exposed Control Rats

	Controls Exposed 40 Days		Thyroid fed Rats Exposed		t	P
	No. of Rats	Mean	No. of Rats	Mean		
Serum Mg (mg%)	15	2.39	9	2.51	.79	>.05
Liver Mg (mg%)	15	20.0	10	23.1	3.31	<.05
Heart Mg (mg%)	15	17.2	10	18.0	.98	>.05
Skeletal Muscle Mg (mg%)	15	24.4	10	25.4	1.10	>.05
% H ₂ O in Skeletal Muscle	6	75.8	44	74.0	1.20	>.05
Blood Volume (% of Body Weight)	4	9.5	4	8.8	.77	>.05
Plasma Volume (% of Body Weight)	4	5.3	4	4.7	1.20	>.05
Thiocyanate Space (% of Body Weight)	4	20.4	3	26.1	1.50	>.05

DISCUSSION

On first inspection of the weight curves it would appear that the males and females exposed to cold are of different populations. The females showed only slight weight gains at the 10 and 20 day periods, but an increased gain was evidenced at the 30 day period and a still greater gain at the 40 day period. These results are essentially in accordance with those of Dugal (Burton and Edholm, 1955) on young, growing rats. Dugal observed an initial loss of weight during the stage of resistance (about the first week), followed by a slow recovery of weight during the stage of acclimatization. The males exposed to cold suffered a substantial decrease in weight at the 10 day period, but at the 20 day period an attempt at recovery was indicated. The weight had decreased at the 30 and 40 day periods, signifying failure of the males to acclimatize in this respect. Apparently the males, who were 16 days older and approximately 150 grams heavier than the females, were unable to overcome the stress of a cold environment due to their age (20 weeks) and average weight (410 grams).

Weiss (1954) observed increased tissue oxygen consumption of liver, diaphragm, heart and kidney in

6 week old rats exposed to 5° C for 10 days but only slightly increased liver tissue oxygen consumption of 6 month old rats under the same stress. Weiss suggested a progressive decrease in metabolic rate and an increasing resistance to acclimatization with advancing age. It is interesting to note at this point the observation of Zarrow and Denison (1956) of a sexual dimorphism with regard to survival in 2° C of mature (60 day old) rats, the females proved to be the hardier. It is difficult to attribute the failure of the males to gain weight to either the weight-age disparity or the sex difference. A combination of the two factors seems more likely.

Statistical comparison of both control and experimental males with respect to the females yielded only two slightly significant differences ($P < .05$), a higher male heart Mg at the 10 day level and a higher female skeletal muscle Mg at the 30 day level. It is therefore assumed in this paper that the two sexes constitute a single population.

The colonic temperatures of the cold exposed animals rose significantly ($P < .01$) from 37.3° C to 38.3° C within the first 16 hours, then fell rapidly to 37.3° C at 71 hours of exposure. By the 11th day the colonic temperatures had again increased significantly ($P < .01$) to 38.7° C after which a steady decline was observed to near

normal levels at the 40 day period. Sellers and You (1950) reported a significant ($P < .01$) elevation in colonic temperature from 37.1°C to 37.7°C of 160 gram rats during 6 days of exposure to 1.5°C . Fregly (1953) reported a significant elevation ($P < .01$) in colonic temperatures from 38.8°C to 39.3°C of 180 gram rats after 10 days of exposure to 5°C and no significant increase after five days.

The external appearance of the animals, as judged by gross observation, resembled those described by Gilson (1950) in 36 of 42 rats exposed to $4-6^{\circ}\text{C}$ for 40 days. Within the first few days of exposure erythema of the feet and ears was evidenced in approximately 75 per cent of the animals. By the 10 day period edema of the tail was observed in about 50 per cent of the animals. The edema persisted in some animals until the terminal point (40th day), while the erythema subsided after about the 20th day. By the 30th day 80 per cent of the remaining animals showed necrotic ears and tail tips. During the final 10 day period all of the animals had suffered a loss of from 20 to 80 per cent of their tails due to spontaneous amputation or chewing. Two of the animals lost considerable blood, as evidenced by low hematocrits (see Table XI). Gilson felt there was a similarity between the acute erythema and edema of cold to the less severe edema and erythema of adrenal

insufficiency, and suggested this as a possible etiological factor in the production of edema in the cold exposed animal. It should be pointed out that no actual quantitative measurements were made in regard to these gross observations.

