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TEMPERATURE DEPENDENCE OF MAGNESIUM STIMULATED ADENOSINE TRIPHOSPHATASE ACTIVITY DURING AGING OF THE CENTRAL NERVOUS SYSTEM OF RAT

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INTRODUCTION

TEMPERATURE dependence of Mg^{++} ATPase has been studied in mammalian brain homogenates¹⁻³ and microsomal preparations⁴. Comparisons of these homeotherms with heterothermic animals⁴ and poikilothermic animals⁵ have shown that ATPases in these animals are less sensitive to low temperatures. The properties of the total ATPase enzyme complex in mammalian brain preparations have been studied in detail⁶⁻⁹ but more attention has been concentrated on the Na^+-K^+ ATPases because of their relationships with the oxidative phosphorylation and active transport processes. Mg^{++} ATPase is responsible for the control of passive permeability of excitable cells¹⁰⁻¹¹ and probably of all cells and cellular organelles¹²⁻¹³. Bowler and Duncan¹⁴ have suggested that Mg^{++} ATPase of the excitable cells has a role in the control and maintenance of the excitability of these cells. It is also known that the development of the receptor potential during the excitation of sense organs reflects a change in the passive permeability of the receptor membrane to cations¹⁵.

Studies on the effect of temperature on Mg^{++} ATPase activity in rat brain during postnatal development is very little, and no data is available on the temperature dependence of the enzyme activity in the aging central nervous system. This paper is a study of the temperature dependence of the magnesium stimulated ATPase activity during the aging of the central nervous system.

MATERIAL AND METHODS

Albino rats (Wistar strain) of 1 day, 3, 13, 44 and 87 weeks age used for the study were maintained at $28 \pm 2^\circ C$ on a commercial diet (Rat and mice feed purchased from Hindustan Lever Ltd., Bombay).

The animals were decapitated and different regions of the brain (Cerebrum, Cerebellum, Medulla and Optic lobes) excised immediately and weighed in precooled beakers of 5 ml capacity. Tissues were homogenised in glass homogenisers in ice-cold 0.13 M Tris-buffer (pH 7.4) in 0.25 M sucrose as described by Tirri *et al.*¹ to give a homogenate which contained, depending upon the age of the animal 14 to 20 mg of tissue per ml. This was centrifuged at 6000 r.p.m. for 30 minutes and the supernatant was used for the study of the enzyme activity.

Incubation medium contained 5 mM ATP in Tris-buffer, 5 mM $MgCl_2$ and 0.2 ml of the enzyme extract all in a volume of 1.8 ml. The above were incubated at 10° , 20° , 30° , 40° and $50^\circ C$ for a period of 30 minutes and the reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid. The tubes were cooled immediately in ice-cold water and centrifuged for 10 minutes at 3000 r.p.m. Inorganic phosphate liberated was estimated spectrophotometrically (Beckman DU-2) by the method of Fiske and Subba Row as described by Leloir and Cardini¹⁶. Protein content in the enzyme extract was estimated by Biuret method¹⁷.

