Neurochemical Research, Vol. 7, No. 1, 1982

MODIFICATIONS IN ENERGY METABOLISM DURING THE DEVELOPMENT OF CHICK GLIAL CELLS AND NEURONS IN CULTURE

G. THOLEY[1], M. LEDIG[2], AND P. MANDEL[2]

[1]*Laboratoire de Biochimie Microbienne*
4, Rue Blaise Pascal
67070 Strasbourg Cédex, France

[2]*Centre de Neurochimie du CNRS*
5, Rue Blaise Pascal
67070 Strasbourg Cédex, France

Accepted August 3, 1981

Developmental changes in lactate dehydrogenase (LDH), enolase, hexokinase (HK), malate dehydrogenase (MDH), and glutamate dehydrogenase (GDH) activities were measured in cultures of pure neurons and glial cells prepared from brains of chick embryos (8 day-old for neurons, 14 day-old for glial cells) as a function of cellular development with time in culture. The modifications observed in culture were compared to those measured in brain extracts during the development of the nervous tissue in the chick embryo and during the post-hatching period. A significant increase of MDH, GDH, LDH, and enolase activities are observed in neurons between 3 and 6 days of culture, whereas simultaneously a decrease of HK values occurs. In the embryonic brain between 11 and 14 days of incubation, which would correspond for the neuronal cultures to day 3 through 6, modifications of MDH, GDH, HK, and enolase levels are similar to those observed in neurons in culture. Only the increase of LDH activity is less pronounced in vivo than in cultivated cells. The evolution of the tested enzymatic activities in the brain of the chick during the period between 7 days before and 10 days after hatching is quite similar to that observed in cultivated glial cells (prepared from 14 day-old embryos) between 6 and 18 days of culture. All tested activities increased in comparable proportions. The modifications of the enzymatic profile indicate that some maturation phenomena affecting energy metabolism of neuronal and glial elements in culture, are quite similar to those occuring in the total nervous tissue. A relationship between the development of the energy metabolism of the brain and differentiation processes affecting neuroblasts and the glial-forming cells is discussed.

INTRODUCTION

Most of our information concerning energy metabolism during brain development is based on observations on mixed cell populations of neurons

0364-3190/82/0100-0027$03.00/0

and glial cells, either in vivo or in vitro. The availability of clonal cell lines of neuronal origin has made it possible to compare the energy metabolism of these cells with that of the mixed cell population in the brain (5, 12, 21, 22). Since the cells used in these studies are of tumoral origin, they may be aberrant in some aspects of their metabolism, especially with regard to the importance of the glycolytic pathway in their metabolism (12, 19, 20).

The progress in technics of primary cultures (12) has facilitated biochemical investigations of specific cerebral cell types. Technics for culturing glial cells are well established (18). Recently, it has also become possible to grow neurons from the brains of 8 day-old chick embryos in culture (14) and to obtain neuronal cells with morphological characteristics of mature cells.

The purpose of the present study is to characterize different aspects of the energy metabolism of glial cells and neurons in culture, during their maturation. The levels of LDH, MDH, GDH, HK, and enolase activities were determined at different times of growth of the cells in culture, and compared to the values measured in whole chick brains at different times of the incubation period or after hatching.

The modifications of the enzymatic profiles observed in the brain are discussed with respect to the results obtained in cultivated cells. A relationship between the development of the energy metabolism of the brain and differentiation processes affecting the neuroblastes and the glial-forming cells is suggested.

EXPERIMENTAL PROCEDURE

Cell Culture Conditions. The neuronal cultures were prepared from the brains of 8 day-old, the glial cell cultures from 14 day-old chick embryos according to the method of Pettmann et al. (14) and Sensenbrenner et al. (18), respectively. They were maintained in DMEM supplemented by 20% fetal calf serum at 37°C and in 5% CO_2 and 95% air atmosphere. The medium was changed every 3 days for the neurons and every 4 days for the glial cells.

Enzymatic Activities. Lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glutamate dehydrogenase (GDH), and hexokinase (HK) activities were determined in a crude cell extract prepared from cultivated neurons or glial cells as described previously (15) or in brain homogenates. For the latter, cerebral hemispheres from chick embryos were dissected, cleaned of their meningeal membranes and homogenized for 2 min with 2 ml of an ice-cold 1 mM EDTA (pH 7.0) solution, in a cooled Potter Elvejhem homogenizer with a teflon pestle. The resultant crude homogenate was treated 3 times with 20 sec sonications separated by 20 sec cooling periods in an ultrasonic disintegrator (power 150 W). The sonicate was then centrifuged at 27,000 *g* for 15 min at 4°C. The supernatant was used as a crude extract.

