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# THE INFLUENCE OF MAGNESIUM ON RESPIRATION, GLYCOLYSIS AND CHOLINESTERASE ACTIVITY IN RAT BRAIN*

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The thermoregulatory behaviour of the higher vertebrates is capable of achieving a fair balance between heat production and heat loss over a rather wide range of environmental temperature. The chief locus of this behaviour is the temperature regulating centre of the hypothalamus (Ranson, 1940). There appears to be a normal set level or range for this centre because under usual conditions the body temperature of homoiotherms lies between 37 and 40° C. (Evans & Starling, 1945). Some temperature-sensitive process or processes within the neurones of the centre must be so organized as to yield this set level. The nature of this process is not known. If it be assumed that some feature of the local cellular metabolism of the centre is a factor determining the level, some information concerning the regulatory process might be obtained by the use of agents which influence both body temperature and cell metabolism.

Magnesium appears to be such an agent. The antipyretic action of magnesium salts has been known for many years (Schütz, 1916; Barbour & Winter, 1928; Winter & Barbour, 1928; Taylor & Winter, 1929; Steadman, Ariel & Warren, 1943; Sunderman & Haymaker, 1947; Sollman, 1948). High serum-magnesium levels are found in hibernating animals (Lustig, Ernst & Reuss, 1937; Suomaleinen, 1938, 1939), in which body temperature is reduced and thermoregulation is impaired but not abolished (Benedict & Lee, 1938; Suomaleinen, 1939). Recently Heagy & Burton (1947, 1948) reported that administration to dogs of subnarcotic doses of magnesium chloride caused lowering of the rectal temperature whether the animal was in an environment that was warm, cool or at normal room temperature. These observations, together with unpublished findings in this laboratory, are in harmony with the working hypothesis that the antipyretic action of magnesium involves a lowering of the set level of the thermoregulatory centre.

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Magnesium is known to play a part in several intracellular processes. For example, it is an important factor in a number of the sequential enzymatic reactions of glycolysis (Cori, 1942; Sumner & Somers, 1943; Barron, 1943) which are common to many types of cells including those of mammalian brain (Banga, Ochoa & Peters, 1939; Ochoa, 1940, 1941; Macfarlane & Weil-Malherbe, 1941; Gerard, 1946). Moreover, magnesium is known to activate cholinesterase (Bodansky, 1946) and to enhance the ability of the metabolic processes to increase the $L$ fraction of the membrane potential of the nerve fibre (Lorente de Nó, 1947). In view of the nature of the antipyretic action of magnesium (Heagy & Burton, 1948) and of the significance of this substance in cell metabolism, it appeared desirable to examine the effects of graded concentrations of magnesium on the metabolism of the thermoregulatory centre. Technical difficulties precluded this direct approach. However, because of the qualitative similarity of the metabolic pattern in the several parts of the central nervous system (Page, 1937), quantitative differences appearing in one region might well be paralleled by changes in a similar direction in other regions. The positive correlation between fall in rectal temperature and apparent general depression of the central nervous system reported by Heagy & Burton (1948) may be cited as evidence favouring this view. Accordingly, the influence of magnesium on respiration and anaerobic glycolysis in rat cerebral cortex slices and on cholinesterase activity in whole rat brain homogenates has been investigated.

## METHODS

Adult albino rats of the Slonaker-Wistar strain were used. The brain was rapidly removed after decapitation and cerebral cortex slices were prepared by the cold moist box technique which has been described previously (Field, 1948; Peiss & Field, 1948). By this procedure the tissue is kept in a cold moist environment from the time of excision until the respirometer flasks, suitably loaded, are placed in a constant temperature bath. Thus in studies on oxygen consumption imbalance between the anaerobic and aerobic phases of metabolism is minimized during the period of tissue manipulation (Fuhrman & Field, 1943, 1945) and in preparation for anaerobic work the overall metabolism is kept at a low level until suitable conditions are provided for the supply of nutrients and the removal of metabolic end-products. Control experiments showed that under the conditions of these experiments the oxygen consumed or the carbon dioxide produced was proportional to the *initial* wet weight of tissue over the range 10–90 mg. Most samples weighed from 40 to 50 mg. Slice thickness was 0·4–0·5 mm. (Field, 1948). Aliquot samples were placed in small weighing bottles and dried to constant weight in an electric oven at 105° C. Manometric measurements were made in a constant temperature bath at 37·5±0·01° C. after 15 min. of thermoequilibration.