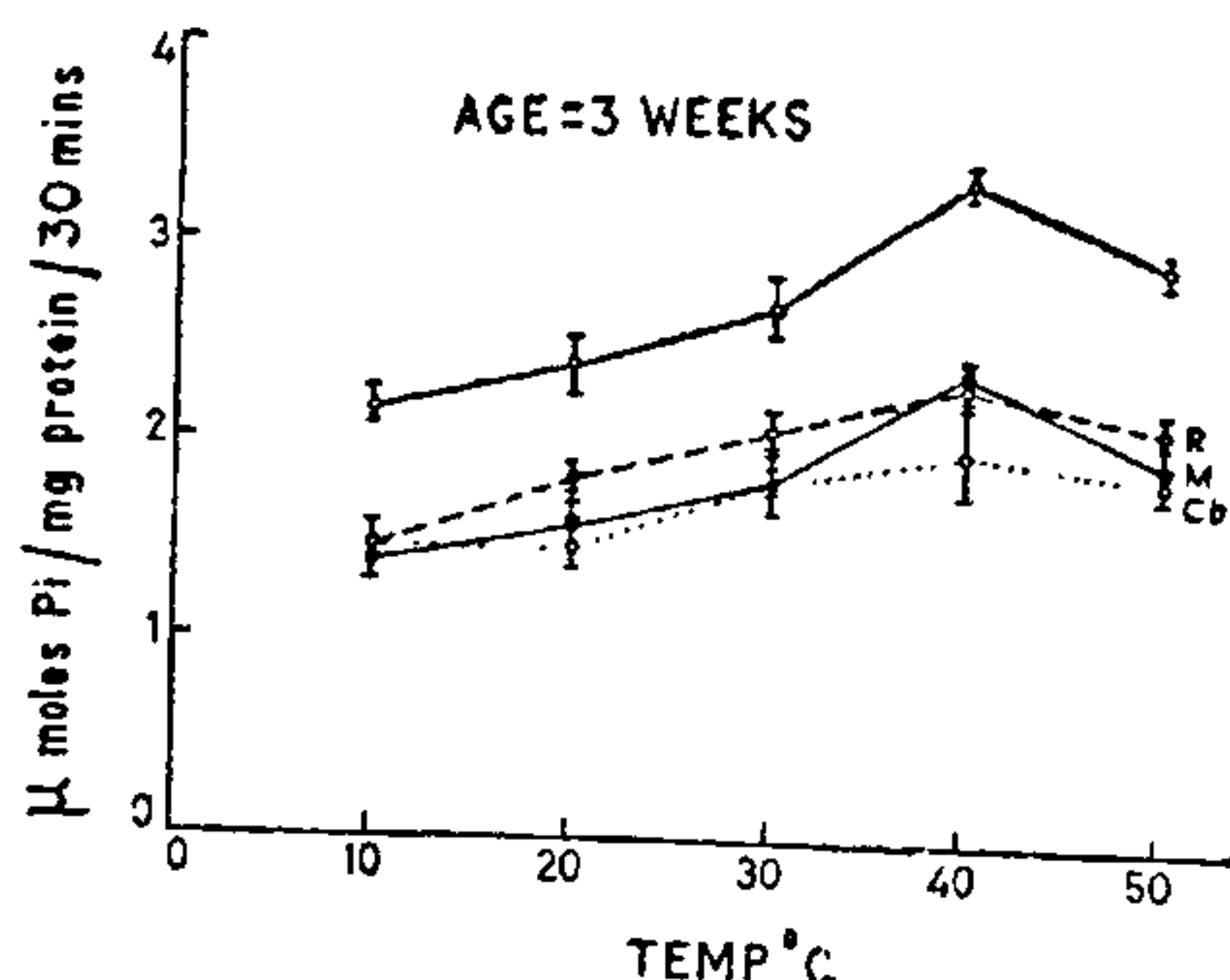
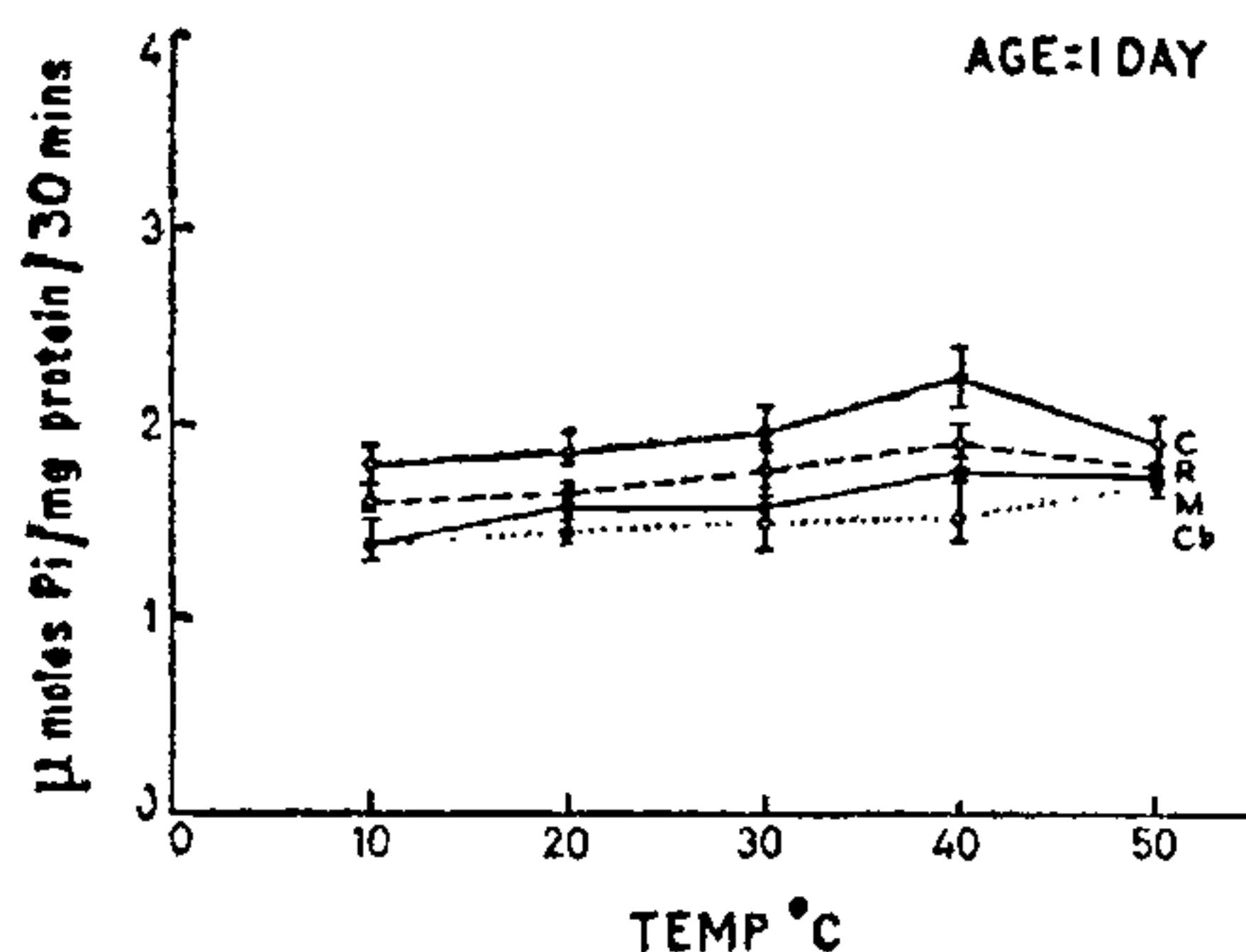
Energy of activation was calculated from the Arrhenius plots as described by Giese¹⁸.

