Studies on the Effect of Hypothermia

IV. The Rise of Serum Magnesium in Rabbits during the Hypothermic States as Shown by the Spectrochemical Method*

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In this paper are presented data demonstrating an increase in the magnesium content of the serum from 12 rabbits of a group whose body temperatures had been lowered during a test of the effect of hypothermia on the Brown-Pearce rabbit epithelioma. Within 2 to 5 hours after the start of the low temperature state (13-22°C) the average increase in serum magnesium was 24 per cent. We believe that this bears some relation to the lethargy reported in another paper of this series.

Serum magnesium has been shown to be increased in several species of animals during normal hibernation (13, 15). It has also been demonstrated by Suomalainen (17-19) that the injection of magnesium and insulin into hedgehogs usually induces hibernation. Thus it appears that magnesium not only plays a significant role in the mechanism of normal hibernation, but also is important in artificially produced pseudohibernation (hypothermic states) and thus is worthy of some study.

METHOD

Normal, young adult, tumor-bearing rabbits of the New Zealand strain were placed in cylindrical wire cages that were immersed in melting ice and water as described in Paper I of this series. The animals were kept on a stock diet of alfalfa and oats. No urine was produced or excreted during the hypothermic state, and when the temperature of the rabbits fell below 25°C, they were in a lethargic and almost completely passive state. Alteration in serum magnesium was studied after the body temperature had been lowered rapidly from the normal 36.5-39.0°C to 13-22°C in a period of approximately 2 hours. Prior to immersion in ice and water, and after the body temperature had been reduced to the desired level, samples of blood of about 1.5 cc. each were taken either from an ear vein or by cardiac puncture. The blood was centrifuged with no anticoagulant added and the serum stored in an ice chest until determinations were made. Specific gravity determinations were made on several samples of serum by weighing a known amount by the method of Brown and Clark (2) to detect alteration in concentration.

METHOD OF MAGNESIUM DETERMINATION

The serum magnesium was determined by a spectrochemical method of analysis quite similar to that developed by Steadman (16) for serum sodium. The magnesium and sodium measurements presented here on rabbits undergoing hypothermia were made simultaneously on the same sample. It was found that the intensity of the magnesium line of 2,779.85 Å could be measured together with the sodium line of 2,680.35 Å, and that the cadmium line of 2,677.60 Å would serve as an internal standard for the magnesium as well as the sodium (16). A single determination was made on 0.25 cc. of serum diluted 1 to 40. With this was mixed 0.25 cc. of cadmium chloride solution, 1 to 4,000 cadmium as the internal standard. This was carefully ashed and the residue picked up in 0.25 cc. of 10 per cent HCl and dried in the crater of the spectrographic carbon, which was made the negative electrode of a low voltage DC arc.

The working or calibration curve of the ratio of the magnesium line intensity in the source to the cadmium line intensity against the quantity of magnesium introduced into the arc in the presence of the other serum minerals is shown in Fig. 1. The calibration curve is not appreciably affected by changes in the amounts of the other minerals, principally sodium, that occur in the present experiments. It may be observed that the curve does not pass through the origin of coordinates. This is because of the magnesium contamination of the carbons. The magnesium contamination is fairly constant in magnitude,

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However, for each lot of spectrographic carbons, and in practice pieces of carbon from the same stick were used for the measurements on each rabbit. Usually 6 determinations were made and averaged for one measurement. These may be done in about 1 hour. The average gives serum magnesium and sodium values with about ±3 per cent standard error. Somewhat different spectrochemical methods for the determination of magnesium in biological fluids have been described by Duffendack and his associates (6) and by Cassen (4).

RESULTS

Table I presents the serum magnesium and sodium values in 12 rabbits whose body temperatures were reduced to various low levels (22–13°C). "Initial serum magnesium" represents the value obtained immediately prior to the production of the hypothermic state. "Final rectal temperature" represents the deep rectal temperature level at which the sample of blood was taken to determine the magnesium during hypothermia, and "Duration of hypothermia" is the interval from beginning of the reduction of the animal's temperature to the time that the blood sample was taken for the magnesium and sodium determinations during hypothermia.

