

side monophosphates (AMP and GMP). Consequently, for form B the activity measured with UMP as phosphate donor (V_{ump}) was little different to that measured with AMP (V_{amp}). Therefore, the ratio V_{ump}/V_{amp} was in this case very low (1.35), whereas it reached a much higher value for form A (65.9).

It appears however that form A represents a very active and aggregate state of nucleoside phosphotransferase, whereas the form B corresponds to a disaggregate and much less

active state. Therefore the value of the ratio V_{ump}/V_{amp} may provide information on the state of aggregation of the enzyme. Thus low values of this ratio seem to indicate a clear preponderance of the disaggregate form B.

- 1 G. Tesoriere, G. Calvaruso and R. Vento, *Experientia* 33, 997 (1977).
- 2 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. biol. Chem.* 193, 265 (1951).

Age-dependent response of lactate dehydrogenase of pituitary of rat to testosterone

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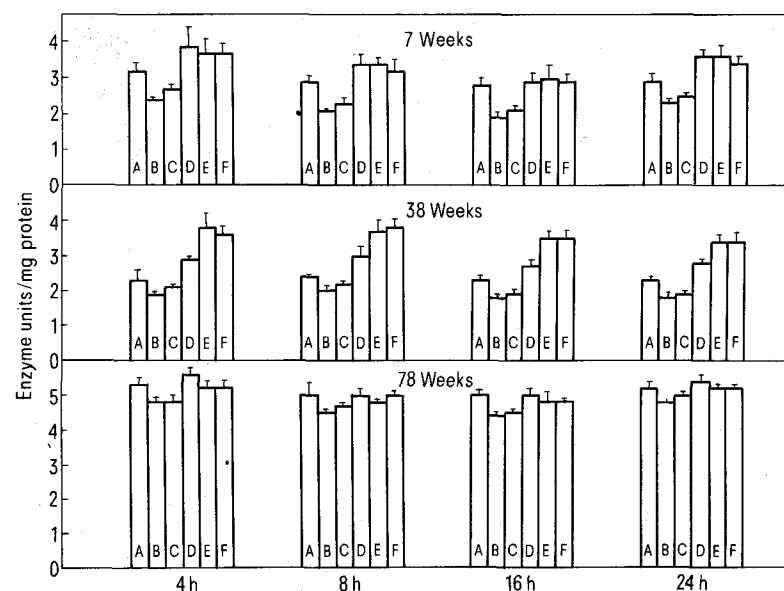
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Summary. The effects of time and various doses of testosterone on the responsiveness of lactate dehydrogenase of pituitary of 7-, 38- and 78-week-old rats were studied. The activity of the enzyme increases in 78-week-old rats. Castration decreases the enzyme activity at all ages. Maximum increase in the enzyme activity is seen with 50 and 100 μ g of testosterone 4 h after administration of hormone to castrated rats. No further time and dose-dependent effect is observed. The magnitude of increase for the enzyme is higher at the age of 38 weeks and decreases in 78-week-old rats.

Alterations in qualitative and quantitative nature of enzymes may be one of the factors contributing to the process of senescence²⁻⁵. The changes in the levels of enzymes can be modulated by the factors like hormones. Age-dependent modulation of certain enzymes of the rat have been reported⁶⁻⁸. The ability to stimulate an adaptive change in the activity of a large number of enzymes is dependent on dose of stimulant, duration of treatment, tissue and age of the animal⁹. Everitt¹⁰ proposed that the process of aging is regulated by hypothalamic-pituitary-peripheral endocrine axis. We report here the time, dose as well as age-dependent changes in the responsiveness of the enzyme, lactate dehydrogenase (LDH; E.C. 1.1.1.27) of pituitary to testosterone. This enzyme catalyses the reversible reaction of pyruvate and lactate of glycolytic pathway.

