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Lactate Dehydrogenase Activity during the Culture of Chick Embryo Neural Retina Cells *in Vitro*

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ABSTRACT. Chick embryo neuroretinal (NR) cells transdifferentiate extensively into lens when cultured for several weeks in (F) medium, but this is largely inhibited when high levels of supplementary glucose (FG) are present. We show here that lactate dehydrogenase activity is promoted in high glucose cultures. Histochemical staining confirms that LDH activity is higher and more intense in FG than in F cultures. The results of LDH isoenzyme studies suggest that late (35-day) FG cultures may attain an "adult type" two-band isozyme pattern, whereas parallel F cultures retain the simple band pattern characteristic of embryonic retina.

Introduction

The appearance of lens-fibre-like cells (lentoid bodies) in long-term monolayer cultures of embryonic chick neural retina (NR) cells is one example of a phenomenon termed transdifferentiation[1-4], used here to describe the conversion of partially differentiated cells from one tissue type (NR) into fully differentiated cells of a foreign tissue type (lens). In this case, the initial retinal tissue (from 8-day embryos) is not yet fully differentiated, but clearly lens cells would never appear during normal neuroretinal development *in vivo*. The lentoid bodies appear mainly after 30 days of NR culture[1,4]. In this system, the extent of NR transdifferentiation is dependent upon: (i) intrinsic factors such as the embryonic stage of the starting NR[5,6] and (ii) extrinsic fators such as medium and substrate conditions[7-10] F medium (MEM supplemented with 10% fetal calf serum) or FH medium (MEM supplemented with 5% horse serum and 5% fetal calf serum) both permit extensive transdifferentiation. In FHG medium (*i.e.*, FH supplemented with glucose to 18 m*M* final), transdifferentiation and δ-crystallin accumulation are blocked[11]. Similarly, in FG medium (*i.e.*, F supplemented with glucose to 28 m*M* final), transdifferentiation and δ-crystallin accumulation are strongly inhibited[7]. Recently, Karim *et al.*[12] have studied glucose

metabolism in transdifferentiating (FH) and glucose-blocked (FHG) cultures. In this paper, we investigate lactate dehydrogenase activity during NR culture in case of F and FG medium.

Similarly, in FG medium (*i.e.*, F supplemented with glucose to 28 m*M* final), transdifferentiation and δ-crystallin accumulation are strongly inhibited[1-4]. Recently, Karim *et al.*[12] have studied glucose metabolism in transdifferentiating (FH) and glucose-blocked (FHG) cultures. In this paper, we investigate lactate dehydrogenase activity during NR culture in case of F and FG medium.

Material and Methods

Material

Fertile eggs were from G.W. Padley Ltd., Grantham, Lincolnshire. Tissue culture media and sera were from GIBCO-EUROPE, and most chemicals from Sigma Ltd.

Methods

(i) *Cell Culture*

Nine-day chick embryo NR cells were cultured for up to 50 days as described previously[8]. Cells were sown at a density of 5×10^6/ml medium comprising Eagle's MEM with Earle's salts, 26 m*M* $NaHCO_3$, 2 m*M* *L*-glutamine, 100 I.U./ml penicillin, 100 μg/ml streptomycin, and 10% foetal calf serum (F medium); FG comprised F medium containing extra glucose to 28 m*M* final (instead of 6 m*M*). The protein content of all cultures was assayed by the method of Lowry *et al.*[13].

(ii) *Lactate Dehydrogenase (LDH) Assays*

Saline extracts of retinal cultures were centrifuged (5000 × g for 10 min), and triplicate samples of the supernatants were assayed for LDH activity as described by Wroblewski and La Due[14].

(iii) *LDH Histochemical Method*

Lactate dehydrogenase (LDH) was demonstrated histochemically by the method of Hess *et. al.*[15], cultures at 15 and 25 days were washed three times with saline, and then 70 per cent alcohol. The cells were then treated with LDH staining solution, 1 *M* sodium lactate, NAD (10 mg/ml), 0.1 *M* sodium cyanide, 0.06 *M* phosphate buffer, 0.9 m*M* nitro blue tetrazolium) for 20 minutes. LDH activity is shown by a purple red colour.