The transient hemoccentration during sudden cold exposure, as reported by D'Amato and Hegnauer (1953) and Rodbard et al (1950), was not shown in this study. The hematocrit values on the 10th, 20th, 30th and 40th day of exposure were not statistically different from the control values. These findings are in accord with those of Garcia (1957) and Deb and Hart (1956).

The water content of the skeletal muscle showed a significant decrease ($P < .05$) at the 20 day level, but returned towards normal by the 40th day. Heroux (1958) reported a decrease in skeletal water and protein of rats after 8 weeks at 6°C , and suggested that the reduction of protein concomitantly with the reduction of muscle water indicates a reduction of growth. The report of Heroux and Gridgeman (1958) on the increased weight of liver, heart, kidney, adrenals and intestines of rats exposed to 4°C for 4 weeks, and the decreased weight of skeletal muscle and skin perhaps suggest a transfer of water and protein from slower to faster metabolizing tissues.

Both plasma and blood volume levels were elevated after 10 days exposure to cold and remained significantly higher than controls for the remainder of the experiment. Since the total body water was not determined, these values were calculated as per cent of body weight. Baker and Sellers (1957) reported increases in blood and plasma volumes of rats exposed to 2° C for 45 days calculated as per cent of body weight or of total body water. Although the reason for increased blood and plasma volumes during acclimatization is not clear, it is interesting to note the work of Heroux (1956) who reported an increased vascularization of the tissues as determined by capillary counts of cold acclimated rats. This suggests a larger blood volume because of a larger capillary bed.

A 30 per cent increase in thiocyanate space was observed at the 10 day period; however, this increase was less evident at the 20 and 30 day levels and had declined to near normal at 40 days. Deb and Hart (1956) reported an increased extracellular fluid (sodium space) in rats after one week of exposure to 6° C relative to body weight, but no increase when calculated as per cent of total body water. After the rise at the one week level, Deb and Hart reported a decline at the three week level, approaching control values at the five week level. It would seem reasonable to expect a sustained elevation

of extracellular fluid with a concomitant increased blood volume.

The increased liver magnesium concentration of the thyroid fed animals exhibited the only significant ($P < .05$) variation in the group of 14 animals during 40 days exposure to cold. It should be pointed out that the two highest values of liver Mg were obtained from the animals that showed greatly reduced hematocrits. It is probable that since the animals had increased their metabolism to their maximum during the cold stress, no further increase could be manifested with the added stress of thyroid feeding. The exogenous thyroid presumably merely served to reduce the production of the hormone by the gland.

It is interesting to note that of the five animals that died during cold exposure four were males that were fed thyroid. All of the thyroid rats that died had lost a substantial amount of blood from their chewed tails. It is difficult to state whether these deaths were due to the thyroid intake or the inability of the animal to acclimatize to a cold environment. The four thyroid deaths occurred between the 25th and 35th days of exposure, the period when acclimatization should occur according to Adolph (1950) and Cottle and Carlson (1956).

The significant elevation of the serum Mg observed at the 10 day period of exposure may not be the highest level reached. If the serum Mg rises as the rectal temperature declines (Platner and Hosko, 1953) the highest level of serum Mg would have occurred at the 3rd day. Since determinations were made only at 10 day increments, the initial rise of serum Mg would not have been manifested. After the 10 day period the serum Mg declined steadily until near normal values were reached at the 40 day period.

