ACTA HISTOCHEM. CYTOCHEM. Vol. 13, No. 2, 1980

DEVELOPMENTAL CHANGE AND REGIONAL DIFFERENCE OF THE LACTATE DEHYDROGENASE ISOZYME PATTERN IN RAT BRAIN

KANKATSU YUN, TAKESHI MATSUO, TAKASHI ORIBE, TSUTOMU TOMIOKA AND TAKAYOSHI IKEDA

1st Department of Pathology, Nagasaki University School of Medicine, Nagasaki 852

Received for publication August 16, 1979

The developmental change of LDH isozyme pattern was studied by disc electrophoresis in brains from embryonic or fetal rats to adult ones. Although a predominance of M-subunit was evident during the prenatal period, a gradual increase of H-subunit was confirmed in rat fetal brains. This tendency grew stronger after birth. Moreover, in regard to regional difference in the LDH isozymic pattern of rat brains, the cerebellum showed the highest H/M ratio, followed by the gray matter of parietal lobe and diencephalon.

Postnatal developmental changes of lactate dehydrogenase (LDH: EC 1.1.1.27), malate dehydrogenase and asparate transferase in the nervous tissue of rats have been reviewed by Bonavita (1), and normal topochemical distribution of LDH isozyme in human adult brain was investigated by Gerhardt *et al.* (12). In recent years, the LDH isozyme in peri-implantation embryos of various animals was studied by several workers (2, 3, 9, 10). But, the continuous developmental changes of LDH from embryonic or fetal brains to postnatal adult ones have not been investigated very much.

This approach should be valid for LDH, of which the patterns of relative concentration of isozymes are specific for each tissue at each stage of development (16). Isozymes, which are a refined expression of the enzymatic differentiation of cells, may give some insight into the basic mechanism of cellular differentiation (17). Their analysis contributes to the knowledge of catalytic mechanisms underlying metabolic regulation in the cells. LDH has been one of the most extensively investigated enzymes in this respect.

It is the purpose of this study to evaluate the developmental change and regional differences in the LDH isozyme pattern of rat brain.

MATERIAL AND METHODS

Adult Wistar rats, obtained commercially (Kuroda Laboratories, Kumamoto) weighing about 230 g were used. The rats were given laboratory chow and tap water *ad libitum*. Animals were mated overnight and the day when sperm was found in the vaginal smears was noted as Day 0 of gestation. At least 5 embryos or fetuses each on Day 10, 12, 14, 16, 18 and 20 of gestation were used for determin-

ation of the developmental changes of LDH isozyme distribution. In addition, at least five offsprings were killed by decapitation at 1, 2 weeks, 1, 3 months and 6 months of age. LDH isozyme distributions in rat fetuses were measured as a whole brain, whereas those of offsprings were taken from anatomically defined regions, namely the gray matter of parietal lobe, the diencephalon and the cerebellum. The brain tissue for LDH determination was rinsed in saline and extraneous tissue and blood vessels were dissected free. The tissue was then frozen and stored at −20°C.

Small blocks of brain tissue were homogenized by hand with a glass rod for 1 min in 5 volumes of 0.9% NaCl. The homogenate was centrifuged at 3000 rpm for 10 min, and 0.2 ml clear supernatant mixed with 0.2 ml, 40% sucrose and a drop of 10 mg/l bromphenol blue was used for samples. Disc electrophoresis was carried out in a 6% acrylamide gel. LDH was visualized in the reaction mixtures described by Dietz and Lubrano (8). After fixation, relative intensity of isozymes was measured by Canalco-model E densitometer.

For analyzing the LDH isozymes, in addition to the relative percentage of M_4 and H_4 fraction, the ratio of H/M subunit was calculated. This served as a convenient index of the degree of shift towards the type of pattern associated with anaerobic glycolysis (7, 19).

Zymograms of every group were analyzed statistically by Fisher test.

RESULTS

1) Developmental changes in LDH isozyme pattern in prenatal rat brain (Figs. 1, 2, 3, Table 1)

A gradual increase of H-subunit during the prenatal periods was confirmed in normal embryos and fetuses. Although M_4 activity occupied most of the LDH activity, a marked decrease occurred with advance of gestation days. The H_4 activity was unobservable on day 10, 12 and 14 of gestation, but although a small amount, a gradual increase of H_4 activity was noted on day 16, 18 and 20 of gestation.