Respiration was measured by the Warburg manometric method (Dixon, 1943; Umbreit, Burris & Stauffer, 1945; Field, 1948). The respirometers were flushed with oxygen before being placed in the constant temperature bath. Thus the gas phase was oxygen. The centre wells contained 5% KOH and were fitted with Whatman No. 40 filter paper wicks. The liquid phase was Ringer-phosphate solution containing 0·011 M-glucose (Krebs, 1933). The sidearms contained graded amounts of magnesium chloride made up in the same solution. The contents of the sidearms were added to the main compartments of the flasks after a 30 min. control period, so that control and 'magnesium added' runs were made with the contents of each flask.

Anaerobic glycolysis was measured by the manometric method (Dixon, 1943; Umbreit *et al.* 1945). The gas phase was 95% $N_2$–5% $CO_2$ which had been passed through a hot copper tube to remove traces of oxygen (Savage & Ordal, 1940). Uniform gassing was effected by passing this mixture through the respirometers in series. After leaving the last respirometer the gas was passed through a Wolff bottle so that the rate of flow could be observed. Care was taken to make sure that a good steady flow of gas was maintained for 10 min. after the respirometers had been placed in the constant temperature bath. The liquid phase was Ringer-bicarbonate solution (Krebs & Henseleit, 1932). This medium had previously been gassed for over an hour with the same oxygen-free mixture used as the gas phase in the respirometers. The medium contained glucose in a final concentration of 0·011 M and magnesium chloride in concentrations ranging up to $43{\cdot}7 \times 10^{-3}$ M. Thus there was no 'pre-addition' control period as in the case of oxygen-consumption measurements. This procedure was followed to avoid the disturbance in the rate of $CO_2$ evolution produced by the handling necessary to make additions from the sidearms of the vessel.

Cholinesterase activity was measured manometrically (Nachmansohn & Feld, 1947; DuBois & Mangun, 1947). The enzyme extract used was the 'Supernatant fraction' of Nachmansohn & Feld (1947). This was prepared from whole rat brain homogenized in ice-cold calcium-free Ringer-bicarbonate solution in a Waring blendor. The main compartments of the respirometer flasks contained the enzyme preparation in the calcium-free Ringer-bicarbonate solution together with the desired amounts of magnesium chloride. The sidearms contained acetylcholinechloride (Merck) made up in the same solution. The contents of the sidearms were added to the main compartments at the end of thermoequilibration. The final concentration of acetylcholine was 0·015 M. The gassing procedure was the same as in the experiments on anaerobic glycolysis.

Respiration and glycolysis data are expressed in the conventional '$Q$' notation. Thus $Q_{O_2}$ and $Q_A^{N_2}$ denote respectively microlitres of oxygen consumed and microlitres of acid produced, measured as gas under standard conditions per mg. *initial* dry weight of tissue per hour (cf. Burk, Sprince, Spangler, Kabat & Furth, 1941). The superscript '$N_2$' denotes the gas mixture 95% $N_2$ + 5% $CO_2$. In all cases $Q_{O_2}$ was constant for several hours and $Q_A^{N_2}$ for 40–60 min. The values of oxygen consumption and glycolysis presented in the figures and tables were calculated from measurements made during steady state periods. Cholinesterase activity is expressed in terms of mg. of acetylcholine hydrolysed in 1 hr. by 1·0 ml. of supernatant fraction from a homogenate containing 100 mg. of fresh tissue per ml. Readings were taken at 5 min. intervals for a period of 40 min.

## RESULTS

*Respiration.* It is shown in Fig. 1 that the respiration of rat cerebral cortex slices was not affected by magnesium-ion concentration up to $8{\cdot}75 \times 10^{-3}$ M. As the magnesium-ion concentration was increased above this level there was a moderate inhibition of respiration, amounting to about 20% at the highest concentration used ($29{\cdot}2 \times 10^{-3}$ M). Thus, under the conditions of these experiments, the concentration of magnesium ion in the liquid phase of the respirometer flasks was not a factor modifying the oxygen consumption of cerebral cortex slices until a concentration well above the physiological range in serum was used (cf. Suomaleinen, 1939).

