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Glucose-6-phosphate Dehydrogenase Activity and Glycogen Content during Chick Neuroretinal Cell Culture

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ABSTRACT. Chick embryo neuroretinal (NR) cells transdifferentiate extensively into lens when cultured for several weeks in low glucose (F) medium, but largely inhibited when high levels of supplementary glucose (FG) are present. Glucose-6-phosphate dehydrogenase activity is higher in FG (28 m*M* glucose final) cultures as compared to F (6 m*M* glucose final) cultures. Another parameter strongly affected by ambient glucose levels is the accumulation of glycogen. Glycogen synthesis increases steadily in FG cultures, but decreases very slightly under F conditions. Glycogen accumulation in FG cultures is largely confined to glial-like (Muller) cells, particularly those underlying clusters of neurons. Thus, high glucose may act in concert with neuronal influences to stimulate or stabilize the normal differentiation of retinal glial cells, whose characteristic features *in vivo* include glycogen synthesis and storage.

Introduction

During long-term monolayer culture, chick embryo neuroretinal (NR) cells lose most of their differentiated characteristics and convert extensively into both melanised pigment cells[1] and crystallin containing lens-fibre-like cells (lentoids[2]). These changes are best described by the term 'transdifferentiation'[3-5]. Several early studies noted the differential effects on NR transdifferentiation of various medium formulations[6,7]. During growth of embryonic chick neural retina cells in standard "monolayer" culture, FG medium (*i.e.* F supplemented with glucose to 28 m*M* final)

inhibits lentoid appearance and δ crystallin accumulation; if the supplementary glucose is omitted from this medium (F; 6 m*M* glucose final), then extensive formation of lentoids is observed and high levels of δ crystallin accumulate[8]. In a slightly different medium formulation (FHG), high glucose blocks transdifferentiate completely[9], whereas low-glucose FH medium allows extensive transdifferentiation. Recently, Karim *et al.*[10] studied glucose metabolism in transdifferentiation (FH) and glucose-blocked (FHG) cultures. In this study, we investigate glucose-6-phosphate dehydrogenase activity and glycogen content in neural retina cultures maintained in F and FG media.

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 chemical from Sigma Ltd.

Methods

i) *Cell Culture*

Nine-day chick embryo NR cells were cultured for up to 50 days as described previously[8,11]. Cells were sown at a density of 5×10^6/ml in 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 was F medium containing extra glucose to 28 m*M* (instead of 6 m*M*). The protein content of all cultures was assayed by the method of Lowry *et al.*[12].

ii) *Glucose-6-phosphate Dehydrogenase (G-6-PDH) Activity*

(a) G-6-PDH activity was determined in saline extracts of cultures using the method of Kornberg and Horecker[13] as modified by Lohr and Waller[14].

(b) *G-6-PDH Histochemical Method.* G-6-PDH 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 G-6-PDH staining solution (1 *M* glycopyranose-6-phosphate disodium, 1*M* NADP, 0.1 *M* sodium cyanide, .2 *M* Tris buffer, 0.9 m*M* nitro blue tetrazolium, 0.5 *M* cobaltous chloride, 0.01 *M* sodium fluoride) for 20 minutes. G-6-PDH activity is shown by a purple red colour.

iii) *Glycogen Measurement and Localization*

(a) The glycogen content of retinal cultures was determined exactly as described by Rousset *et al.*[16], using the anthrone reagent. These assays were calibrated using known amounts of glucose alongside the test solutions of glycogen.

(b) The localisation of glycogen in retinal cultures was demonstrated histochemically using the periodic acid – Schiff (PAS) reagent as described by Hotchkiss[17]. PAS-positive (*i.e.*, glycogen-containing) cells are stained bright purple red.

