

Influence of Retinal Extracts and Fibroblast Growth Factor on Lentoidogenesis in the Chick Embryo Retinal Glial Cell Cultures

S.A. KARIM*, F.M. AL-KUDSI* and D.I. DE POMERA**

**Department of Biology, Faculty of Science,*

King Abdulaziz University, Jeddah, Saudi Arabia

***Department of Life Science, University of Nottingham,
University Park, Nottingham, U.K.*

ABSTRACT. Extract prepared from embryonic chick retina stimulates growth and particularly lentoidogenesis when added as a supplement to retinal glial (RG) cultures *in vitro*. This effect is especially marked when using a medium (H) containing 5% horse serum, where growth factors are likely to be limiting. The number of lentoids in such cultures increases with the concentration of extract. Using extracts from earlier and later stages of retinal development, there is an age-dependent decline in the extent to which lentoidogenesis is stimulated. It is suggested that these efforts are mediated by growth factors present in the retinal extract acting upon Muller glial cells or their precursors in the RG cultures. In support of this suggestion, we show that purified fibroblast growth factor (but not epidermal growth factor) exerts similar effects on δ -crystalline in accumulation and number of lentoids.

Introduction

It is known that the transformation of the cells from one kind to another is called transdifferentiation. Okada *et al.* showed that neural retina cultures can transdifferentiate into lentoids (small lens)^[1], and from here the term lentoidogenesis. Depomerai and Gali^[2] found that lentoidogenesis was promoted in 9 days embryonic neural retina culture by adding 9 days chick embryo extracts but this promotion decreases in the presence of adult chicken serum or even new born bovine serum. On

the other hand, adding retina extract to a medium with low percentage of lentoidogenesis promotes lentoidogenesis, and increases the number of lentoids.

Numerous *in vitro* studies showed that retinal extract can stimulate the growth of lens epithelial cells^[3,4]. Recently, Karim and Depomerai^[3] reported that a crude retinal extract prepared from chick embryos is able to promote transdifferentiation into lens. Similar effects result from supplementation with purified fibroblast growth factor^[3,5].

The present study reports the influence of retinal extracts and fibroblast growth factor on lentoidogenesis in the chick embryo retinal glial cell cultures.

Materials and Methods

Materials

Fertile eggs were obtained from home raised chicken. Tissue culture media and sera were from Gibco-Europe, and most chemicals from Sigma.

Methods

(i) Cell culture

Nine-day chick embryo NR cells were cultured for up to 50 days^[6]. Cells were sown at a density of 5×10^6 /ml medium comprising Eagle's MEM with Earle's salts, 26 mM NaHCO_3 , 2 mM L-glutamine, 100 I.U./ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 5% horse serum. NR cultures were stripped of > 90% of neuronal N cells by treatment with 50 μm -Chinoform-ferric chelate between the days 3 and 7 *in vitro* according to the method of Ohtsuka *et al.*^[7]. The resultant cultures are composed of monolayer sheets of epitheloid glial-like G-cells (Muller glia and their precursors) Fig. 1.

(ii) Retinal extract preparation

Retinal extract was prepared as described by Arruti and Courtois^[6]. Retinas from recently killed chick embryos (8, 12 and 18 days old) were homogenized in an equal volume of phosphate buffered saline. After centrifugation at 500 Xg for 30 minutes, the supernatant was passed through a succession of Millipore filters of decreasing pore diameter (.2, 0.45 and 0.22 μm).

(iii) Quantification of δ -crystalline

Haemagglutination-inhibition assays were performed as described previously by Depomerai *et al.*^[8], using monospecific anti- δ -crystalline antiserum (11) and indicator sheep red blood cells coated with total newly-hatched-chick lens proteins (rich in δ -crystalline) extracts of cultures.

Results

Figure 2 compares the appearance of lentoids in 45-day cultures maintained in HR_2 and HR_3 . Lentoids are prominent in the HR_1 and HR_2 cultures (Fig. 2a), but few if any could be found in HR_3 and H cultures (Fig. 2b).