For enolase activity the cultivated cells were disrupted using a Kontes minibombe and

the brain hemispheres with a Potter Elvejhem homogenizer. The crude suspension obtained in both cases was centrifuged for 1 hr at 100,000 *g* and the cytosol was used for the enzymatic assay.

Enolase activity was determined by monitoring initial velocity measurement at 240 nm spectrophotometrically according to the method of Marangos et al. (13). LDH activity was measured by the method of Bergmeyer and Bernt (3), GDH was determined by the method of Schmidt (17), hexokinase as described previously (16).

One unit of enzymatic activity was defined as the amount of enzyme which transformed 1 μmol substrate/min at 25°C. Specific activity is expressed as units/mg protein. Protein was measured according to the method of Lowry et al. (10).

RESULTS

Changes in LDH, HK, Enolase, MDH, and GDH Activities in Cultivated Neurons, Glial Cells, and in Chick Brains. Figure 1 shows the developmental pattern of LDH, HK, enolase, and Figure 2 that of MDH and GDH activities of neurons and glioblasts determined at various times of culture and that of chick brain between the 8th day of incubation and the 15th day after hatching.

During the pre-hatching period, between 8 and 20 days of incubation, significant increases of MDH, GDH, and enolase activities are observed in extracts of chick embryo brain. The magnitude of the increase during this period is 2–5 fold. The increase of LDH activity during the same period is considerably less. A decrease of HK activity by about 30% is observed simultaneously.

In cultivated neurons, a progressive increase of the activities of LDH, MDH, GDH, enolase and a decrease of HK activities are observed between 3 and 6 days of culture (time periods equivalent to embryonic ages of 11 through 14 days). The magnitude of the increase during this period is 2–4 fold and the decrease of HK activity approximately 30%.

During the post-hatching period, two phases in the levels of the tested enzymatic activities can be observed. Between the 1st and the 8th day after hatching, the following evolution of the tested enzymatic activities is observed: MDH and enolase are high on the day of hatching, and thereafter these activities decrease sharply. Simultaneously, LDH, GDH, and HK activities are not significantly modified.

A second phase starting 8 days after hatching seems apparent. It is characterized by a drastic increase of all tested enzymatic activities: LDH, enolase, HK, MDH, and GDH. From 8 through 15 days after hatching, the magnitude of the increase of the tested enzymatic activities is between 50 and 100%, that of HK amounting to more than 200%.

Cultivated glioblasts (day 7 through 18) also present 2 phases in their evolution of the enzymatic activities. This time period could be considered