(iv) *LDH Agarose Gels*

Horizontal agarose gel electrophoresis was performed at 4°C for 3-4 hours in 1% aqueous agarose; the gel size was 62 mm × 124 mm, and the thickness 2.0 to 1.5 mm. The buffer solution was 62 m*M* Tris, 50 m*M* sodium hippurate, 16.7 m*M* hippuric acid. All samples were mixed with approximately 1 ml of sample buffer (0.0625 *M* Tris-HCl pH 6.8, 0.23% W/V SDS, 10% V/V glycerol, 50 m*M* 2-mercaptoethanol, 0.001% W/V bromophenol blue). Four μg of soluble protein (extracted from cultures) were placed in the wells, and the current switched up to 20 mA. Elec-

trophoresis was carried out until the bromophenol blue marker reached the bottom of the gel. The gels were then stained for LDH activity at 37°C with the above staining solution and fixed in 7% acetic acid.

Results

Chick embryo neuro-retinal (NR) cells transdifferentiate in both lens and pigment cells after 4-5 weeks *in vitro* when cultured in Eagles minimal essential medium containing 10% foetal calf serum (F), but lentoid appearance and δ-crystallin accumulations are inhibited if supplementary glucose is present (FG) (Fig. 4).

As shown in Fig. 1, LDH activity is consistently higher in FG cultures than in parallel F cultures, the difference being greatest at 35 and 42 days and smaller at 7, 14, 21 and 28 days. Figure 2 shows the staining of LDH activity in 15 and 25 day NR cultures maintained in F as compared to FG media. There is strong LDH staining in FG cultures (Fig. 2A and C), whereas this is both sparser and weaker in F cultures (Fig. 2B and D). Staining is most intense within or beneath clusters of neuronal cells. After agarose gel electrophoresis (Fig. 3), neuroretinal LDH activity appears to comprise a single band, rather than the several anticipated. The position and intensity of this band do not alter much during embryonic development *in vivo* (Fig. 3, Lanes 1 and 2), though the increased width of the band in adult stages may suggest a second isozyme. Figure 3 lanes 4 to 7 show LDH isozyme patterns obtained from F and FG cultures at different stages. The activity of LDH band (S) in Fig. 3 is high in 15-day F cultures (lane 7) but decreases somewhat by 35 days (lane 6). The LDH band from 35-day FG cultures (but not that from F cultures) is very broad, suggesting the presence of two (or more) isozymes (Fig. 3.4). Given the different run conditions, this may correspond to the adult *in vivo* pattern seen in Fig. 3, lane 3; if this is so, then the 35-day F cultures would appear to retain an embryonic LDH isozyme pattern.

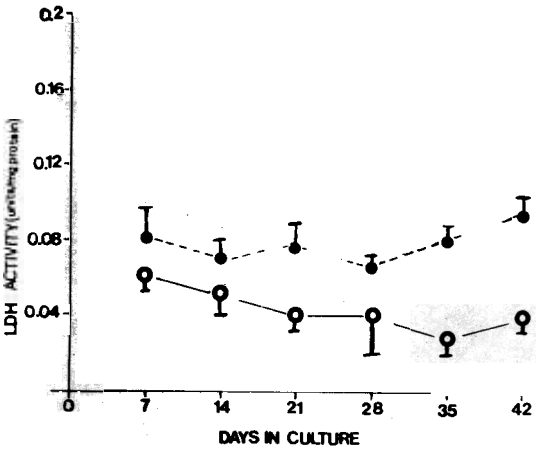


FIG. 1. LDH activity in monolayer cultures of 9-day chick embryo NR cells. LDH activity was measured as described in the Methods; each point gives the mean and standard error derived from at least four replicate assays on retinal homogenates from two sets of cultures. ● - - - - ●, FG; o ——— o, F.