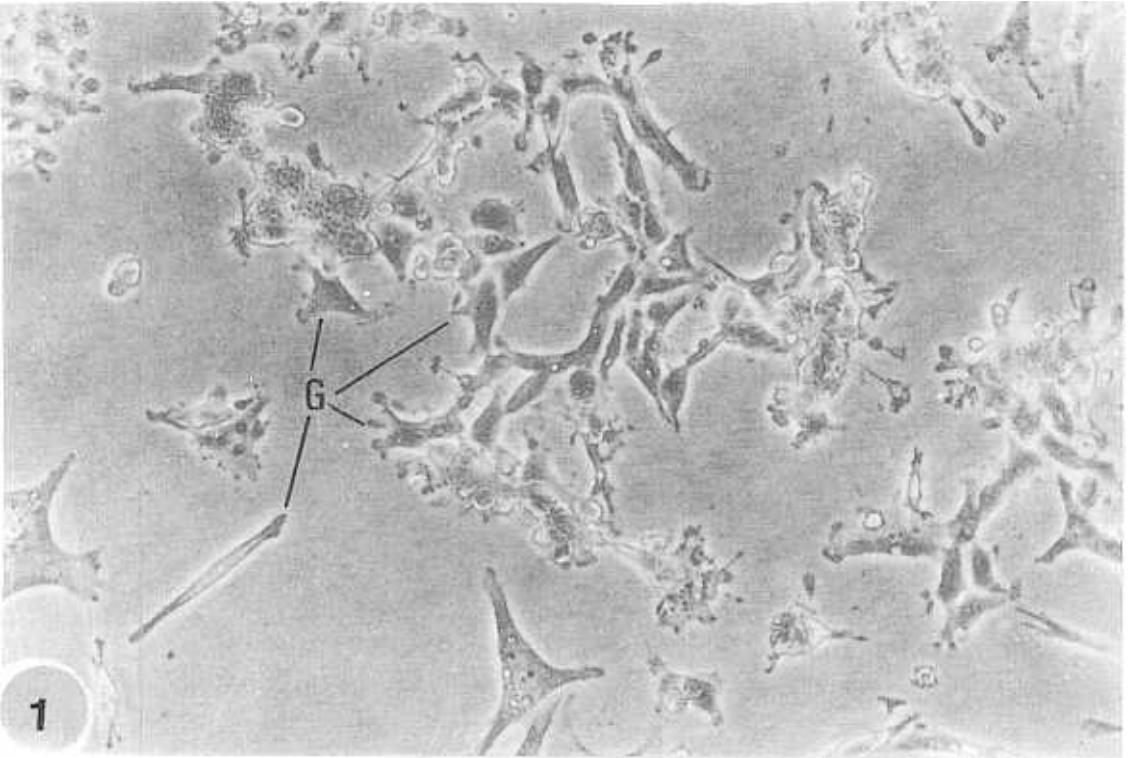


FIG. Phase contrast photograph showing glial cells in culture after removal of the neuronal cells by chionoform treatment ($\times 120$).

Figure 3 shows the number of lentoids formed on the 45th day of the chick embryonic retina glial cell culture when using different stages of the retinal extracts. As seen in the figure, the number of lentoids in HR₁ was the greatest, while it became less in HR₂ and the lowest levels were in HR₃ and H cultures. Finally, we have tested the effects of two purified growth factors (FGF and EGF) on δ -crystalline accumulation and number of lentoids in RG cultures using H medium. The concentrations used were those shown to give half-maximal responses to FGF in terms of proliferation (3 mg/ml) and fibre differentiation (40 mg/ml) in cultures of rat lens epithelial cells^[5]. Slight stimulation of δ -crystalline accumulation (Fig. 5) and lentoids number (Fig. 4) was observed using the lower concentration (3 mg/ml) of FGF as a supplement for H medium, while EGF had no significant effect at either concentration. A much more dramatic stimulation of δ accumulation (about 7 fold) and lentoids number about (10-fold) was obtained with higher concentration of FGF (40 mg/ml).