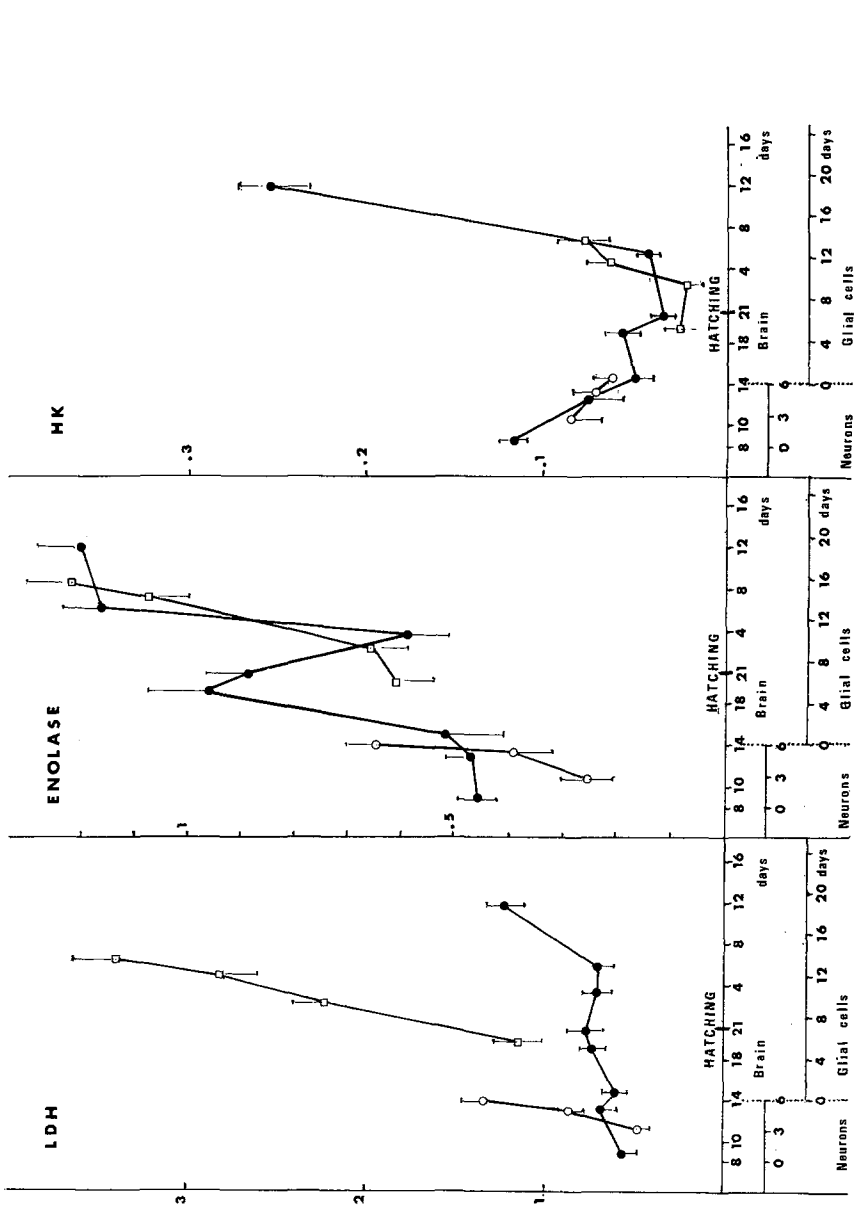

FIG. 1. Developmental profile of LDH (lactate dehydrogenase), enolase, and HK (hexokinase) activity in cultivated neurons (○), glial cells (□), and chick brains (●). The neurons were cultivated for periods between 3 and 6 days and the glial cells for periods between 7 and 18 days. These periods are equivalent to embryonic ages of 11 and 14 days and to chick ages of the day of hatching and 11 days post-hatching respectively. Each point is a mean of 5 to 9 determinations ± SEM.