Materials and methods. Wistar strain albino rats were maintained under standard laboratory conditions; 7-, 38- and 78-week-old male rats were used. The rats of each age were divided into 6 sets each with 30-36 rats. 1st set served as normal rats. The remaining 5 sets of each age were

castrated bilaterally under ether anaesthesia. They were kept for 21 days. On the 22nd day, the rats of 2nd set were administered with vehicle solution. This group served as control to the hormone-treated rats. The rats of 3, 4, 5 and 6 sets were given i.p. with 10, 50, 100 and 200 μ g testosterone/100 g b.wt respectively at 16.00 h. The hormone was dissolved in 10% ethanol-normal saline solution. The rats of each set were killed by cervical dislocation at 4, 8, 16 and 24 h after administration of the hormone; 5-6 rats were sacrificed from each set at the time mentioned above. The pituitary was taken out and 2% homogenate was prepared with 0.1 M phosphate buffer, pH 7.4 in a cold room. The homogenate was centrifuged for 30 min at $14,000 \times g$ at 0°C . The supernatant was used for spectrophotometric assay of LDH¹¹. The enzyme activity was expressed as units/mg protein (specific activity) after determining the protein content in the supernatant¹². The assay mixtures of normal and castrated rats were incubated with testosterone (10^{-7} M, 10^{-6} M, 10^{-5} M) to find out whether the effects of this hormone administration on



Time, dose and age-dependent response of LDH of pituitary to testosterone. A normal rats; B castrated rats (C); C C+testosterone 10 μ g; D C+testosterone 50 μ g; E C+testosterone 100 μ g; F C+testosterone 200 μ g. Bars indicate SD.

enzyme were due to direct action of the hormone on the enzyme or not.

Results and discussion. The activity of LDH is lower in the pituitary of 38-week-old rat than in the 7-week, and increases in 78-week-old rats (figure). Such type of increase in the LDH activity has also been found in the kidney, liver and lung of male mouse^{13,14}. Castration decreases the enzyme activity of pituitary at all ages, thereby indicating that androgens may be one of the factors that regulate the enzyme in this gland. Testosterone increases the enzyme activity in the pituitary of castrated rats of all 3 ages. Our studies on time-dependent response of LDH of pituitary to the hormone reveals that maximum response is 4 h after administration of hormone. The hormone treatment for 8, 16 and 24 h has no significant effect as compared to that of 4 h. Studies of Stifel et al.¹⁵ on dose and time response of rat jejunal glycolytic enzymes, phosphofructokinase, pyruvate kinase and fructose diphosphatase, to oral sex hormones indicate that changes in the enzyme activities occur from 4 h after following intubation with hormone, and reach a maximum at 16 h. In our studies, however, the maximum response is seen 4 h after administration of hormone. The discrepancy between our and Stifel et al.¹⁵ findings may be due to difference in the mode of administration of hormone. Also, pituitary may be one of the sensitive tissues to sex hormones. The maximum effects on the activity of LDH are observed with 50 and 100 µg of testosterone, and thereafter no significant dose-dependent effect is observed. The finding is in agreement with that of Stifel et al.¹⁵, who have observed that, in rat jejunum, the activities of phosphofructokinase, pyruvate kinase and fructose diphosphatase show significant adaptive changes with various doses of testosterone ranging from 1 to 100 µg. The maximum effect was observed with 50 µg of testosterone. As far as age is concerned, the magnitude of increase in enzyme activity is higher in 38- and 7-week-old rats. However, the 100 µg testosterone causes about 2fold increase in the enzyme activity than that of 50 µg in castrated 38-week-old rats. But in 7-week-old rats, both doses of the hormone have almost the same effect. This shows that the response of the enzyme to hormone increases in 38-week-old rats. Such type of higher induction of enzyme has also been reported for soluble alanine aminotransferase of the liver of rat¹⁶. However, the magnitude of response of LDH

to hormone decreases considerably in 78-week-old rats. Such an age-dependent impairment in the magnitude of induction of cytoplasmic malate dehydrogenase¹⁷, ornithine aminotransferase and glucose 6-phosphatase of the liver⁸, thymidine kinase and deoxythymidylate synthetase of salivary gland¹⁸ and pyruvate kinase of cerebral hemisphere¹⁹ has also been reported in the rat. Our in vitro studies on the effects of various doses of testosterone on LDH do not reveal any significant changes in the activity of enzyme of normal and castrated rats. It is, therefore, not necessary to give data. Thus it is seen that the response of this enzyme in this gland is dose and age-dependent.