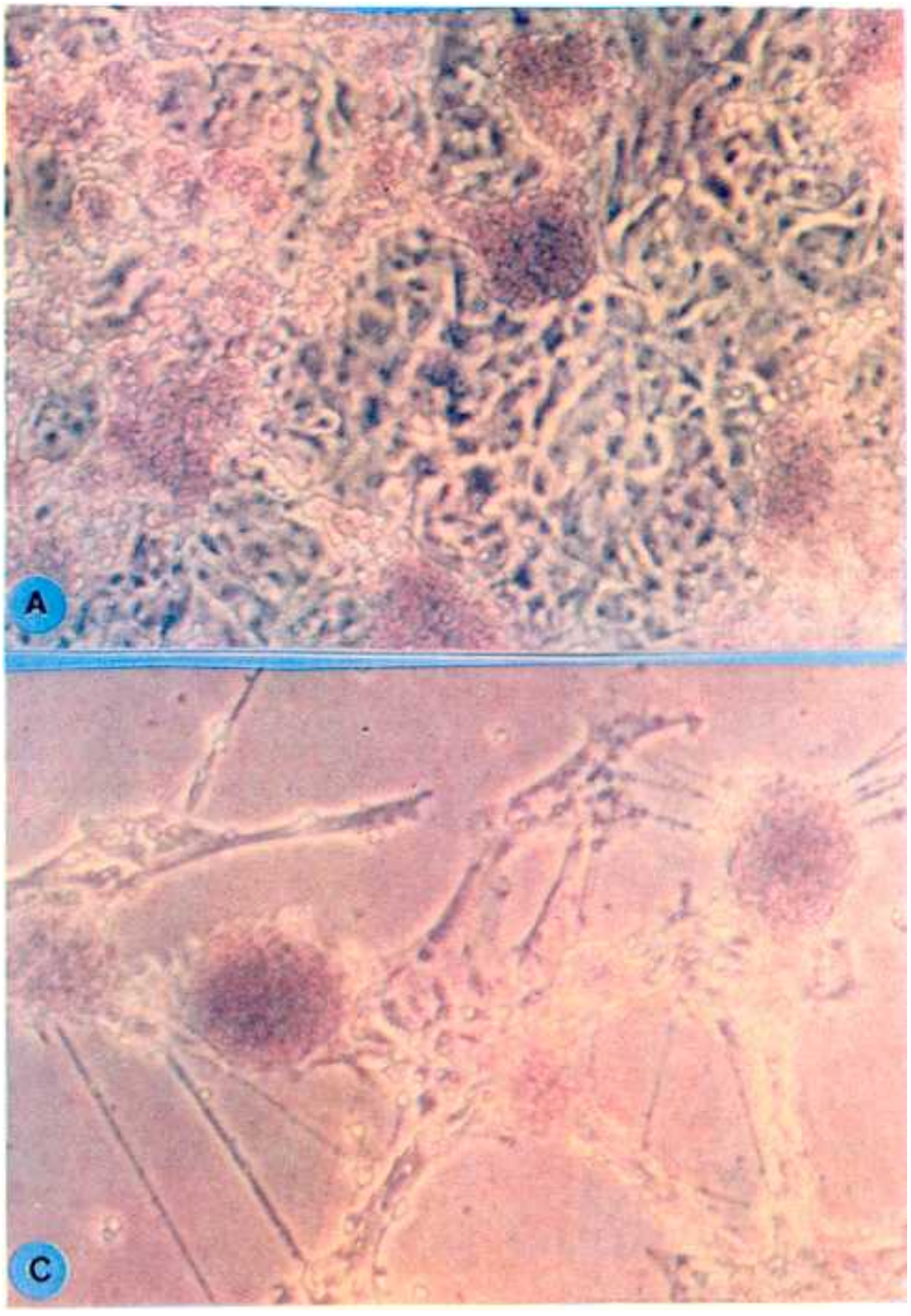