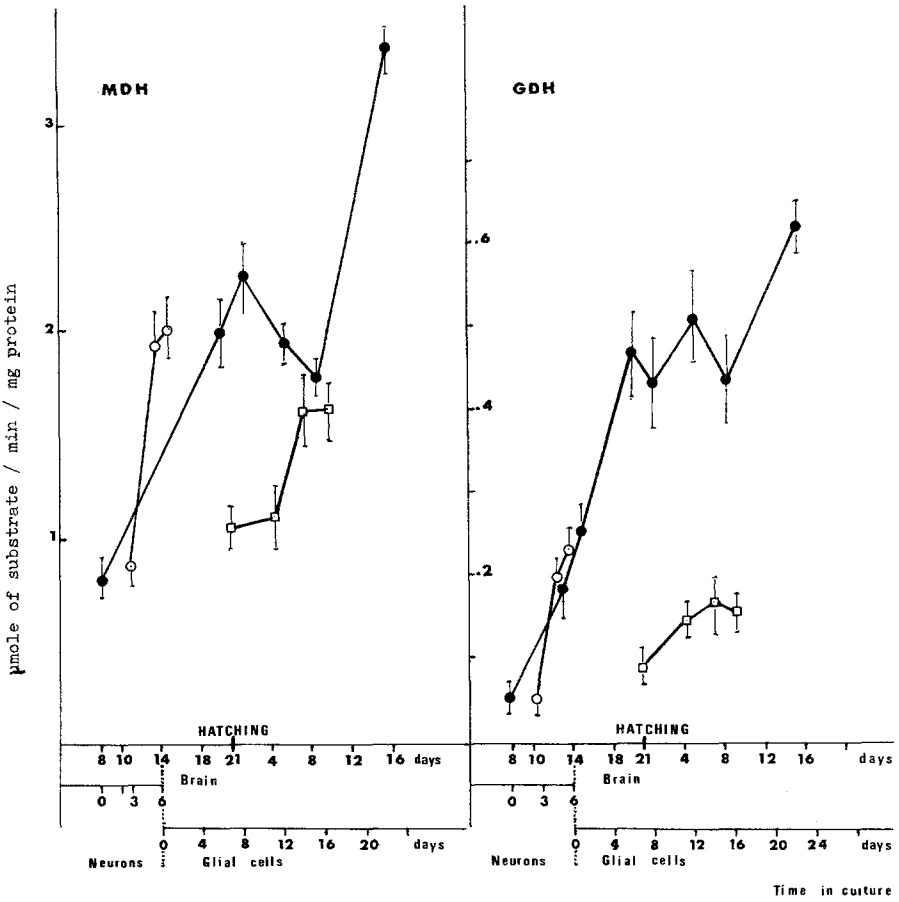

FIG. 2. Developmental profile of MDH (malate dehydrogenase) and GDH (glutamate dehydrogenase) activity in cultivated neurons (○), glial cells (□), and chick brains (●). The neurons were cultivated for periods between 3 and 6 days and the glial cells for periods between 7 and 18 days. The periods are equivalent to embryonic ages of 11 and 14 days and to chick ages of the day of hatching and 11 days post-hatching respectively. Each point is a mean of 5 to 9 determinations ± SEM.

equivalent in the chick brain to a period between the day of hatching and 11 days post-hatching. Between the 7th and the 11th day of culture, corresponding to the logarithmic growth phase of the cell, only the activities of LDH and GDH are significantly increased; the levels of the other enzymes are not modified. From 11 through 18 days of culture (stationary phase of growth) LDH, MDH, HK, and enolase activities are significantly increased; only the level of GDH is not modified during this period.

TABLE I

RATIOS OF MALATE DEHYDROGENASE/HEXOKINASE, GLUTAMATE DEHYDROGENASE/HEXOKINASE, AND ENOLASE/HEXOKINASE ACTIVITIES CALCULATED FOR NEURONS, GLIAL CELLS AT VARIOUS TIMES OF CULTURE AND FOR TOTAL BRAIN BETWEEN THE 8TH DAY OF INCUBATION AND THE 8TH DAY AFTER HATCHING

Ratio	Neurons			Glial Cells			Brain age					
	Time of culture			Time of culture			Embryo			Chick		
	3 days	5 days	6 days	6 days	10 days	15 days	8 days	13 days	20 days	1 day	8 days	15 days
Malate dehydrogenase / Hexokinase	10	27	29	41	50	28	7	28	33	71	41	13
Glutamate dehydrogenase / Hexokinase	0.05	0.27	0.33	0.32	0.67	0.27	0.50	0.42	0.88	1.34	1.00	0.24
Enolase / Hexokinase	3	6	10	24	30	19	4	6	16	28	28	5

At different times of culture or of brain development, the ratios of MDH/HK, GDH/HK, and enolase/HK activities were calculated for neurons, glial cells, and brain (Table I). A progressive increase of the ratios is observed in neurons during the entire culture period and in brains until hatching. In the post-hatching chick brain, a decrease of the calculated values occurs during the supposedly corresponding period of growth. Glial cells in culture present an increase of the values until 10 days of culture, with a decline of the values thereafter.

DISCUSSION

An increase of different enzyme activities linked to brain energy metabolism has already been observed during maturation of primary cultures of rat glial cells (21). In the present study, we compared the modifications of the levels of different enzymes involved in the energy metabolism during the maturation of chick neurons and glial cells in culture. Isolated neurons and glial cells express in culture several distinct features also observed in the developing brain tissue (6). If we accept the view that the relative levels of the tested enzymes reflect the metabolic importance of these enzymes, modifications of the energy metabolism occuring in parallel with cell differentiation in culture can be evaluated by this approach. Furthermore, any modifications observed in culture can be compared to those occuring during the development of nervous tissue in vivo.