Discussion

The ability to promote transdifferentiation of neural retina cells into lens is shared

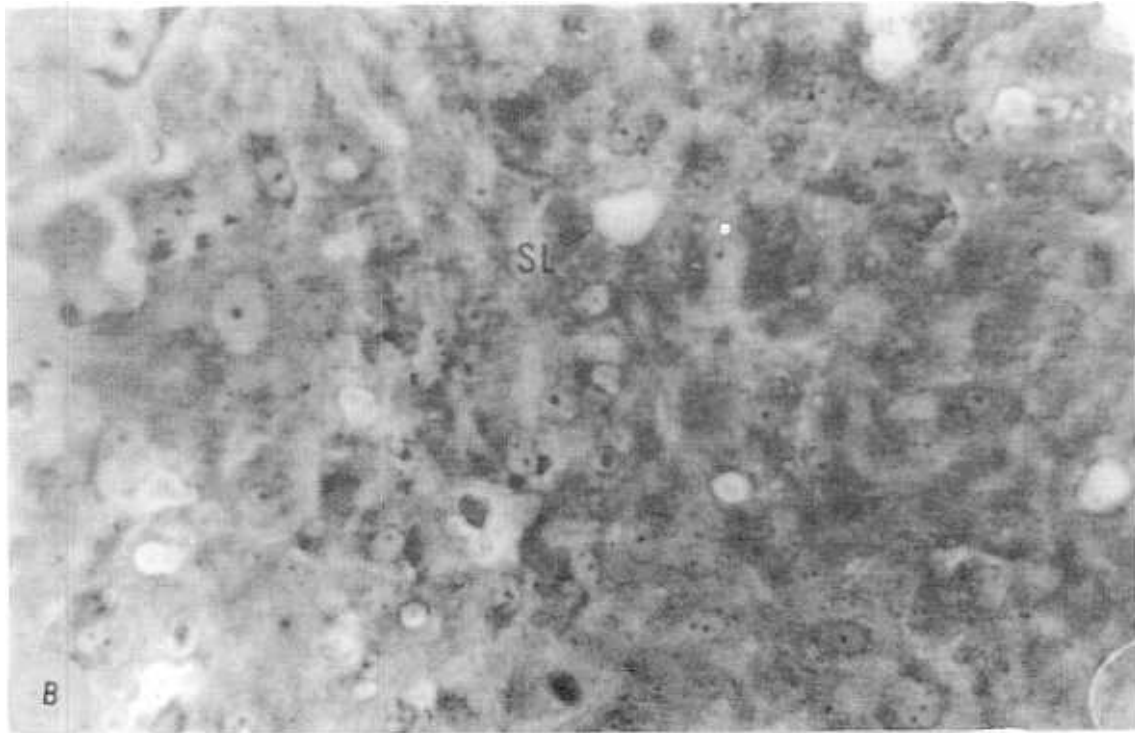
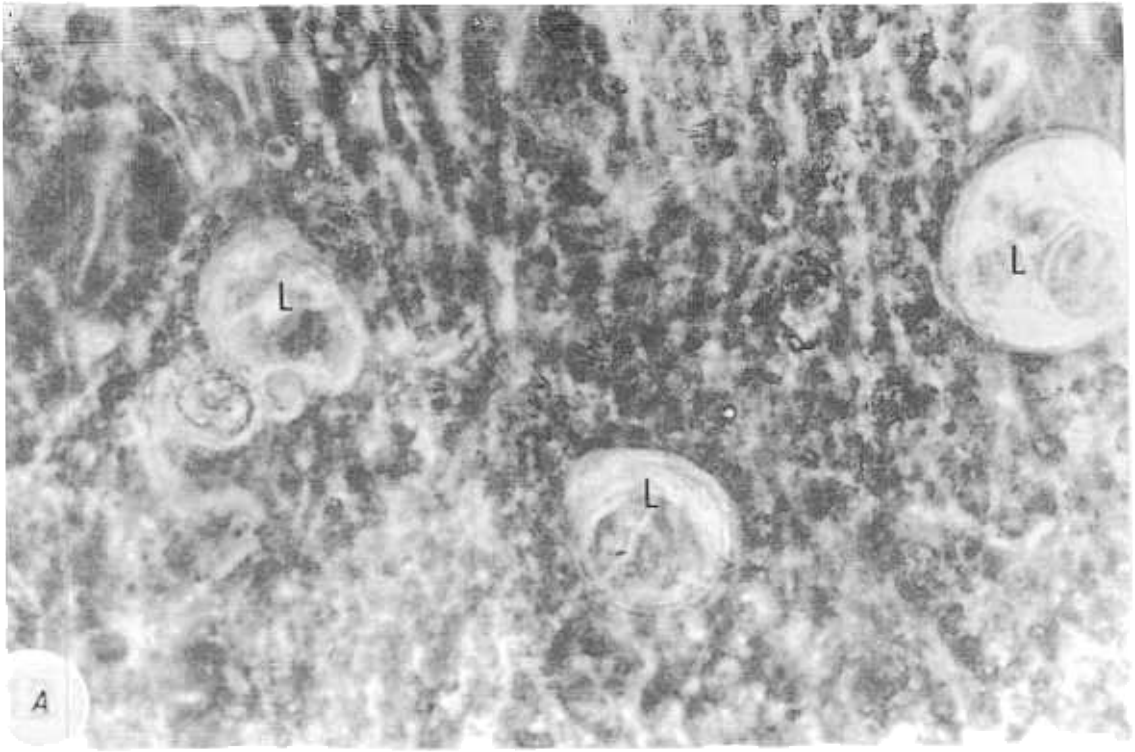