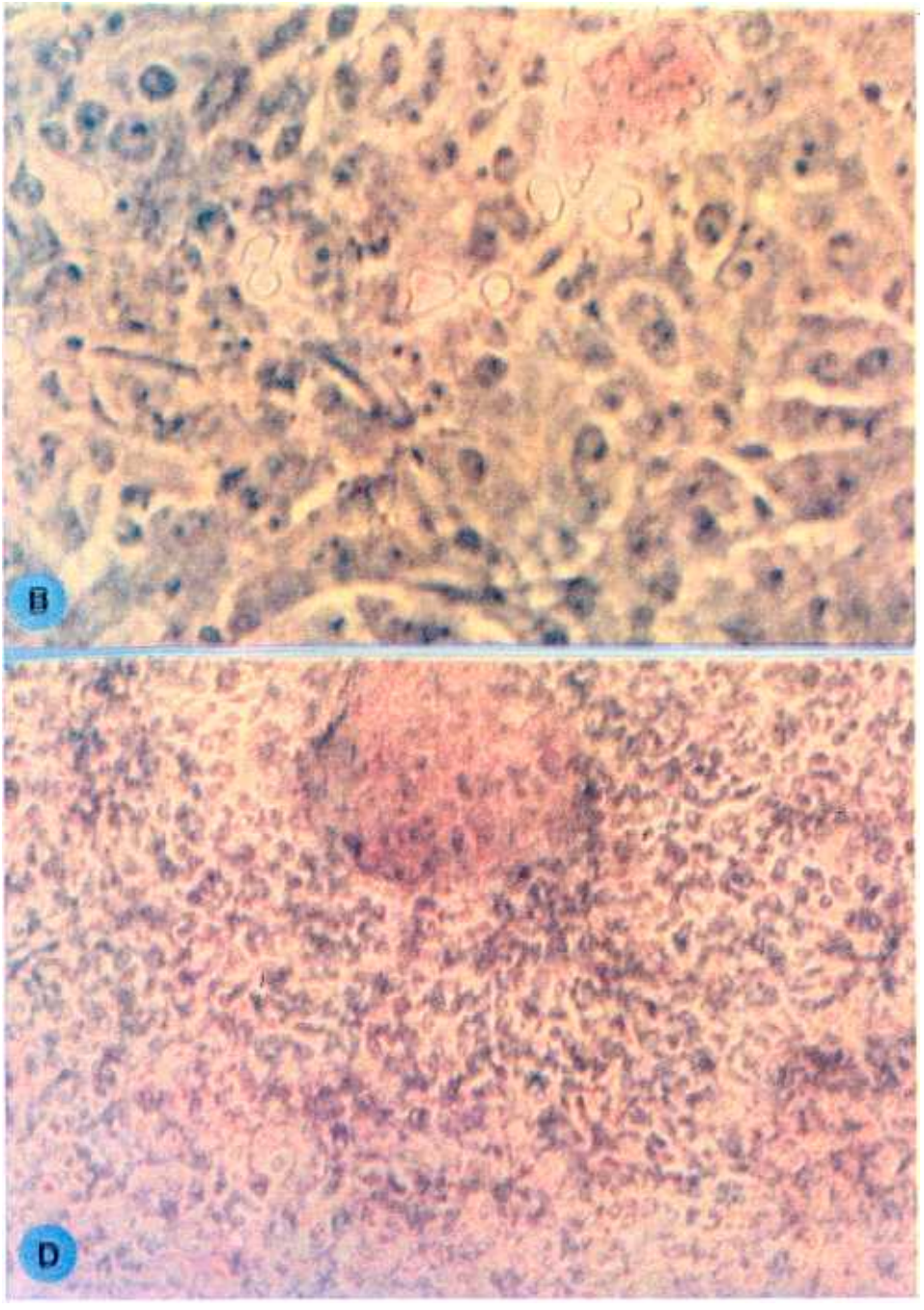