An approximately 2-fold increase of enolase, MDH, GDH, and LDH was observed in neurons between 3 and 6 days of culture; a decrease of HK activity of about 30% occured simultaneously. Neurons in culture still divide on the 3rd day, but have a complete neuronal network by the 6th day (14). The observed modifications of the enzymatic profile certainly reflect metabolic aspects of maturation of the neurons. We have already observed that cultured neurons contain predominantly the aerobic isoenzymatic form of LDH, indicating probably a more aerobic metabolism in these cells (22). The net increase of the values of the MDH/HK and GDH/HK ratios in the neurons between 3 and 6 days culture reflect a shift of the energy metabolism towards a more aerobic form. The lowering of the HK activity levels during this period seems more intriguing, considering that metabolism of glucose is impossible without hexokinase, and a lowering in the level of hexokinase activity could indicate a reduction in glucose consumption. A possible explanation of a such relative deficiency in hexokinase activity of differentiated neurons is that the relative levels of hexokinase in the neurites of the mature cells are lower than in the perikarya, as observed in rat cerebellar cortex cells (9).

The modifications of the MDH, GDH, and HK values in the embryonic brain between 11 and 14 days of incubation are comparable to those observed in neurons in culture for day 3 through 6, i.e. ages equivalent to the embryonic ages. Only the increases of LDH and enolase activities are less pronounced in vivo than in cultivated cells. The high values observed in this case may be related to the partially hypoxic conditions present in culture which induce enzymatic activities involved in the anaerobic glycolytic metabolism. The increase of MDH, GDH, and enolase, and the decrease of HK activities observed in the brain continue until 20–21 days of incubation. Immediately after hatching a significant decrease of MDH and enolase occurs, the lowest values are observed in 5–7 day-old chicks, HK, and GDH activities do not change significantly during this period.

The evolution of the tested enzymatic activities in the brain of the chick between 7 days before and 10 days after hatching has to be compared to those observed in cultivated glial cells (derived from 14 days old embryos) between 6 and 18 days culture. All the tested activities were increased to the same extent (2–4-fold) in the glial cells during the logarithmic phase (6–11 days) and the stationary phase (18 days) of growth. Thus, we can postulate that the speed of evolution of the glial cells is similar in vivo and in culture.

This would mean that the majority of the glial cells in the brain of the 20 day-old embryo is still in a proliferating state, with low values of MDH, GDH, enolase, and HK. This compares favorably with the activities of differentiated neurons also observed in the brain just after hatching. The parallelism in the increase of HK and enolase observed in the brain of 2 to 8 days old chicks, and in glial cells between 10 and 18 days culture probably express a maturation phenomenon affecting specifically this type of cells. A similar observation of glial maturation during the same period has been made by Cicero et al. (4) in the avian optic tectum by following the evolution of the glial specific protein S-100.

The variations of the MDH/HK, GDH/HK, and enolase/HK ratios during the post-hatching period, the increase of the GDH/HK and enolase/HK values (also observed during the maturation of neurons in culture) and the decrease of the MDH/HK ratio (similar to the decrease observed in cultivated glial cells) indicate that differential modifications of the energy metabolism occur in neuronal and glial elements.

Some aspects of these maturations phenomena can be observed in primary cultures of neurons and glial cells. Whether a such differential distribution of enzymatic activities involved in the energy metabolism of neurons and glial cells during nervous tissue maturation is of any functional significance in the metabolic relationship between neurons and glial cells remains to be determined.

ACKNOWLEDGMENTS

We wish to thank Dr. B. Roth-Schechter for reviewing the manuscript and her helpful suggestions. The expert technical assistance of Mrs. M. A. Laluet, Mrs. M. Ziessel and Miss G. Schmidt are gratefully acknowledged. This research was supported by grant INSERM-ATP 81-79-113.