Results

Figure 1 shows that G-6-PDH activity is consistently higher in FG than in F cultures, though the maximal differences occur between 14 and 21 days, being much smaller in early (5 day) and late (35-42 days) cultures. As shown in Fig. 2, G-6-PDH activity is more strongly stained in FG cultures (Fig. 2A and C) as compared to F cultures (Fig. 2B and D), and appears to be localised in or beneath neuronal cells. *In vitro*, glycogen levels (Fig. 3) increase steadily (from 7 until about 42 days) in FG cultures, but decline and then increase slightly in F cultures. The differences (between FG and F cultures) in glycogen levels become maximal at 35-42 days (Fig. 3).

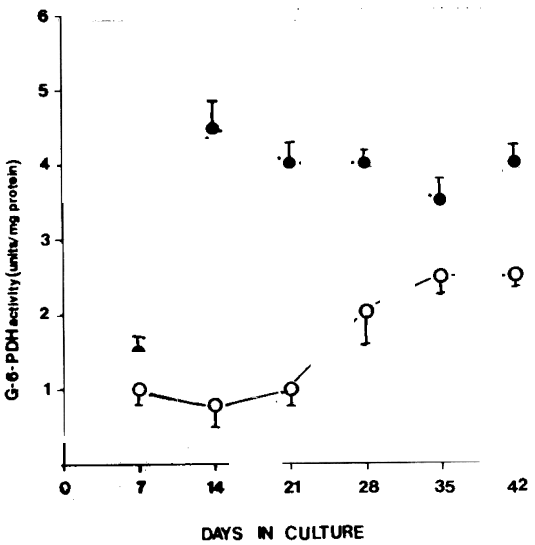


FIG. G-6-PDH activity in 9-day monolayer NR cultures. G-6-PDH was assayed as described in Methods; each point represents the mean and standard error derived from at least four replicate assays on two sets of cultures. ● ····· ●, FG; o ——— o, F.

The histochemical localisation of glycogen in neural retina cultures is shown in Fig. 4. In general, staining is more intense throughout in FG as compared to F cultures, in accordance with the biochemical assay (Fig. 3). At early stages of culture, intense staining is associated principally with clusters of neuronal cells (Fig. 4A).

Discussion

Chick embryo neuroretinal (NR) cells transdifferentiate into both lens and pigment cells after 4-5 weeks (1 and 2) when cultured in Eagle's minimal essential medium containing 10% foetal calf serum (F), but lentoid appearance and δ crystallin accumulation are both strongly inhibited when supplementary glucose is present (FG)[8,11].

One of the principal pathways of glucose metabolism is that involving glucose-6-phosphate dehydrogenase (pentose phosphate pathway). G-6-PDH activity was