It is noted that in every case there is a definite increase in the serum magnesium. The values vary from 11 to 38 per cent above the initial level with an average of 24 per cent rise due to hypothermia. There is no apparent relationship between the magnitude of the increased magnesium concentration and either the rectal temperature or the duration of hypothermia, nor presence or absence of the tumor in these experiments.

The serum specific gravity values for rabbits 9 and 10 were 1.0287 before and 1.0285 after for No. 9, and 1.0312 before and 1.0325 after hypothermia for No. 10. These values are in the upper range of normal and not in the dehydration level. Since there is no significant change in the specific gravities in these 2 rabbits, the increase in magnesium concentration apparently is not due to loss of serum water.

The serum sodium changes, on the other hand, show considerable variability and do not correspond to the changes in magnesium. However, it may be noted that within the experimental error and with the exception of No. 1 and No. 8, all sodium values under the influence of hypothermia tend to approach the normal level for human serum, 330 mgm. per 100 cc. In No. 9 the serum specific gravity remained unchanged at the level of normal hydration yet the serum sodium value rose 12 per cent from a rather low normal level, while in the other animal, No. 10, which was slightly less well hydrated, the sodium values rose somewhat less (7 per cent). These two observations, and the spread in change of sodium serum values from −20 per cent to +19 per cent of the original value, suggest that the electrolyte-water balance in the hypothermic state warrants further study.
SUomalainen (17–19) noted that during the normal hibernation of hedgehogs there is a decrease of blood sugar and adrenalin, and that the liver glycogen remains constant. The injection of adrenalin into hibernating hedgehogs did not affect the magnesium, but doubled the blood sugar. Injection of magnesium caused the hedgehogs to develop a pronounced decrease in body temperature that was counteracted when calcium was injected.

Taylor and Winter (20) also observed that when magnesium was injected into patients with fever there was a drop of 1° F. in temperature with each 2 mgm. per 100 cc. increase in serum magnesium.

Hibernation may be induced in normally hibernating animals by administering magnesium and insulin (17). Hibernation in hedgehogs may be induced also by injecting insulin and placing them in a cold chamber. In the insulin hibernation, as in natural hibernation, serum magnesium increases from 40 to 60 per cent, while serum calcium shows a very slight decrease (11).

Suomalainen (17–19) has further demonstrated an increase in serum magnesium in normally sleeping hedgehogs and human subjects. Although it is apparent that magnesium is intimately linked with the phenomenon of normal hibernation, the exact mechanism of the shift of magnesium from its usual stores, mainly bone and muscle although it occurs in all the glands and cells of the body (8, 15), into the circulation, and the role that it plays in hibernation are not known.

It has been observed in this laboratory that during hypothermia rabbits become lethargic and approach a phase simulating hibernation, although rabbits do not ordinarily hibernate. Furthermore, notable hypoglycemia (blood sugar of 30 to 40 mgm. per 100 cc.) has been observed in 4 of these rabbits, associated with the increase in serum magnesium. Apparently the lethargic pseudohibernation of true homeothermic animals during hypothermia is intimately linked with increase in serum magnesium and decrease in blood sugar (3, 5, 7, 12).