FIG. 2. LDH staining in monolayer NR cultures maintained in F and FG media. The LDH staining was performed as described in Methods.
Frames (A) and (C): FG cultures after 15 and 25 days *in vitro*, × 100 magnification.



Frames (B) and (D): F cultures after 15 and 25 days *in vitro*, × 100 magnification.

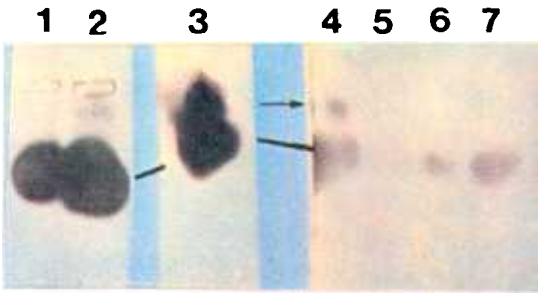


FIG. 3. Lactate dehydrogenase (LDH) isozyme patterns from NR cells *in vivo* and *in vitro*. Agarose gel electrophoresis was performed as described in Methods.

Lanes (1), (2) and (3): LDH isozymes from fresh NR cells at 8 and 17 days of embryonic development, and at an adult stage, respectively.

Lanes (4) and (5): LDH isozymes from FG cultures after 35 and 15 days *in vitro*.

Lanes (6) and (7): LDH isozymes from F cultures after 35 and 15 days *in vitro*.

Discussion

Our present results show that lactate dehydrogenase (LDH) activity (Fig. 1 and 2) is higher in FG cultures as compared to F cultures. Glycolysis was found to be greatest in retinal tissue maintained in high glucose media (2-4 g/L of glucose[16]. Karim *et al.*[12] have showed that lactate dehydrogenase (LDH) activity and lactate production *in vitro* are both much higher in FHG (high glucose) cultures as compared to FH (low glucose) cultures. As seen in Fig. 2, LDH activity is higher, and gives more intense staining in FG cultures than in F cultures. This activity appears to be localized mainly in or beneath cluster of neurons. There is an intriguing suggestion in the results of our LDH isoenzyme study (Fig. 3, lane 4) that late (35-day) FG cultures may attain an "adult type" two-band isozyme pattern, whereas parallel F cultures retain the simpler band pattern characteristic of embryonic retina. The lower pH of the maintenance medium, seen after incubation of retinas at high glucose concentration, probably results from increased lactate production due to more glycolysis[12,17]. De Pomerai and Gali[8] found that at pH 6.8, the appearance of lens crystallin is retarded and choline acetyl transferase (CAT) activity persists for longer, whereas at pH 8.0 crystallins appear earlier and CAT activity declines rapidly. One effect of using high glucose medium (FG) is to lower the pH over a 2-3-day period due to accumulation of lactic acid[18] *via* metabolism from glucose (Fig. 1 and 2) The role of cell interactions (*e.g.*, between neurons and glia, or neurons and target cells) cannot be assessed in cultues of a single cell-type, although such interactions are known to be significant for the expression of differentiated cell functions among retinal glial (Muller) cells[19] and cholinergic neuronal cells[20]; this may also be true for transdifferentiation[7,8]. Tholey *et al.*[21] measured LDH activity in neurons and glial cells from chick embryo brain in culture, and compared these to the LDH activity of brain tissue *in vivo*. They found a two-fold greater increase of LDH

activity in neurons between 3 and 6 days of culture, as compared to cultures of glial cells or brain tissue *in vivo*. This suggests that neuronal cells may use available glucose for energy to a greater extent than glial cells, although the glial cells act in many