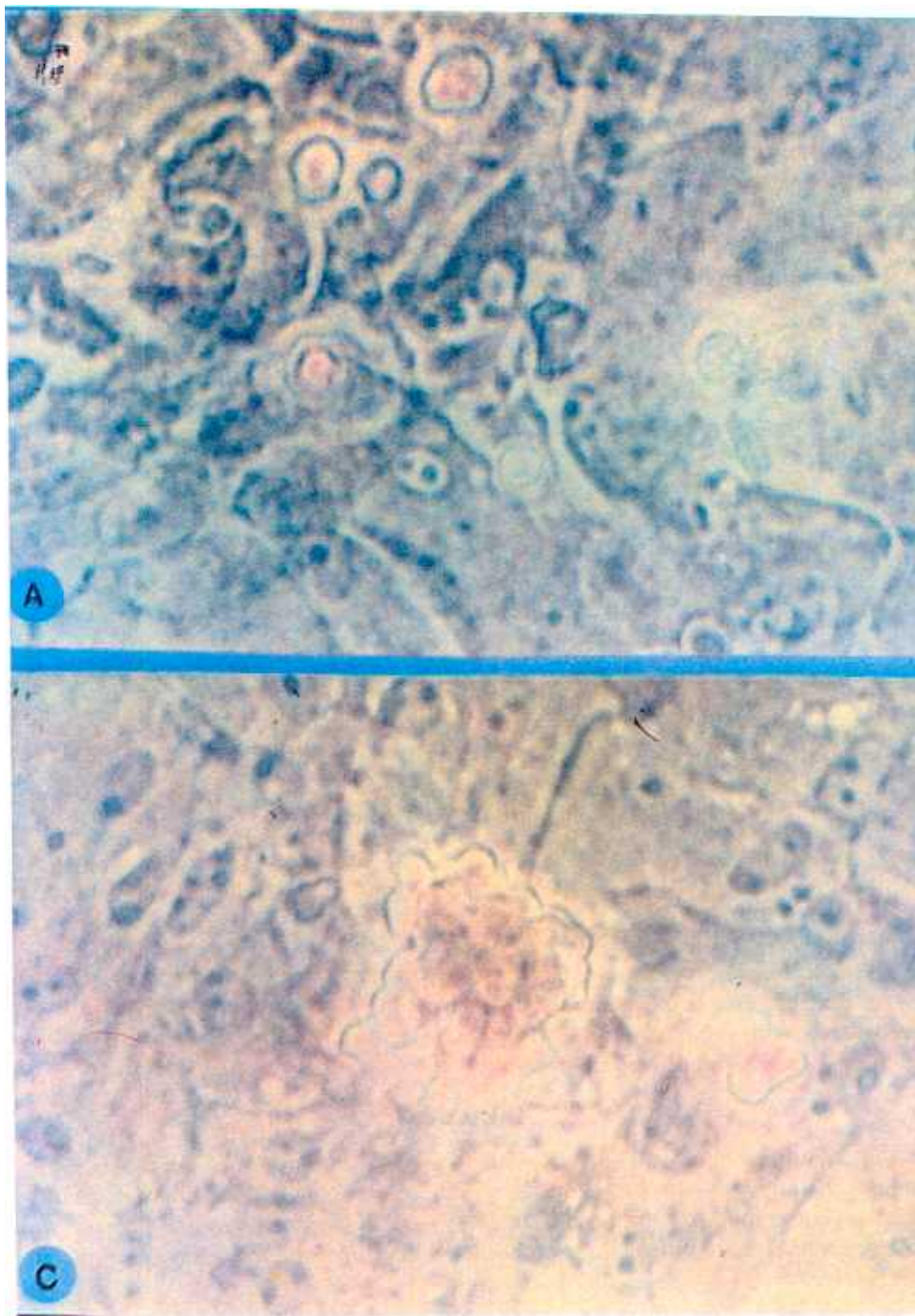
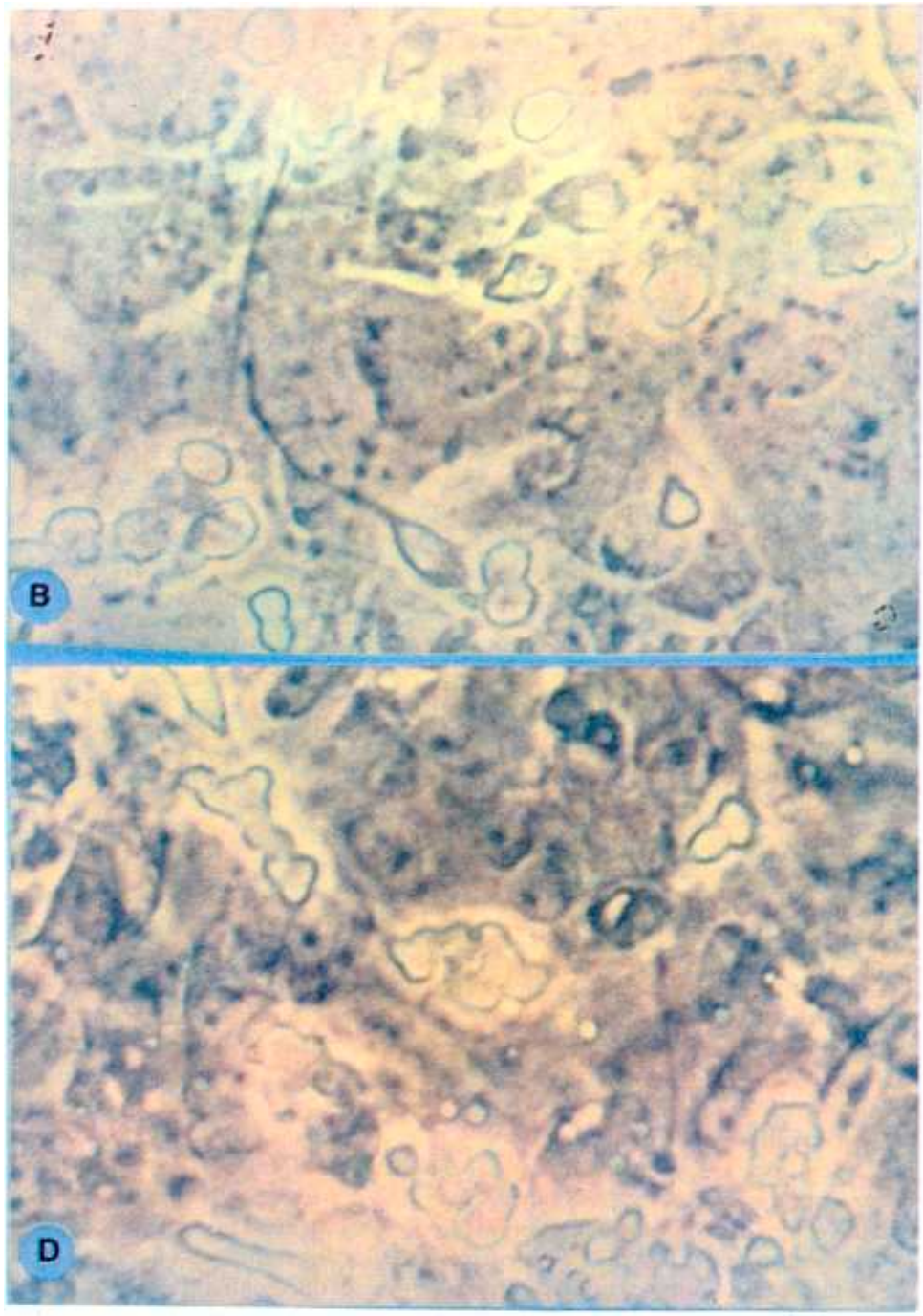