2) Developmental changes of LDH isozyme pattern in postnatal rat brain (Figs. 4, 5, 6, Table 2)

The postnatal rat brain was examined in three different regions, namely cerebellum, diencephalon and cerebral gray matter of parietal lobe, which showed H/M ratios higher than 1.0 from an early period. The H/M ratio of cerebellum was consistently higher than those of diencephalon and cerebral gray matter from 1 week after birth to adulthood. While the H/M ratio of diencephalon was higher than that of cerebral gray matter at 1 week of age, they were equal about 2–3 weeks of age and thereafter the diencephalon ratio was consistently lower than the cerebral gray matter ratio. Stochastically, there were no significant differences among the H/M ratios of three regions of rat brain at 1 week of age.

At 2 weeks and 1 month of age, the H/M ratio of the cerebellum was significantly higher than that of diencephalon ($P<0.05$), but the H/M ratio of cerebral gray matter was not significantly correlated with those of diencephalon and cerebellum.

At 3 months of age, the H/M ratio of the cerebellum was significantly higher

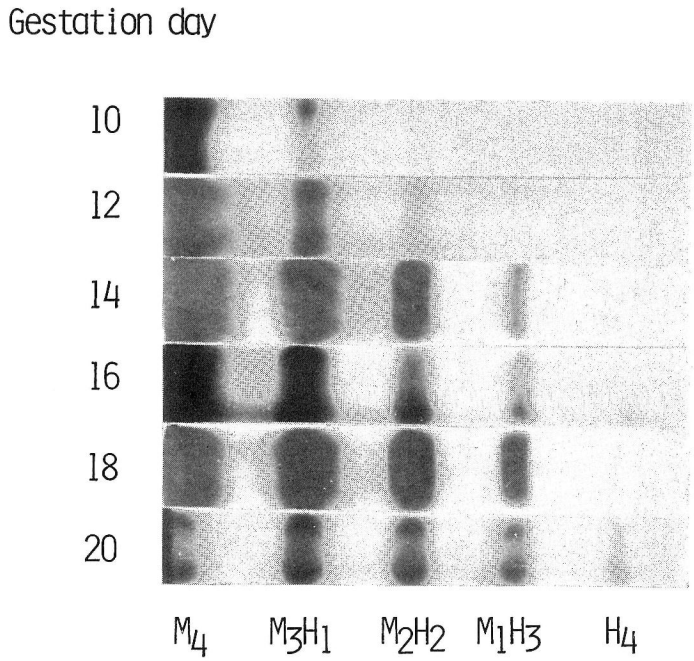

FIG. 1. Developmental change of LDH zymograms in rat fetal whole brains.

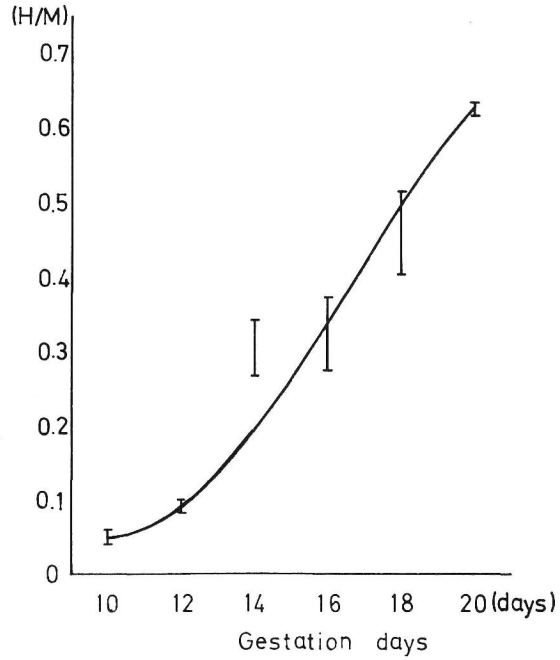

FIG. 2. Developmental change of H/M ratio in rat fetal whole brains.