However, the values of serum Mg indicated above do not express the real increase in serum Mg. The plasma and blood volumes were diluted from 25-35 per cent during cold exposure. The actual increase of serum Mg computed from the increased blood volume at the 10 day period is 4.28 mg. per cent, representing an actual increase of 90 per cent as opposed to the apparent increase of 37 per cent. At the 20 day period the actual increase is 73 per cent, at the 30 day period 43 per cent, and at the 40 day period 37 per cent. Thus the serum Mg would still be significantly elevated after 40 days of cold exposure. The actual serum Mg rise as computed from the increased plasma volume, was only slightly less than the amount calculated from increased blood volume.

The heart and skeletal muscle magnesium also rose

significantly at the 10 day period, but fluctuations thereafter were insignificant. The liver magnesium failed to show any alteration until the 40 day period, at which time it was significantly lower ($P < .05$). The increase in the heart and muscle Mg may be similar to the increase in Mg, in that the highest concentration had occurred before sampling. The tissue Mg elevations are doubtlessly secondary effects of the increased serum Mg, as Fenn and Haege (1942) reported that muscle cells can release or absorb Mg depending on the concentration in the serum. The failure of liver tissue to exhibit increased Mg concentration is not clear.

The report of Conway and Hingerty (1946) on increased skeletal muscle and serum Mg in adrenalectomized rats coupled with the observation of Katsh et al (1954) of adrenal insufficiency on the basis of ascorbic acid content of cold exposed rats during the first day of exposure may offer a plausible explanation for the observed Mg rise during cold exposure.

Hingerty (1957) showed a 17 per cent increase of Mg in skeletal muscle of the rat 3 days after adrenal ectomy, while the increase in Mg in this work was 11 per cent after 10 days in the cold. This suggests that the skeletal muscle magnesium was not at its highest level at the 10 day period, but in reality was declining.

Heroux and Hart (1954a) reported the increase of adrenal activity after the first day of cold exposure which reaches a maximum in about 20 days and then returns to the pre-acclimation levels. Since the adrenal insufficiency manifested during the first day of cold exposure is rapidly replaced by increased adrenal activity, the water retention and increased blood volume and thiocyanate space may be due to strong sodium retention from an overactive adrenal.

However, Da Costa, Booker, Maloney, Jones and Froix (1953) reported a lag between the onset of the stress period and the changes in the electrolytes that should follow adrenal hormone release. Thus there appears to be a period of time during which the effects of adrenal insufficiency might be strongly manifested, during which the adrenal is functioning at its maximum in order to maintain an increased metabolism. The observation of Katsh et al (1954) of the failure of the hypophysis to attain normal ascorbic acid levels until after 10 days of cold exposure suggests that, although the adrenals are hyperactive at this time, the animal is still suffering from a modified form of hormonal insufficiency and therefore an electrolyte imbalance would be expected.

The lowering of the body temperature appears to

decrease corticoid output according to Egdahl et al (1955). The lack of an instrument which can measure slight alterations in body temperatures without disturbing the animal is perhaps the cause of failure to demonstrate a reduction in body temperature upon exposure to cold. Hibernators, whose body temperature is substantially reduced while hibernating, exhibit some symptoms of adrenal insufficiency, such as reduced metabolism, decreased oxygen consumption, and increased serum magnesium.

The report of Baker and Sellers (1957) of significant increases in plasma potassium at 1, 3, 16, and 192 hours after exposure to cold, with the values then falling to pre-exposure levels, coincide fairly well with the results of this experiment. The observation of Zwemer and Sullivan (1934) on increased plasma Mg and potassium as a result of adrenal insufficiency in cats suggests that these two ions may be under a similar influence of the adrenals during exposure to cold.