- 1 To whom all correspondence should be addressed. The authors are grateful to Professor M.S. Kanungo, Biochemistry Laboratory, Department of Zoology, Banaras Hindu University for the suggestions and encouragement. K.V.G. thanks the PL-480 for a research fellowship.
- 2 P.D. Wilson, *Gerontologia* 19, 79 (1973).
- 3 J.R. Florini, *Exp. Aging Res.* 1, 137 (1975).
- 4 M. Rothstein, *Mech. Age Dev.* 4, 325 (1975).
- 5 M.S. Kanungo, in: *Interdisciplinary Topics in Gerontology*, p. 100. Ed. R.G. Cutler. Karger, Basel 1976.
- 6 R.C. Adelman, *Exp. Geront.* 6, 75 (1971).
- 7 V.K. Moudgil and M.S. Kanungo, *Biochim. biophys. Acta* 329, 211 (1973).
- 8 Y.E. Rahman and L. Periano, *Exp. Geront.* 8, 93 (1973).
- 9 R.C. Adelman, in: *Enzyme Induction*, p. 303. Ed. D.V. Parke. Plenum Press, London and New York 1975.
- 10 A.V. Everitt, *Exp. Geront.* 8, 265 (1973).
- 11 A. Kornberg, in: *Methods in Enzymology*, p. 441. Ed. S.P. Colowick and N.O. Kaplan. Academic Press, New York and London 1955.
- 12 E.W. Sutherland, C.F. Cori, R. Haynes and N.S. Olsen, *J. biol. Chem.* 180, 825 (1949).
- 13 P.D. Wilson and L.M. Franks, *Gerontologia* 17, 16 (1971).
- 14 P.D. Wilson, *Gerontologia* 18, 36 (1972).
- 15 F.B. Stifel, R.H. Herman and N.S. Rosensweig, *Biochim. biophys. Acta* 208, 387 (1970).
- 16 S.K. Patnaik and M.S. Kanungo, *Biochem. biophys. Res. Commun.* 56, 845 (1974).
- 17 M.S. Kanungo and B.S. Gandhi, *Proc. nat. Acad. Sci., USA* 69, 2035 (1972).
- 18 G.S. Roth, K. Karloy, V. Britton and R.C. Adelman, *Exp. Geront.* 9, 1 (1974).
- 19 G.B.N. Chainy and M.S. Kanungo, *J. Neurochem.* 30, 419 (1978).

Stable and transmissible dicentric chromosome with terminal centromeres in ascites cells of mouse sarcoma 180¹

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Summary. The occurrence of a stable and transmissible dicentric chromosome with 2 terminal centromeres has been reported in the ascites form of mouse sarcoma 180 cells which is chromosomally hypotetraploid. The number of such dicentrics is 2 in all endoreduplicated cells. The probable mode of anaphase separation of the dicentric has been discussed.

A great deal of verbiage has been spent on the problem of the stability of dicentric chromosomes in the karyotypes of plants and animals. It has been suggested by most of the pioneer investigators that dicentric chromosomes are usually unstable, since theoretically there are 2 different centromeres on the same chromatid that migrate to opposite poles during cell division instituting a bridge-breakage-fusion cycle². This suggestion is quite consistent with the observed mode and behaviour of artificially induced dicentric chromosomes because in all known cases such dicentrics were found unstable. On the contrary, evidences are now accumulating on the spontaneous occurrence and

successful transmission of dicentric chromosomes in natural populations as well as in different tissue culture lines³⁻⁹. These findings are now casting doubt on the concept of dicentric chromosome instability in natural populations. The present communication is a report on the occurrence of a stable dicentric chromosome with 2 distinct terminal C-positive heterochromatic zones in the ascites cells of mouse sarcoma 180 (MS-180). Chromosomes from the ascitic fluid of MS-180 were prepared after 96 h of transplantation by following colchicine-saline citrate acetic-alcohol-flame drying technique¹⁰. C-banding was performed by slight modification¹¹ of the