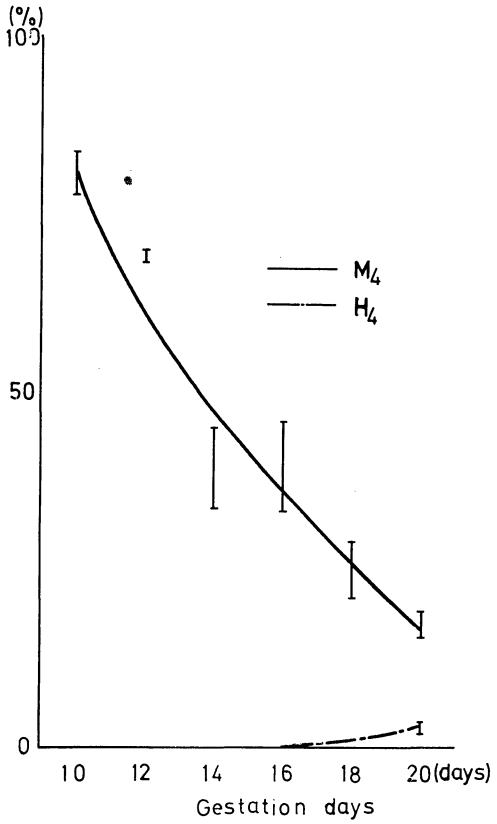

FIG. 3. Developmental change of relative M_4 and H_4 activity in rat fetal whole brains.

TABLE 1. *Developmental change of H/M ratio, relative M_4 and H_4 activity in rat fetal whole brains.* (*mean±SD*)

Gestation day	Number of samples	H/M ratio	M_4 (%)	H_4 (%)
10	5	0.050±0.008	80.7±3.1	0
12	5	0.093±0.001	69.1±0.7	0
14	5	0.306±0.037	36.8±3.2	0
16	5	0.325±0.051	39.6±6.5	0.20±0.02
18	5	0.459±0.012	25.0±4.0	0.71±0.06
20	5	0.632±0.005	17.4±1.8	2.7 ±0.8

than those of diencephalon and cerebral gray matter ($P<0.01$). The H/M ratio of the cerebral gray matter was significantly higher than that of diencephalon ($P<0.01$).

In adult rats, the H/M ratio of the cerebellum was significantly higher than those of diencephalon and cerebral gray matter ($P<0.001$). The H/M ratio of cerebral gray matter was significantly higher than that of diencephalon ($P<0.05$).

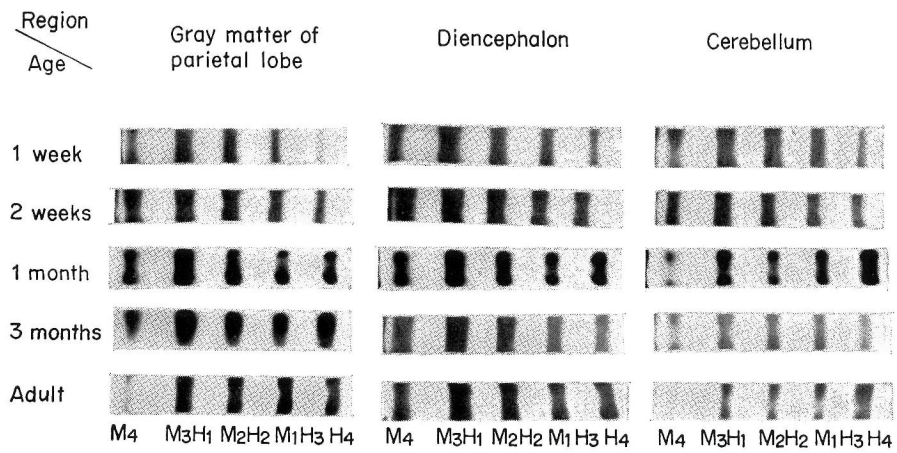

FIG. 4. Developmental change of LDH zymograms in postnatal rat brains.

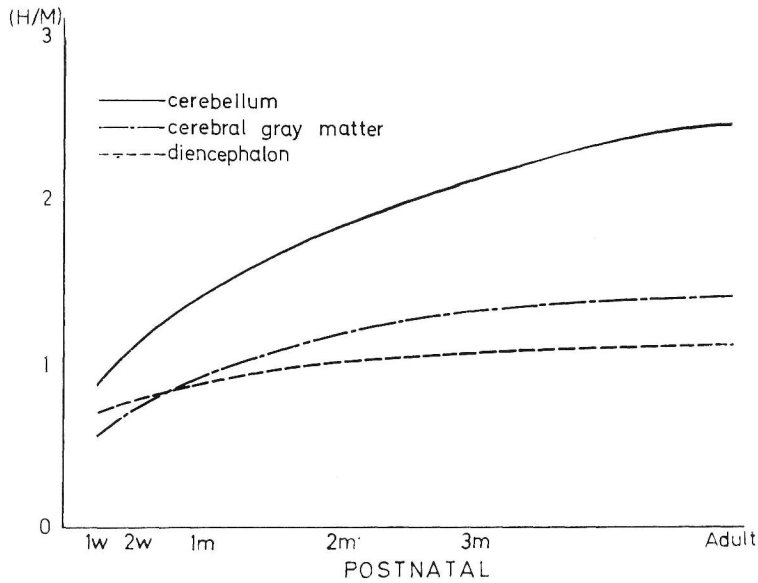

FIG. 5. Developmental change in H/M ratio in postnatal rat brain.