FIG. 2. G-6-PDH staining in monolayer NR cultures maintained in F and FG media. The G-6-PDH staining was performed as described in Methods. Frames (A) and (C) – FG cultures after 15 and 25



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

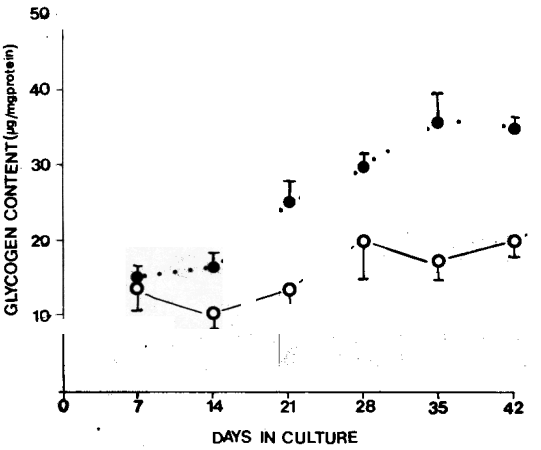


FIG. 3. Glycogen content in monolayer cultures of 9-day chick embryo NR cells. Glycogen levels were measured biodumically using the anthrone reagent (see Methods). Each point gives the mean and standard error derived from at least four replicate assays on two sets of cultures. ● ····· ●, FG; o ——— o, F.

found to be high during the early stages of NR culture in FG as compared to F medium (Fig. 1). Although the localisation of G-6-PDH activity cannot be specified precisely from our histochemical staining, the data in Fig. 2 suggest its presence mainly in the overlying neuronal cells, which are non-dividing (Fig. 2).