*Anaerobic glycolysis.* Fig. 2 illustrates the effects of graded concentrations of magnesium ion on anaerobic glycolysis in rat cerebral cortex slices. It is evident from inspection of Fig. 2 that anaerobic glycolysis is decidedly influenced by the level of magnesium-ion concentration in the suspension medium. As this level was varied over the range 0 to $12{\cdot}5 \times 10^{-3}$ M there was a progressive rise

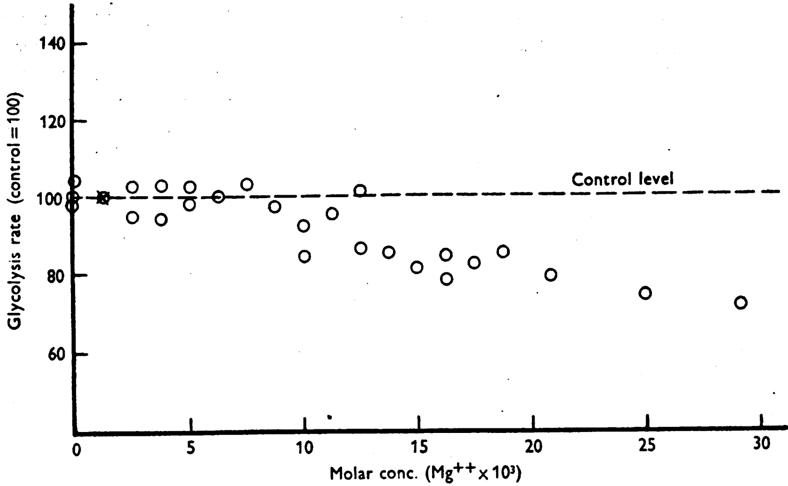


Fig. 1. Effect of graded concentrations of magnesium on the oxygen consumption of rat cerebral cortex slices. Suitable amounts of magnesium chloride, made up in 0·2 ml. Krebs Ringer solution, were added from the sidearms of the respirometer flasks after a 30 min. control period. Main compartments of the flasks contained 1·8 ml. of Krebs Ringer solution without magnesium. Oxygen consumption was a rectilinear function of time both before and after additon of magnesium. Further details are given in Tables 1 and 2.

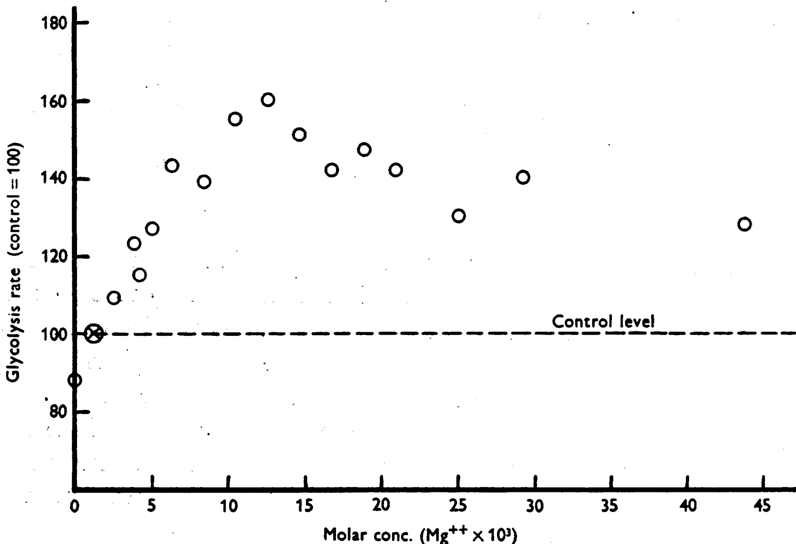


Fig. 2. Effect of graded concentrations of magnesium on anaerobic glycolysis in rat cerebral cortex slices. Main compartment of the respirometer flasks contained 2·0 ml. of Krebs Ringer-bicarbonate solution, with varied concentrations of magnesium chloride. No additions were made from sidearms since such additions disturb the rate of glycolysis for a time. Rate of glycolysis was fairly constant for the first 40 min. All calculations are based on readings taken during this 'steady state' period. Further details are given in Table 2.

in the rate of glycolysis to a maximum 60% above the rate of the control (containing $1\cdot19\times10^{-3}$ M-magnesium). Further increase in magnesium concentration led to a decrease in glycolysis from this maximum, but at the highest concentration tested ($43\cdot7\times10^{-3}$ M) the glycolytic rate was still approximately 30% above the control level.

When the data on which Fig. 2 is based were treated statistically it was found that the variations in $Q_A^{N_2}$ due to concentration of magnesium are highly significant (Snedecor, 1946; analysis of variants). Similar treatment revealed that the interaction of differences in readings with time were not significant, i.e. that steady states were obtained.