As shown in Fig. 6, the pattern of change of relative M_4 and H_4 activity between cerebral gray matter and diencephalon was similar, although M_4 activity was higher than H_4 activity until 1 month, when they became comparable. Thereafter the former was lower than the latter. On the other hand, in the cerebellum, although M_4 activity was higher than H_4 activity until 10 days of age, thereafter the former was lower than the latter which then occupied about half of the total LDH activity subsequently.

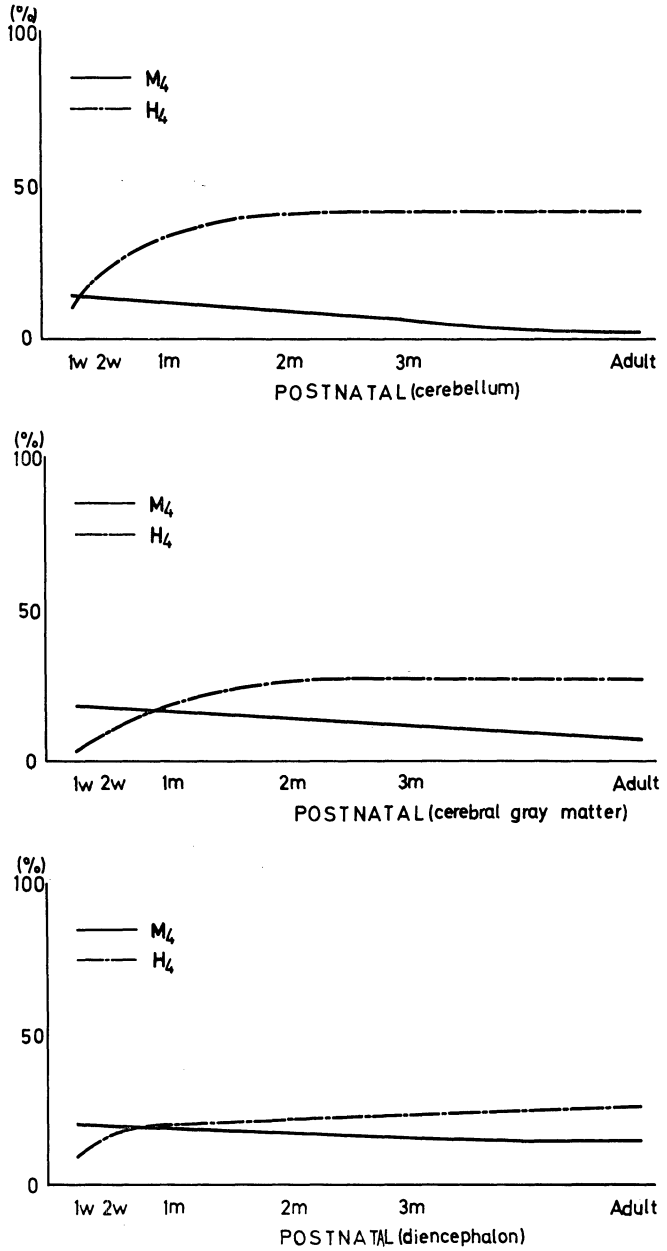

FIG. 6. Developmental change of relative M_4 and H_4 activity in postnatal rat brains.

DISCUSSION

LDH exists in multiple molecular forms, that is, as isozymes have been summarized by Dawson *et al.* (7). The LDH isozymes differ from one another physically,

TABLE 2. *Developmental change of H/M ratio, relative M_4 and H_4 activity in postnatal rat brains. (mean±SD)*