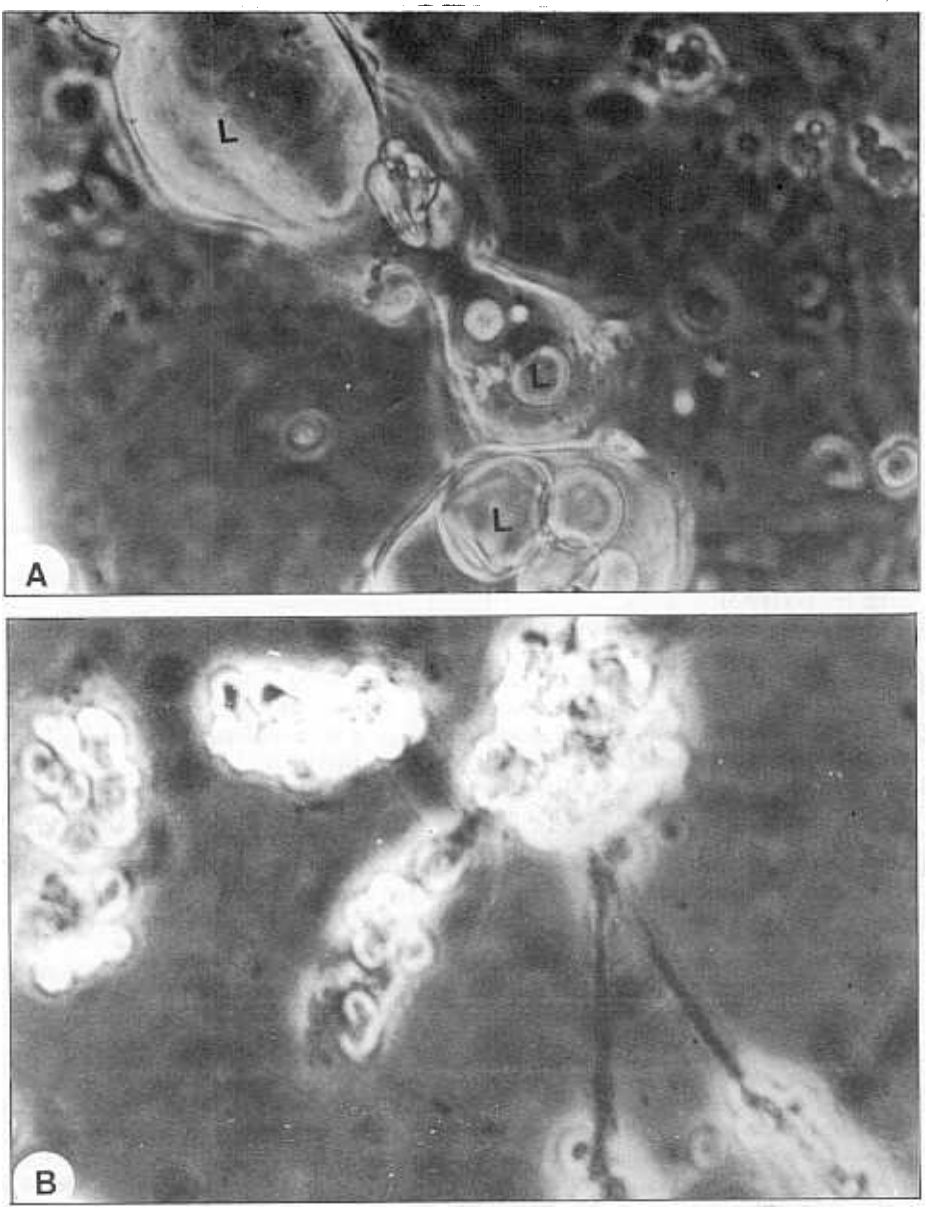


FIG. 4. The appearance of lentoids in F cultures at 33 days, compared with their absence or inhibited in parallel FG cultures. All fields were photographed under a phase contrast microscope, and the magnification is 100 ×. (A) F culture; (B) FG culture; L indicates a lentoid structure.

ways as metabolic "support" cells from the neurons of the retina[17]. This suggestion would appear to be supported by the apparent localization of LDH activity within clusters of neuronal cells (Fig. 2), although we cannot at present exclude the possibility that LDH may in fact be present in the glial cells underlying such clusters (as in the case of glycogen)[12].