The pathway of glycogen synthesis from glucose in different chick embryo tissues including retina has been extensively studied. This pathway is found in retina *in vivo* and in culture[18-21]. In neural retina (NR) cultures, glycogen content increases to high levels in high glucose (FG) medium (Fig. 3) between 35 and 42 days of culture, while it decreases slightly in low glucose (F) medium during the same period. *In vivo,* glycogen production is largely confined to the glial Muller cells in retinal tissue[22]. Karim *et al.*[10] have demonstrated histochemically that this is also true *in vitro* (*i.e,* glycogen product is largely confined to Muller cells). This is most obvious at later stages of culture (Fig. 4C), but earlier the glycogen staining appears to be associated with clusters of neuronal cells (Fig. 4A), although in reality this substance is confined to the glial cells underlying such clusters[10]. Both this and the other evidence strongly suggest that many aspects of normal glial cells differentiation are prompted by the influence of retinal neurons, both *in vivo* and *in vitro*. Previously, it has been shown that the extent of transdifferentiation *in vitro* declines with increasing embryonic age of the starting NR[23]. This can be interpreted as reflecting the increased commitment of NR glial cells to follow a normal retinal pathway of differentiation, so precluding their transdifferentiation into lens. In dense monolayer NR cultures supplemented with hydrocortisone (HC), the glial marker enzyme glutamine synthetase (GSase)

can be induced transiently between about 4 and 16 days[24]. Moreover, HC reduces the extent of subsequent transdifferentiation into lens in these same cultures. It is plausible that glial cells directed towards normal differentiation (manifested, *e.g.*, as GSase expression) under the influence of HC *in vitro* may be precluded from changing over to a lens pattern of differentiation. Probably neuronal cells assist in this effect, since HC-induction of GSase activity is largely confined to those glial cells underlying clusters of neurons in dense NR cultures[25]. We suggest that the basis of the high-glucose on transdifferentiation[9,11] is fundamentally similar. Neuronal influences and high glucose conditions act together to promote glycogen production in NR cultures.

References

[1] **Itoh, Y., Okada, T.S., Ide, H.** and **Eguchi, G.**, The differentiation of pigment cells in cultures of chick embryonic neural retina, *Dev. Growth Differ* **17:** 39-50 (1975).

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

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

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

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

[6] **Clayton, R.M., Depomerai, D.I.** and **Pritchard, D.J.**, Experimental manipulation of alternative pathways of differentiation in cultures of embryonic chick neuroretina, *Dev. Growth Differ.* **19:** 319-328 (1977).

[7] **Agata, K., Kondoh, H., Takagi, S., Nomura, K.** and **Okada, T.S.**, Comparison of neuronal and lens phenotype expression in the transdifferentiating cultures of neural retina with different culture media, *Dev. Growth Differ.* **22:** 571-577 (1980).

[8] **De Pomerai, D.I.** and **Gali, M.A.**, Influence of serum factors on the prevalence of normal and foreign differentiation pathways in cultures of chick embryo NR cells, *J. Embryol. Exp. Morphol.* **62:** 291-308 (1981).

[9] ————. A switch for transdifferentiation in culture: Effects of glucose on cell determination in chick embryo neuroretinal cultures, *Dev. Biol.* **93:** 534-538 (1982).

[10] **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 6-crystallin accumulation, *Cell Differ.* **22:** 29-46 (1987).

[11] **De Pomerai, D.I.** and **Gali, M.A.**, Determination of chick neuro-retinal 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).

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

[13] **Kornberg, A.** and **Horecker, B.**, Glucose-6-phosphate dehydrogenase. *In:* **Colowick, S.** and **Kaplan, N. (ed.)**, *Methods in Enzymology*, Vol. 1, Academic Press, New York, pp. 323-327 (1955).