Postnatal age	Number of samples	Region								
		cerebral gray matter			diencephalon			cerebellum		
		H/M	M_4	H_4	H/M	M_4	H_4	H/M	M_4	H_4
1 week	5	0.51 ±0.05	21.4 ±4.0	3.3 ±1.7	0.67 ±0.05	19.1 ±1.9	9.4 ±0.6	0.87 ±0.02	14.8 ±2.8	10.8 ±1.2
2 weeks	5	0.73 ±0.05	15.4 ±4.3	9.5 ±1.4	0.81 ±0.11	19.1 ±3.5	14.3 ±1.9	1.05 ±0.07	9.9 ±3.1	16.5 ±1.7
1 month	6	0.98 ±0.16	14.6 ±4.8	17.4 ±0.7	0.88 ±0.06	21.8 ±4.2	20.4 ±3.6	1.53 ±0.45	13.6 ±8.7	33.1 ±6.8
3 months	5	1.29 ±0.32	10.6 ±6.3	28.5 ±8.0	0.97 ±0.10	15.6 ±3.7	21.6 ±7.1	2.11 ±0.18	7.2 ±3.8	42.7 ±11.7
Adult	13	1.42 ±0.42	7.6 ±4.2	25.4 ±5.9	1.12 ±0.24	14.6 ±6.6	25.8 ±5.6	2.48 ±0.46	2.5 ±2.1	42.5 ±5.7

chemically, immunologically and in their biological activity (16). As reported by Markert and Urspring (17), LDH of heart type (H_4) is strongly inhibited by excess pyruvate whereas the muscle type enzyme (M_4) is not so strongly inhibited. This difference indicates that the H-subunit enzyme is geared for function in aerobic tissue, such as the heart and kidney, and that the M-subunit is not strictly dependent on aerobiosis.

In the present study, developmental changes of LDH isozyme distribution of rat brain from Day 10 of gestation to 6 months after birth were examined. During the prenatal period, although the predominance of M-subunit was evident, a gradual increase of H-subunit was confirmed in rat fetal brains. This indicated that a gradual shift occurred from anaerobic glycolysis towards aerobic glycolysis. This tendency was also identified after birth. The major differentiation of the isozyme patterns occurred during the perinatal and juvenile period of rat life, that is, from fetus to about 2 weeks of age, which corresponds to the period when the zonation of the cortical plate is nearing completion and, neuronal and glial cells proliferate rapidly (15).

Engel and Petzoldt (9) studied the LDH isozyme pattern in pre-implantation and post-implantation embryos of the mouse, rat, guinea-pig and Syrian hamster. They showed that prior to implantation only H_4 was present, whereas after implantation only M_4 was demonstrable and additional isozymes appeared subsequently. They demonstrated that in rodent species the total pre-implantation LDH activity was generally based on the maternally transmitted H-subunit, while the activation of the embryonal LDH genes started only with implantation and that the gene for M-subunit was activated first, with the gene for H-subunit following later on. Their observations of the early developmental change of LDH isozyme pattern are very relevant to our present data in regard to the ontogenetic developmental change of LDH isozyme pattern.

In regard to whether or not there were regional differences in the isozymic composition within the rat central nervous system after birth, Gerhardt *et al.* (11) determined in human, rabbit and guinea-pig brains and demonstrated in guinea

pig that the cerebellum showed the highest H/M ratio, followed by frontal cortex, superior colliculus, chiasma and retina in order of decreasing H/M ratio. Bonavita (1) also examined the developmental changes of LDH isozyme pattern in postnatal rat nervous tissue, and demonstrated that the H/M ratio of diencephalon was lower than that of any other region of brain.

Our results confirmed the previous measurement of Gerhardt *et al.* and Bonavita, that is, the present data showed that the H/M ratio of cerebellum was consistently higher than in both cerebral gray matter and diencephalon from an early stage after birth. The H/M ratio of cerebral gray matter was lower than that of diencephalon until about 10 days after birth, and thereafter the former was consistently higher than the latter.

Since the H/M ratio of cerebellum was higher than other part of brain, a more steady supply of energy would be required in the cerebellum. This observation is closely related to the physiological function of the cerebellum. Zivkovic and Djuricic (21) determined the activity of LDH and its isozyme in cerebral cortex thalamus, cerebellar cortex and pons, and suggested that the 4- to 5-fold greater H_1M_3 and M_4 activity in cerebral cortex and thalamus compared with cerebellar cortex and pons might be interpreted as a sign of the unequal participation of the oxidized form of NADP. Pasantes-Morales *et al.* (18) suggested that low H/M ratio was indicative of high activity of the hexose monophosphate pathway of glucose utilization.

On the other hand, Bonavita (1) could not identify the H_4 activity until a few days after birth, while it was demonstrated from Day 16 of gestation in the present study. This observation indicated that the isozymic differentiation of perinatal rat brain LDH was earlier than he had indicated.