The source of Mg during cold exposure is not clear. Wolff (1939) observed that red blood cells have the ability to absorb or release Mg ion depending on the concentration in the plasma. Platner (1950) showed some evidence of permeability of erythrocytes to magnesium with an observed decrease in corpuscular magnesium concomitant with a rise in serum Mg. Thus one of the pos

sible origins of the increased serum Mg upon exposure to cold is the red blood cell. Another likely source is the diet, since the food intake is increased appreciably when animals are exposed to cold. However, this source loses import when considering immediate alterations in serum Mg concentration. A third possible source is the skeleton, but Terkildsen (1952) presumed that Mg was fixed and thus unable to contribute materially in the rapid movements observed here. Terkildsen also suggested the improbability of decreased Mg excretion being the source of the elevated serum levels after observation of only a slight elevation of serum Mg levels upon ligation of both ureters for 24 hours in the rabbit.

Hingerty (1957) suggested that the observed increase in plasma and muscle potassium after adrenalectomy were secondary effects, the cause being essentially renal and "presumably associated with loss of a normal inhibition by cortical hormones of potassium reabsorption". The parallelism of Mg and potassium during adrenal insufficiency again suggests a similar mechanism for Mg. Thus the more likely source, according to experimental observations, appears to be the red blood cell, but the well established syndrome of potassium retention under conditions of adrenal insufficiency and the possible retention of Mg should not be excluded.

Assuming that the work of Conway and Hingerty is correct and that Mg increases in the plasma and skeletal muscle with adrenal insufficiency, and the ascorbic acid content of the adrenals and the pituitary indicate the presence of adrenal insufficiency during the first stages of cold exposure, it is suggested that the increased Mg levels observed in this study are the result of a form of adrenal insufficiency. If the concentrations of serum Mg were determined in serum and tissues during the very first stages of cold exposure and correlated with adrenal function, perhaps a quantitative measurement of adrenal insufficiency could be positively related to the magnesium rise reported here.

The role of Mg in acclimatization is probably that of an ion whose mobility is altered by variations in humoral control during exposure to cold. It is suggested that the increased Mg concentrations exhibited by hypothermic animals and animals exposed to cold are in reality a secondary effect of the primary endocrine alterations. However, the possibility that increased magnesium concentrations are the result of a reduction of peripheral temperatures of the body should not be excluded. Temperatures of the limbs and tail can be considerably lower than core temperatures and furthermore it is known that Mg is released from the skin when an animal is exposed to acute hypothermia.

SUMMARY

1. Serum magnesium determinations were obtained on 38 albino rats under normal conditions and the mean value was 2.25 mg per cent.
2. Liver, heart, and skeletal muscle magnesium determinations were obtained on 32 albino rats under normal conditions and the mean values were 22.0 mg per cent, 16.8 mg per cent, and 24.8 mg per cent respectively.
3. Determinations made on albino rats exposed to 3.5° C ± 1.5° C for periods of 10, 20, 30 and 40 days revealed a) significant increases in serum, heart, and skeletal muscle magnesium at the 10 day level, b) a gradual decline of serum magnesium from the high level at 10 days but still significantly elevated at 40 days, c) significant increases in plasma and blood volumes throughout the entire period of exposure, d) significant increase in thiocyanate space at the 10 day level, gradually declining to near normal value at the 40 day level, and e) a significant decrease in skeletal muscle water content at the 20 day period, returning to near normal by 40 days.

4. Initial adrenal insufficiency upon exposure to cold is suggested as a possible cause of increased magnesium concentrations.
5. Hematocrits were determined on 22 albino rats under normal conditions and the mean value was 44.3 per cent.
6. Total water content of skeletal muscle was obtained on 16 albino rats under normal conditions and the mean value was 76.1 per cent.
7. Plasma volume, blood volume and thiocyanate space were determined on 11 albino rats under normal conditions. The mean values expressed as per cent of body weight were 4.0 per cent, 7.3 per cent, and 19.9 per cent respectively.
8. The loss of weight by older, heavier male rats during exposure to cold as compared to the females appeared to be due to the weight-age dissimilarity and sex difference.

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