Glucose effects on cellular differentiation patterns are not confined to NR cells in culture, but are also found in several other systems. (i) An involvement of glucose metabolism in the regulation of differentiation of cultured cells has been suggested from the results obtained with the human colon carcinoma cell line HT-29. When these differentiated cells are repassaged in the presence of 25 m*M* glucose, they revert to an undifferentiated phenotype[22,23]. (ii) Elimination of glucose from medium containing nerve growth factors does not support fibre outgrowth. Fibre outgrowth occurs only when glucose is added to the medium. Our findings thus contribute to a growing body of evidence suggesting a key role for simple metabolites such as glucose in a variety of differentiation processes.

References

[1] **Okada, T.S., Itoh, Y., Watanabe, K.** and **Eguchi, G.**, Differentiations of lens in cultures of neural retina cells of chick embryos, *Dev. Biol.* **45:** 318-329 (1975).

[2] **Okada, T.S.**, Cellular metaplasia or transdifferentiation as a model for retinal cell differentiation, *Curr. Top. Dev. Biol.* **16:** 349-380 (1980).

[3] ————. Recent progress in studies of the transdifferentiation of eye tissue *in vitro, Cell Differ.* **13:** 177-183 (1983).

[4] **De Pomerai, D.I.**, The transdifferentiation of neural retina into lens *in vitro, Zool. Science.* **5:** 1-19 (1988).

[5] **De Pomerai, D.I.** and **Clayton, R.M.**, Influence of embryonic stage on the transdifferentiation of chick neural retinal cells in culture, *J. Embryol. Exp. Morphol.* **47:** 179-193 (1978).

[6] **Nomura, K.** and **Okada, T.S.**, Age-dependent change in the transdifferentiation ability of chick neural retina in cell culture, *Dev. Growth Differ.* **21:** 161-168 (1979).

[7] **De Pomerai, D.I.** and **Gali, M.A.**, Determination of chick neuroretinal cells in culture: Serum factors acting between 12 and 20 days of culture influence the extent of subsequent lens cell formation, *Dev. Growth Differ.* **23:** 229-236 (1981).

[8] ————. Influence of serum factors on the prevalence of normal and foreign differentiation pathways in cultures of chick embryo WR cells, *J. Embryol. Exp. Morphol.* **62:** 291-308 (1981).

[9] ————. Embryonic serum factors required for transdifferentiation of chick embryo neuroretinal cells in culture, *Dev. Growth Differ.* **24:** 233-243 (1982).

[10] **Gali, M.** and **De Pomerai, D.I.**, Differential effects of culture media on normal and foreign differentiation pathways followed by chick embryo neuroretinal cells *in vitro, Differentiation* **25:** 238-246 (1984).

[11] **De Pomerai, D.I.** and **M.A. Gali**, A switch for transdifferentiation in culture: Effects of glucose on cell determination in chick embryo neuroretinal cultures, *Dev. Biol.* **93:** 534-538 (1982).

[12] **Karim, S.A., Flor-Henry, M.** and **De Pomerai, D.I.**, Glucose metabolism in transdifferentiating and glucose-blocked cultures of chick embryo neuroretinal cells: An inverse relationship between glycogen and δ-crystallin accumulation, *Cell Differ.* **22:** 29-46 (1987).

[13] **Lowry, O.H., Rosebrough, N., Farr, A.** and **Rondall, R.**, Protein measurement with the Folin-phenol reagent, *J. Biol. Chem.* **193:** 165-175 (1951).

[14] **Wroblewski, F.** and **La Due, J.**, Lactic dehydrogenase activity in blood, *Proc. Soc. Exp. Biol. Med.* **90:** 210-213 (1955).

[15] **Hess, R., Scarpelli, D.G.** and **Pearse, A.G.**, Cytochemical localization of pyridine nucleotide-linked dehydrogenase, *Nature, Lond.* **181:** 1531-1532 (1958).