Our study demonstrated that the analysis of LDH isozyme pattern contributes to the evaluation of the degree of organic differentiation in at least the brain.

REFERENCES

1. Bonavita, V.: Developmental changes of enzyme pattern in the nervous tissue. In Protides of the Biological Fluids, ed. by H. Peeters, Elsevier, Amsterdam, 1965, p. 163.
2. Brinster, R. L.: Lactate dehydrogenase activity in the preimplanted mouse embryo. *Biochim. Biophys. Acta* 110; 439, 1965.
3. Brinster, R. L.: Lactate dehydrogenase in preimplantation rat embryo. *Nature* 214; 1246, 1967.
4. Cahn, R. D., Kaplan, N. O., Levine, L. and Zwillung, E.: Nature and development of lactic dehydrogenase, the two major types of this enzyme form molecular hybrids which change in make up during development. *Science* 136; 962, 1962.
5. Cepica, S., Valenta, M. and Pavlu, V.: Prenatal changes of lactate dehydrogenase isoenzyme patterns and activity in bovine tissues. *Physiol. Bohemoslov* 25; 551, 1976.
6. Chvapil, M.: Lactate dehydrogenase isoenzyme pattern and total LDH activity in the lung and liver of rats chronically exposed to low and high oxygen concentration. *Life Sci.* 17; 761, 1975.
7. Dawson, D. M., Goodfriend, T. L. and Kaplan, N. O.: Lactic dehydrogenase, function of the two types rates of synthesis of the two major forms can be correlated with metabolic differentiation. *Science* 143; 929, 1964.
8. Dietz, A. A. and Lubrano, T.: Separation and quantitation of lactic dehydrogenase isoenzymes by disc electrophoresis. *Analyt. Biochem.* 20; 246, 1967.

9. Engel, W. and Petzoldt, U.: Early developmental changes of the lactate dehydrogenase isoenzyme pattern in mouse, rat, guinea-pig, Syrian hamster and rabbit. *Humangenetik* 20; 125, 1973.
10. Epstein, C. J., Kwok, L. and Smith, S.: The source of lactate dehydrogenase in preimplantation mouse embryo. *FEBS Letters* 13; 45, 1971.
11. Gerhardt, W. and Andersen, H.: Lactate dehydrogenase isoenzymes of brain: comparative studies of the visual pathway. In Protides of the Biological Fluids, ed. by H. Peeters, Elsevier, Amsterdam, 1965, p. 173.
12. Gerhardt, W., Clausen, J., Christensen, E. and Riishede, J.: Lactate dehydrogenase isoenzymes in the diagnosis of human benign and malignant brain tumors. *J. Nat. Can. Inst.* 38; 343, 1967.
13. Gilcrease, D. and Justus, J. T.: Isozymic pattern of lactate dehydrogenase in whole embryo and adult tissues of Mexican axolotl. *Comp. Biochem. Physiol. (B)* 50 (2B); 289, 1975.
14. Ikeda, T., Okamoto, N. and Satow, Y.: Differentiation of the LDH-isozyme in normal and abnormal development of the rat. *Hiroshima J. med. Sci.* 18; 13, 1969.
15. Kohno, S.: Personal communication.
16. Markert, C. L. and Moller, F.: Multiple forms of isozyme: tissue, ontogenetic and species specific patterns. *Proc. Nat. Acad. Sci.* (Wash) 45; 753, 1959.
17. Markert, C. L. and Urspring, H.: The ontogeny of isozyme pattern of lactate dehydrogenase in the mouse. *Develop. Biol.* 5; 363, 1962.
18. Pasantes-Morales, H., Kletki, J., Urban, P. F. and Mandel, P.: Changes in the lactate and malate dehydrogenase isoenzyme patterns of chicken embryo brain and retina. *J. Neurochem.* 19; 1183, 1972.
19. Sherwin, A. L., Leblanc, F. E. and McCann, W. P.: Altered LDH isoenzyme in brain tumors. *Arch. Neurol.* 18; 311, 1968.
20. Sorensen, A. R.: The utilization of lactate in mouse oocyte maturation and first cleavage. *Biol. Reprod.* 7; 139, 1972.
21. Zivkovic, R. V. and Djuricic, B. M.: Regional distribution of lactate dehydrogenase isoenzymes in adult rat brain. *Experientia* 31; 1258, 1975.