In another paper of this series (1) it is shown that hypothermia first produces a decided increase in thyroid function with considerable increase in basal metabolism, followed by a definite decrease in thyroid function with a decrease in basal metabolism at times to as low as –97 per cent of normal. The increased metabolism (+130 to 185 per cent) when hypothermia is first instituted is associated with signal shivering and often voluntary muscular activity. This violent muscular activity is probably an attempt by the animal to maintain the normal body temperature and must be associated with an active carbohydrate metabolism. It has been demonstrated that magnesium plays an extensive role in carbohydrate metabolism (9, 12, 13). Schmidt and Greenberg (13) believe that it is necessary for the activity of the enzyme phosphatase. Meyerhof (10) has shown that zymohexase is catalyzed by magnesium. Cori and her associates (5) point out that magnesium functions in muscle by accelerating the conversion of glucose-1-phosphate to glucose-6-phosphosphate, the first step in the breakdown of glycogen to lactic acid. Scott and Packer (14) have demonstrated by means of the electron microscope that the magnesium content in muscle is very high, and almost entirely in the cell, with a small part in the tissue spaces and none in the sarcoplasm. Following active muscular stimulation there is a decrease in the muscle magnesium.

It may be that the muscle magnesium is utilized in the breakdown of carbohydrate for the production of heat, and following its utilization is accordingly shifted from the muscle into the circulation.

It is well known that an increased concentration of blood magnesium elicits soporific effects, and that in high concentrations anesthesia is produced. A mild sedative or hypnotic result follows when the serum level reaches 5 mgm. per 100 cc. At 18 to 21 mgm. per 100 cc. profound coma sets in (9, 11). Wolff (22) has found that in rabbits the corpuscles contain far more magnesium than the plasma, and that there is no parallelism between the two. The magnesium in the corpuscles is fixed, does not diffuse, and remains rather constant. The plasma level, on the other hand, undergoes the considerable variation of 2.0 to 4.0 mgm. per 100 cc. (8, 21, 22). The significance of the corpuscular magnesium content is not manifest. The lethargy observed in rabbits following hypothermia, therefore, and the decided drowsiness complained of by human beings subjected to considerable chilling may be due to increased serum magnesium, and possibly is related also to the accompanying hypoglycemia.

One might thus trace the sequence of events following the induction of hypothermia as follows: Its onset is associated with definite thyroid hyperactivity and violent muscular activity in an attempt to maintain body temperature. This is accompanied by a notably increased carbohydrate metabolism resulting in a hypoglycemia. Magnesium, a coenzyme of carbohydrate metabolism, is utilized in the metabolism of carbohydrates and shifted from its muscle and other stores into the circulation. The increased serum magnesium contributes to the lethargic state (pseudohibernation) noted in hypothermia after the shivering stops, usually below 32° C. The quiescent soporific condition of prolonged hypothermia is possibly a result of the increase in serum magnesium and a decrease in blood sugar. The serum magnesium remains at
an elevated level because of possible failure of the kidney (anuria). There is no evidence that any alterations produced by low temperature in the testicular tumors (2 to 5 cc. volume) of 4 rabbits analyzed in this series contributed in any way to the changes noted in either the magnesium or sodium levels. The initial values in tumor-bearing rabbits, however, were all within normal limits for their species.

CONCLUSIONS

1. A spectrochemical method was used to determine serum magnesium and serum sodium levels at the beginning and after 2 to 5 hours of the hypothermic state in 8 normal and 4 tumor-bearing rabbits.

2. The initial serum magnesium level averaged 2.63 mgm. per 100 cc. (2.32 to 2.95 mgm.)

3. The serum magnesium showed a rise of 24 per cent (11 per cent to 38 per cent) above the initial values after the animals had been from 2 to 5 hours in the hypothermic state.

4. There did not seem to be any relationship between serum magnesium levels or their change and the duration of the low temperature (2 to 5 hours) nor its level (13–22° C.), nor to the serum sodium values, nor to the serum specific gravity in 2 animals.

5. From other data reported elsewhere in this series, there did seem to be a relation between the elevation of the serum magnesium and the depressed thyroid activity, hypoglycemia, low oxygen consumption, and the lethargic state during hypothermia. This relationship is not entirely clear and warrants further study.

6. The cessation of kidney function may have prevented the excretion of magnesium, or the cold state may have prevented its return to its storage sites.

7. There is no clear explanation of the wide fluctuations in the serum sodium (−20 per cent to +19 per cent) found in the hypothermic states in these 12 rabbits.

REFERENCES


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