[16] **Kornbleuth, W., Yardoni-Yaron, E.** and **Wertheimer, E.,** Glucose utilization of the retina: Influence of various media, *Archs. Opthalmol. New York.* **50:** 45-59 (1953).

[17] **Reif, L.** and **Adler, A.,** Effects of glucose concentration on cortisol induction of glutamine synthetase in chick embryo retinas, *Current Eye Res.* **2:** 117-122 (1982).

[18] **Weiss, S., Lester, T., Kalter, S.** and **Heberling, R.,** Chemically defined serum-free media for the cultivation of primary cells and their susceptibility to viruses, *In Vitro* **16:** 616-628 (1980).

[19] **Linser, P.** and **Moscona, A.,** Induction of glutamine synthetase in embryonic neural retina: Localization in Muller fibers and dependence on cell interactions, *Proc. Natl Acad. Sci. U.S.A.* **76:** 6476-6480 (1979).

[20] **Ramirez, G.** and **Seeds, N.,** Temporal changes in embryonic nerve cell recognition correlate with cholinergic development in aggregate cultures, *Devel. Biol.* **60:** 153-162 (1977).

[21] **Tholey, G., Ledig, M.** and **Mandel, P.,** Modification in energy metabolism during the development of chick glial cells and neurons in culture, *Neurochem. Res.* **7:** 27-36 (1981).

[22] **Pinto, M., Appay, M.D., Simon-Assmon, P., Chevalier, G., Dracopoli, N., Fogh, J.** and **Zeweibaum, A.,** Enterocytic differentiation of cultured human colon cancer cells by replacement of glucose by galactose in the medium, *Biol. Cell.* **44:** 193-196 (1982).

[23] **Zweibaum, A., Pinto, M., Chevalier, G., Dussaube, E., Triadou, N., Lacroix, B., Haffen, K., Brun, J.** and **Rousset, M.,** Enterocytic differentiation of a sub-population of the human colon tumor cell line HT-29 selected for growth in sugar free medium and inhibition by glucose, *J. Cell. Physiol.* **122:** 21-29 (1985).

نشاط إنزيم اللاكتات دهيدروجيناز أثناء زراعة خلايا شبكية عصبية جنينية في الزجاج

صالح عبد العزيز كريم و ديفيد دي بوميرا

قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز ، جدة ، المملكة العربية السعودية ؛ و قسم علم الحيوان ، جامعة نوتنجهام ، نوتنجهام ، المملكة المتحدة

المستخلص . تتحول خلايا الشبكية العصبية بعد عدة أسابيع من زراعتها إلى خلايا عدسية وذلك في بيئة F (بيئة تحتوى على نسبة بسيطة من الجلوكوز 6 m*M*) وتتثبط عملية التحول هذه عند زراعة نفس الخلايا في بيئة FG (بيئة تحتوى على نسبة عالية من الجلوكوز 28 m*M*) .

وباستقصاء نشاط إنزيم اللاكتات دي هيدروجيناز بطرق مختلفة تبين أن نشاطه يكون عاليا في البيئة التي تحتوى على نسبة عالية من الجلوكوز (FG) ، وعند استخدام طريقة الصبغ الكيميائي للأنسجة تأكد أن إنزيم اللاكتات دي هيدروجيناز يوجد بنسبة عالية ومكثفة في بيئة FG عنه في بيئة F . وتوضح نتائج التحليل بجهاز الإلكتروفورسيس أن هناك شريطين (two bands) من الإنزيمات الشبيهة يتكونان عند زراعة هذه الخلايا في بيئة FG عند عمر ٢٥ يومًا من الزراعة ، وذلك كما هو موجود لنفس الخلايا في الطور البالغ ، في حين في بيئة F ، وفي نفس العمر ، يتكون شريط إنزيم شبيه واحد وذلك كما هو موجود في خلايا الشبكية الجنينية . وتدل هذه النتائج على ارتفاع معدل الأيض في خلايا الشبكية مستخدمة الجلوكوز المتوافر بتراكيز عالية في بيئة FG .