RESULTS

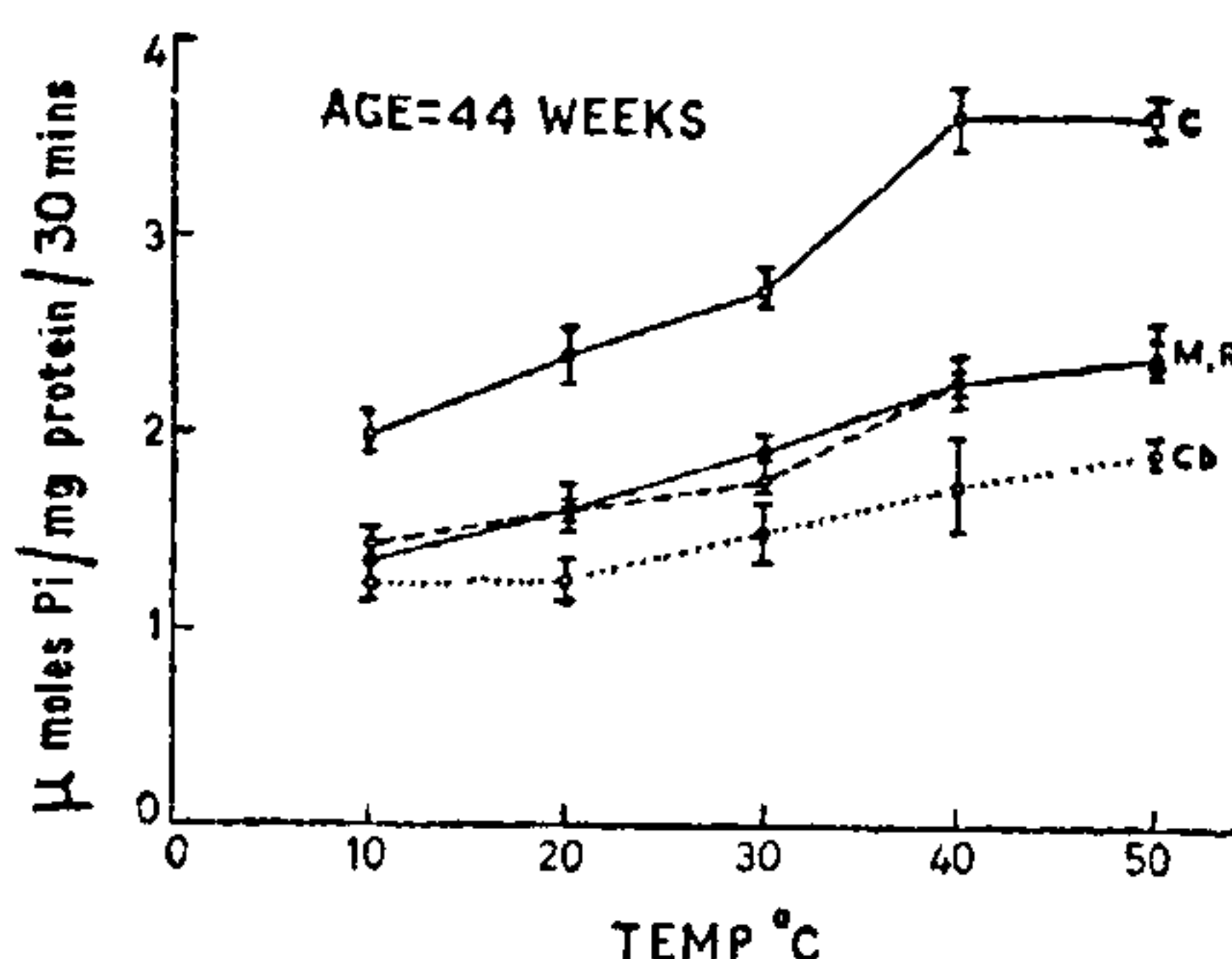
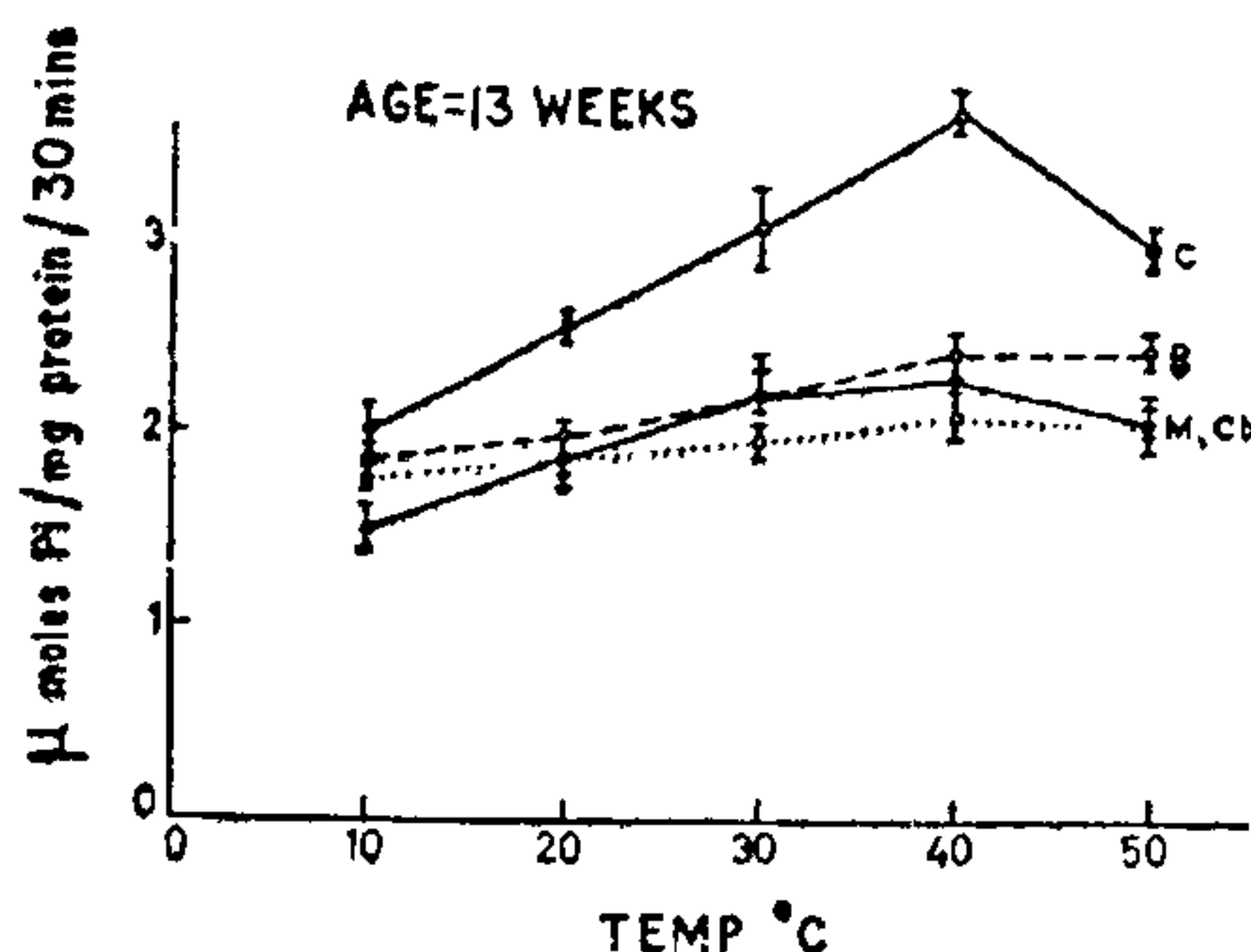
An incubation period of 30 minutes was used for all extracts, regardless of their activity. This period was chosen on the basis of our earlier studies³. Figures 1-5 show the activity-temperature relationships of Mg^{++} ATPase in 1 day, 3, 13, 44 and 87 weeks old rats respectively. It is seen that Mg^{++} ATPase is temperature insensitive in cerebellum, medulla and optic lobes of 1 day old animals