[14] **Lohr, G.** and **Waller, H.**, Glucose-6-phosphate dehydrogenase. *In:* **Bergmeyer, H.U. (ed.)**, *Methods of Enzymatic Analysis*, Academic Press, New York, pp. 636-643 (1974).

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

[16] **Rousset, M., Zweibaum, A.** and **Fogh, J.**, Presence of glycogen and growth related variations in 58 cultured human tumor cell lines of various tissue origins, *Cancer Res.* **41:** 1165-1170 (1981).

[17] **Hotchkiss, R.D.**, A microchemical reaction resulting in the staining of polysaccharide structure in fixed tissue preparations, *Arch. Biochem.* **16:** 131-191 (1948).

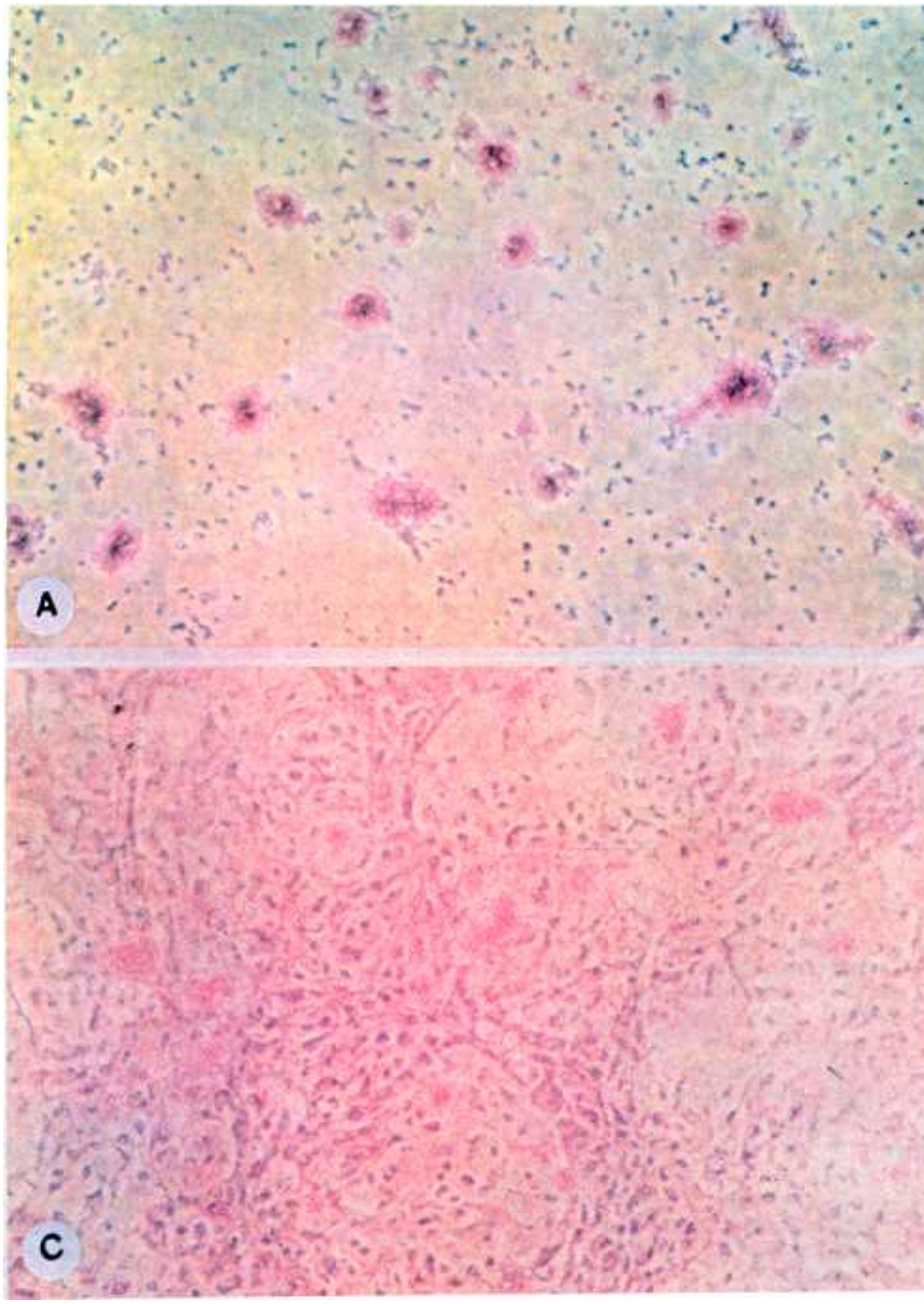
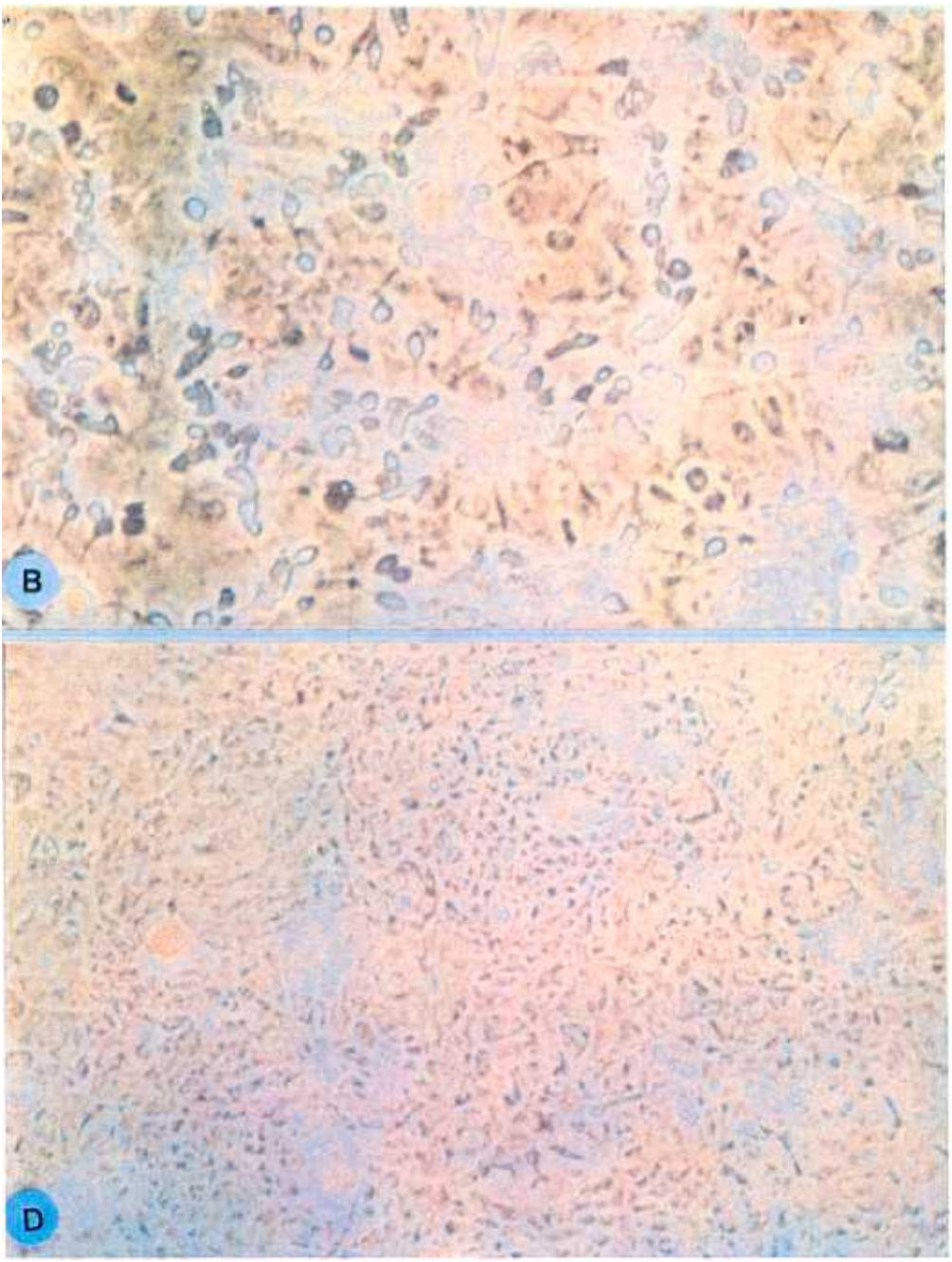