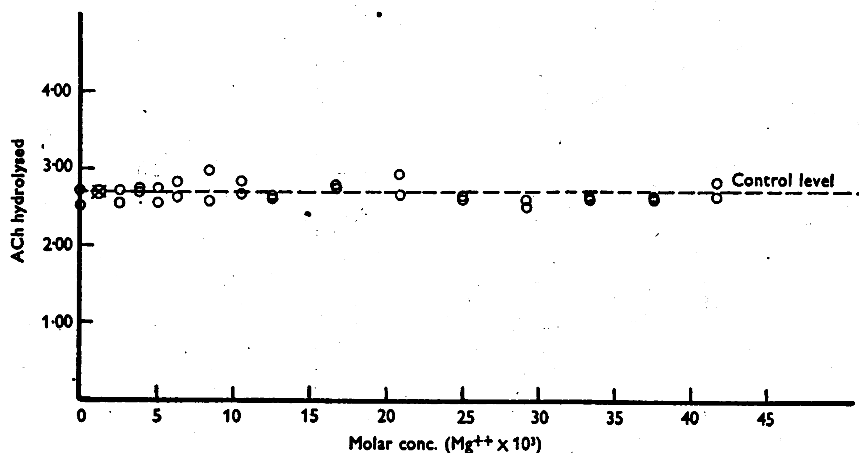


Fig. 3. Effect of graded concentrations of magnesium on the hydrolysis of acetylcholine by the supernatant fraction of whole rat brain homogenates. Main compartment of the respirometer flasks contained 0·2 ml. of the supernatant fraction of the homogenate and 1·15 ml. of calcium-free Krebs Ringer-bicarbonate solution, made up to contain the desired amounts of magnesium chloride. Sidearms contained 0·15 ml. of acetylcholine chloride (Merck) in a final concentration of 0·015 M. After thermoequilibration the contents of the sidearms were added to the main compartments. Crossed circle represents the control level ($1\cdot19\times10^{-3}$ M-magnesium). Open circles denote the means of duplicate determinations at the indicated concentrations of magnesium.

*Cholinesterase activity.* Under the conditions of these experiments the cholinesterase activity of the supernatant fraction of whole rat brain homogenate (Nachmansohn & Feld, 1947) was not affected by the concentration of magnesium in the suspension medium over the range 0 to $41\cdot7\times10^{-3}$ M. This is shown in Fig. 3.

*Collected data.* The data used in the construction of Figs. 1–3, together with the absolute values of the means of the control observations and additional statistical information, are presented in Tables 1 and 2. By means of these figures it is possible to convert any of the percentage values of respiration,

glycolysis or cholinesterase activity to the corresponding absolute rates, thus facilitating comparison with other observations.

TABLE 1. Summary of data showing the effect of graded concentrations of magnesium on the rate of anaerobic glycolysis, the rate of oxygen consumption and the rate of acetylcholine hydrolysis in rat brain

(Control level is at a magnesium concentration of $1{\cdot}19 \times 10^{-3}$ M. Figures in parentheses represent the number of determinations. All other values are means of duplicate determinations.)

| Molar conc. ($Mg^{++} \times 10^{-3}$) | $Q_A^{N_2}$ as % of control | $Q_{O_2}$ as % of control | ACh hydrolysis as % of control |
|---|---|---|---|
| 0·00 | 88 (6) | 100, 98, 104 | 97·5 |
| 1·19 | 100 (30) | 100 (62) | 100·0 (12) |
| 2·50 | 109 (3) | 94, 102 | 98·0 |
| 3·75 | 123 (3) | 94, 103 | 100·5 |
| 4·17 | 115 (3) | — | — |
| 5·00 | 127 (3) | 98, 102 | 98·5 |
| 6·25 | 143 (6) | 100 | 101·5 |
| 7·50 | — | 103 | — |
| 8·33 | 139 (6) | — | 103·5 |
| 8·75 | — | 97 | — |
| 10·00 | — | 84, 92 | — |
| 10·42 | 155 (6) | — | 102·0 |
| 11·25 | — | 95 | — |
| 12·50 | 160 (6) | 86, 101 | 97·5 |
| 13·75 | — | 85 | — |
| 14·58 | 151 (3) | — | — |
| 15·00 | — | 81 | — |
| 16·25 | — | 78, 84 | — |
| 16·67 | 142 (3) | — | 103·0 |
| 17·50 | — | 82 | — |
| 18·75 | 147 (6) | 85 | — |
| 20·83 | 142 (3) | 79 | 104·0 |
| 25·00 | 130 (3) | 74 | 97·0 |
| 29·17 | 140 (3) | 71 | 94·5 |
| 33·33 | — | — | 97·5 |
| 37·50 | — | — | 97·5 |
| 41·67 | — | — | 101·5 |
| 43·75 | 128 (3) | — | — |

TABLE 2. Means and other statistics of absolute values of control $Q_{O_2}$, $Q_A^{N_2}$ and cholinesterase activity (ACh activity)

(Units defined in section on methods.)