in the temperature range of 10–50° C, whereas the enzyme from the cerebrum shows a slight increase in the temperature sensitivity in the temperature

range of 30–40° C. The insensitivity in the enzyme activity is noticed in the temperature range of 30–50° C in all the regions of the brain studied



FIGS. 1–2. Fig. 1. Activity-temperature curves for Mg^{++} ATPase in the CNS of 1 day old rat. Fig. 2. Activity-temperature curves for Mg^{++} ATPase in the CNS of 3 weeks old rat.



FIGS. 3–4. Fig. 3. Activity-temperature curves for Mg^{++} ATPase in the CNS of 13 weeks old rat. Fig. 4. Activity-temperature curves for Mg^{++} ATPase in the CNS of 44 weeks old rat.

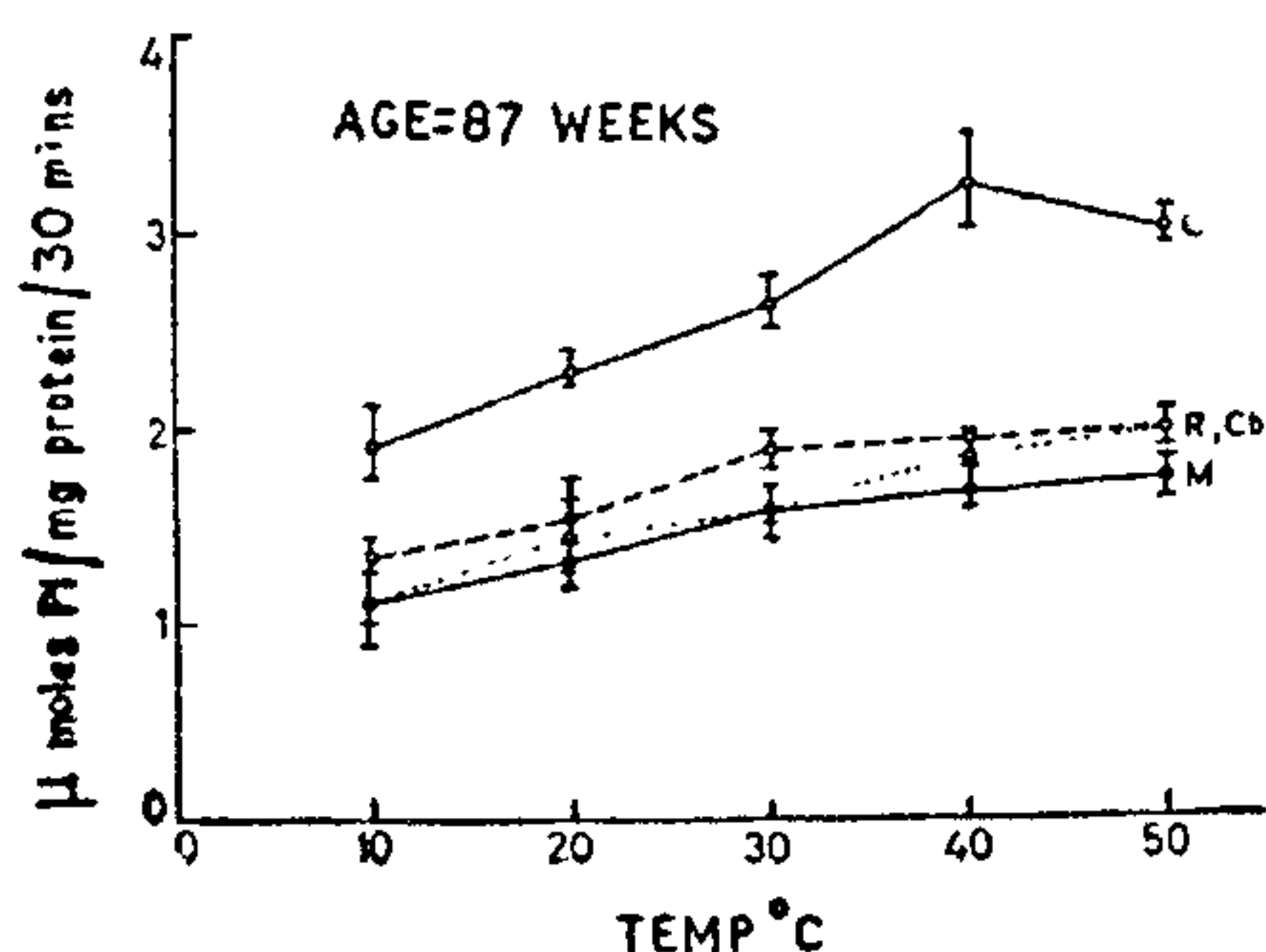


FIG. 5. Activity-temperature curves for Mg^{++} ATPase in the CNS of 44 weeks old animal. c=Cerebrum; Cb=Cerebellum; M=Medulla; R=Optic lobes.

except in the cerebrum of 87 weeks old rats. Mg^{++} ATPase of the cerebrum remains temperature sensitive at all the ages studied, the sensitivity being maximum at the age of 13 weeks.