FIG. 4. Photographs of 9-day chick embryo. NR cells in monolayer culture, stained to illustrate the variation of glycogen content in low-glucose (F) and high-glucose (FG) cultures. PAS reagent was used for the histochemical demonstration of glycogen, as detailed in Methods. Frames (A) and (C) –



FG cultures after 20 and 30 days *in vitro*, × 80 magnifications. Frames (B) and (D) – F cultures after 20 and 30 days *in vitro*, × 80 magnifications.

[18] **Kent, S.P.**, Further observations on the effect of X-irradiation on the glycogen content and histology of the rabbit retina, *Radiat. Res.* **10** 380-385 (1959).
[19] **Kuwabara, T.** and **Cogen, D.**, Retinal glycogen, *Arch. Opthal.* **66:** 680-688 (1961).
[20] **Mucalh, M.M.** and **Coimbra, A.**, Electron microscope radioautographic study of glycogen synthesis in the rabbit retina, *J. Cell. Biol.* **47:** 263-275 (1970).
[21] **Mizuno, K.** and **Sato, K.**, Reassessment of histochemistry of retinal glycogen, *Exp. Eye Res.* **21:** 489-497 (1975).
[22] **Rhodes, R.H.**, Ultrastructure of Muller cells in the developing human retina, *Graefe's Arch. Clin. Opthalmol.* **221:** 171-178 (1984).
[23] **De Pomerai, D.I.** and **Clayton, R.M.**, Influence of embryonic stage of the transdifferentiation of chick neural retinal cells in culture. *J. Embryol. Exp. Morphol.* **47:** 179-193 (1978).
[24] **De Pomerai, D.I., Carr, A., Soranson, J.** and **Gali, M.A.**, Pathways of differentiation in chick embryo neuro-retinal cultures, *Differentiation* **22:** 6-11 (1982).
[25] **Linser, P.** and **Moscona, A.**, Hormonal induction of glutamine synthetase in cultures of embryonic retinal cells: requirement for neuron-glia contact interaction, *Dev. Biol.* **96:** 529-534 (1983).

نشاط إنزيم الجلوكوز-٦-فوسفات دي هيدروجيناز والمحتوى الجليكوجيني خلال زراعة الخلايا الجنينية لطبقة الشبكية العصبية

صالح عبد العزيز كريم و ديفيد دي بوميرا

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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 في بيئة FG (بيئة تحتوى على نسبة عالية من الجلوكوز 28 m*M*) . تبين من الدراسة أن نشاط إنزيم جلوكوز-٦-فوسفات دي هيدروجيناز يكون عاليا في بيئة FG مقارنة ببيئة F . وكذلك فإن تكوين الجليكوجين وتراكمه يزيد أثناء زراعة الخلايا الجنينية للشبكية العصبية في بيئة FG ويقل في بيئة F . وتُفسر زيادة تراكم الجليكوجين بوجود خلايا مولر (Muller cells) وخاصة الموجودة تحت الخلايا العصبية المتجمعة (المتكتلة) . وهكذا ، فإن النسبة العالية للجلوكوز في بيئة FG يمكن أن تعمل ، مع تأثيرات الخلايا العصبية ، على تحسين التمايز الطبيعي لخلايا مولر التي من خصائصها تكوين الجليكوجين وتخزينه داخل الكائن الحي (*in vivo*) .