REFERENCES

1. BALAZS, R. 1970. Carbohydrate metabolism. Pages 1–29, *in* A. LAJTHA (ed.), Handbook of Neurochemistry, Plenum Press, New York.
2. BERGMEYER, H. V., and BERNT, E. 1974. Lactat dehydrogenase—U.V. Test mit pyruvat und NADH, Pages 607–612, *in* BERGMEYER, H. V. (ed.), Methoden der Enzymatischen Analyse. Verlag Chemie 3d Ed., Weinheim, Bergstr.
3. BERGMEYER, H. V., and BERNT, E. 1974. Malat dehydrogenase—U.V. Test, Pages 649–653, *in* BERGMEYER, H. V. (ed.), Methoden der Enzymatischen Analyse, Verlag Chemie 3d Ed., Weinheim, Bergstr.
4. CICERO, T. V., COWAN, W. M., and MOORE, B. W. 1970. Changes in the concentration of the two brain specific proteins, S-100 and 14-3-2, during the development of the avian optic tectum. Brain Res. 24:1–10.
5. CIESIELSKI-TRESKA, J., THOLEY, G., WURTZ, B., and MANDEL, P. 1976. Enzymic modifications in a cultivated neuroblastoma done after bromo deoxyuridine treatment. J. Neurochem. 26:465–469.
6. DIEBLER, M. T., FARKAS-BARGETON, E., and WEHRLE, R. 1979. Development changes of enzymes associated with energy metabolism and the synthesis of some neurotransmitters in discrete areas of human ureo cortex. J. Neurochem. 32:429–435.
7. DITTMANN, L., HERTZ, L., SCHOUSBOE, A., FOSMARK, H., SENSENBRENNER, M., and MANDEL, P. 1973. Energy metabolism of nerve cells during differentiation. Exptl. Cell. Res. 80:425–431.
8. DITTMANN, L., SENSENBRENNER, M., HERTZ, L., and MANDEL, P. 1973. Respiration by cultivated astrocytes and neurons from the cerebral hemispheres. J. Neurochem. 21:191–198.
9. ITOH, T., and QUASTEL, J. H. 1970. Acetoacetate metabolism in infant and adult rat brain in vitro. Biochem. J. 116:641–655.
10. LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
11. MANDEL, P., CIESIELSKI-TRESKA, J., HERMETET, J. C., HERTZ, L., NISSEN, C., THOLEY, G., and WARTER, F. 1973. Some histochemical, biochemical and pharmacological aspects of differentiation of neuroblastoma cells of mouse. Pages 223–230, GENAZZANI, E., and MERKEN, H. (ed.), Central Nervous System—Studies on metabolic Regulation and Function, Springer Verlag, New York.
12. MANDEL, P. 1978. The value of nerve cell cultures for neurochemical research. Proc. Europ. Soc. Neurochem. 1:489–523.
13. MARANGOS, P. J., ZOMZELY-NEURATH, C., and YORK, C. 1976. Determination and characterization of neuron specific protein (NSP) associated enolase activity. Biochem. Biophys. Res. Commun. 68:1309–1316.
14. PETTMANN, B., LOUIS, J. C., and SENSENBRENNER, M. 1979. Morphological and biochemical maturation of neurones cultivated in the absence of glial cells. Nature, 281:378–380.

15. ROTH-SCHECHTER, B. F., THOLEY, G., and MANDEL, P. 1979. Development and mechanism of barbiturate tolerance in glial cell cultures. Neurochem. Res. 4:83–97.
16. ROTH-SCHECHTER, B. F., WINZERITH, M., THOLEY, G., DIERICH, A., and MANDEL, P. 1979. Effect of pentobarbital on mitochondrial synthesis in cultured glial cells. J. Neurochem. 33:669–676.
17. SCHMIDT, E. 1962. Glutamat dehydrogenase, Page 752, H. U. BERGMEYER (ed.), Methoden der Enzymatischen Analyse, Verlag Chemie Weinheim, Bergstr.
18. SENSENBRENNER, M. 1977. Dissociated brain cells in primary culture, Pages 191–213, *in* FEDOROFF, S., and HERTZ, L. (eds.), Cell, Tissue and Organ Cultures in Neurobiology. Academic Press, New York.
19. THOLEY, G., CIESIELSKI-TRESKA, J., WURTZ, B., and MANDEL, P. 1972. Variations de diverses activités enzymatiques liées au métabolisme glucidique dans des cellules de neuroblastome en prolifération et en différenciation. C.R. Acad. Sci. Paris, 275:1715–1718.
20. THOLEY, G., WURTZ, B., CIESIELSKI-TRESKA, J., and MANDEL, P. 1972. Enzymatic activities modified during multiplication and differentiation of neuroblastoma cells. Nature, New Biology, 239:180–181.
21. THOLEY, G., ROTH-SCHECHTER, B. F., and MANDEL, P. 1980. Development of glial cells in primary cultures: energy metabolism and lactate dehydrogenase isoenzymes. Neurochem. Res. 5:847–854.
22. THOLEY, G., ROTH-SCHECHTER, B. F., and MANDEL, P. 1981. Activity and isoenzyme pattern of lactate dehydrogenase in neurons and astroblasts cultured from brains of chick embryos. J. Neurochem. 36:77–81.