Table I gives the energies of activation of Mg^{++} ATPase in different regions of the brain. Medulla and cerebrum show only one peak at the age of 3 weeks, whereas in cerebellum and optic lobes two peaks appear in the energy of activation, one at the age of 3 weeks and the other at 44 weeks' age, the former peak being higher in both these regions. However, lowest energy of activation in all the regions studied was found in 1 day old animals.

DISCUSSION

It is clear from the results that in 1 day old animals the enzyme shows temperature insensitivity

TABLE I
Energies of activation in the CNS of rats

Age of the animal	Energy of activation Cals/mol			
	Cerebrum	Cerebellum	Medulla oblongata	Optic lobes
1 day	676.3	514.012	1142.515	914.012
3 weeks	3884.5	3327.5	3884.5	2742.0
13 weeks	2056.5	1599.5	2513.5	1371.0
44 weeks	2513.5	2513.5	2513.5	2285.0
87 weeks	1828.0	1828.0	2056.5	1828.0

in the temperature range of 10–50° C in all the regions studied except in the cerebrum where the sensitivity increases in the temperature range of 30–40° C. The temperature sensitivity of the enzyme becomes well defined in 3 weeks old animals in the temperature range of 10–40° C with an activity maxima at 40° C. The temperature insensitivity shown by 1 day old animals in the temperature range of 10–50° C is obviously of importance in the functioning of the central nervous systems of young rats which develop effective thermoregulation only at the age of 2–3 weeks.

In 1 day, 3 weeks and 13 weeks old animals the enzyme shows a steady increase in specific activity with a rise in temperature with a maxima at 40° C in all the four regions studied. Above 40° C, activity either decreases or remains unchanged, whereas in 44 and 87 weeks old animals the peak is shifted in the temperature range of 40–50° C in all the regions. Here the existence of different maxima in the apparent activity of enzyme at different stages of life may be explained by conformational changes in the enzyme, or probably it may be an enzymatically based adaptation of the central nervous system during the aging process.

Studies on the temperature dependence of ATPase (Magnesium stimulated) in the whole brain homogenates of the developing rats by Tirri *et al.*¹ have shown that the maximum activity occurs at 37–41° C and also a discontinuity between 15–20° C. They have also shown a decrease in the temperature sensitivity between 20–30° C in the animals of 1 to 11 days age. The maximum enzyme activity at 40° C during development and the temperature insensitivity in 1 day old animals, shown by Tirri *et al.*¹, is in conformity with the present results. However, no discontinuity in the enzyme activity as shown by Tirri *et al.*¹ was observed in the present study at any stage of life.

The temperature independence of the enzyme activity in the range of 30–50° C in all the regions of the brain except the cerebrum of 87 weeks old animals is of importance to the aging animals as they tend to lose the capacity of effective thermoregulation.

Studies on the Na^+-K^+ ATPases which are of importance in the oxidative phosphorylation and active transport, which would throw light on the temperature dependence of the energy transferring processes in the central nervous system of the developing and aging animals, are in progress.

ACKNOWLEDGEMENTS

We are extremely grateful to Dr. A. R. Kasturi Bai, Head of the Zoology Department, Bangalore University, for her encouragement and interest in the work. We are deeply indebted to Late Prof. K. Pampapathi Rao for suggesting the problem. Financial support from the Department of Atomic Energy, Government of India, is gratefully acknowledged.

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