| | $Q_{O_2}$ | $Q_A^{N_2}$ | ACh activity |
|---|---|---|---|
| No. of animals | 12 | 12 | 6 |
| No. of samples | 62 | 30 | 12 |
| Mean | 10·94 | 7·53 | 2·69 |
| Range | 9·27–12·80 | 5·03–11·05 | 2·52–2·84 |
| Standard deviation | 0·830 | 1·731 | 0·0826 |

## DISCUSSION

Concentration-action curves (Clark, 1933), illustrating the influence of graded concentrations of magnesium in the suspension medium on the oxygen consumption and anaerobic glycolysis of rat cerebral cortex slices and on the cholinesterase activity of the supernatant fraction of whole rat brain homogenate, have not been described previously, as far as the authors know. However, data of this sort are available for rat diaphragm respiration (Stadie &

Zapp, 1947), the respiration of minced pigeon-breast muscle (Elsden, 1939) and for glycolysis in rat brain extracts (Geiger, 1940).

The present results on the respiration of cerebral cortex slices are in agreement with those of Stadie & Zapp (1947) on diaphragm in that concentrations of magnesium above $10 \times 10^{-3}$ M depress oxygen uptake. It is interesting to note that inhibition of the respiration of rat cerebral cortex slices begins at magnesium concentrations corresponding quite closely with serum-magnesium levels producing deep anaesthesia in the rabbit ($7 \cdot 38$ to $8 \cdot 41 \times 10^{-3}$ M; Taylor & Winter, 1929). However, serum-magnesium levels which impair temperature regulation in the dog (*c.* 10 mg. % or $4 \cdot 1 \times 10^{-3}$ M; Heagy & Burton, 1948) lie in the range of concentrations of magnesium in the suspension medium which do not influence rat cerebral cortex slice respiration (Fig. 1). Thus the present observations on the influence of graded concentrations of magnesium on the respiration of cerebral cortex slices do not provide a satisfactory clue as to a possible influence of subnarcotic doses of magnesium on the metabolism of the thermoregulatory centre. The same comment holds for the results of the measurements of cholinesterase activity as a function of the concentration of magnesium in the suspension medium (Fig. 3).

The most interesting lead is provided by the observations on anaerobic glycolysis as a function of the concentration of magnesium in the suspension medium. It is shown in Fig. 2 that magnesium concentrations of 3 to $7 \times 10^{-3}$ M caused a striking increase in glycolysis in cerebral cortex slices. These concentrations correspond to the range of serum magnesium levels which cause a decrease in rectal temperature in the dog, possibly by lowering the set level of the thermoregulatory centre (Heagy & Burton, 1948; Hall *et al.* unpublished observations). Thus if there is a key regulatory process in the cells of the centre which is affected by the concentration of magnesium, this process may be one of the reactions of the anaerobic phase of carbohydrate breakdown. However, no proof is available that this is so. The evidence is suggestive only, and it is quite possible that the increase in anaerobic glycolysis of cerebral cortex slices with increase in the concentration of magnesium ion in the suspension medium is in no way related to the effects of magnesium on temperature regulation.

SUMMARY

1. The oxygen consumption and anaerobic glycolysis of rat cerebral cortex slices and the cholinesterase activity of the supernatant fraction of whole rat brain have been measured at different levels of magnesium concentration in the suspension medium.

2. Magnesium concentrations up to $8 \cdot 75 \times 10^{-3}$ M did not affect the rate of oxygen consumption of cerebral cortex slices suspended in Krebs Ringer-phosphate solution. Higher concentrations, up to $29 \cdot 2 \times 10^{-3}$ M, caused moderate lowering of the rate of oxygen uptake.

3. There was an increase in the rate of anaerobic glycolysis in rat cerebral cortex slices suspended in Krebs-Henseleit Ringer-bicarbonate solution as the magnesium concentration in the medium was raised from 0 to $12{\cdot}5 \times 10^{-3}$ M. The maximum rate, attained when the magnesium concentration was $12{\cdot}5 \times 10^{-3}$ M, was 60% above the rate at the control level of magnesium ($1{\cdot}19 \times 10^{-3}$ M). Higher concentrations lowered the rate of glycolysis from the peak value, but at $43{\cdot}7 \times 10^{-3}$ M glycolysis was still about 30% above the control rate.

4. Concentrations of magnesium over the range of 0 to $41{\cdot}7 \times 10^{-3}$ M did not influence the cholinesterase activity of the supernatant fraction of whole rat brain homogenate made up in calcium-free Ringer-bicarbonate solution.

5. It is suggested that the influence of magnesium on anaerobic glycolysis in central nervous system tissue may provide a clue to the mechanism of the influence of magnesium on temperature regulation in homoiotherms.

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