FIG. 2. Phase contrast photograph showing typical field from 45 day cultures. (a) in HR_2 media (10% of 12 day chick embryo retina extract ($\times 200$)). (b) in HR_3 media (10% of 18 day chick embryo retina extract) ($\times 200$).

L : Lentoids

SL : Small lentoids.

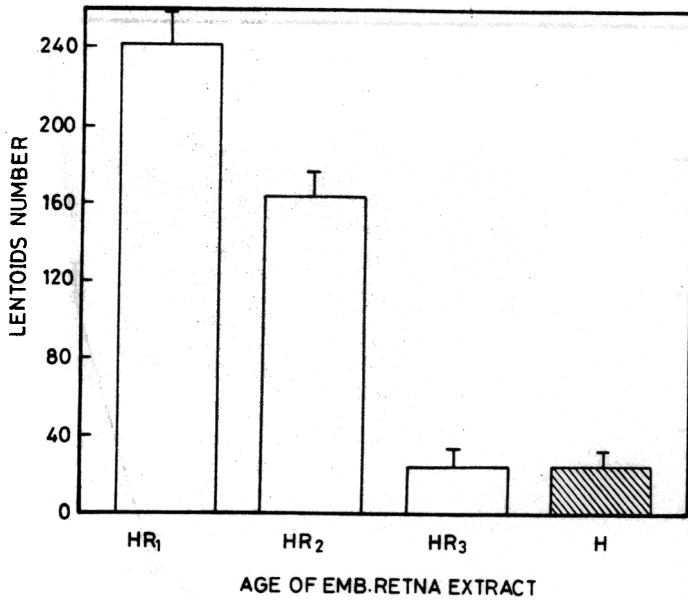


FIG. 3. Number of lentoids formed during embryonic glial cell cultures of retina in HR₁, HR₂, HR₃ and H medium at 45 days of cultures. Each point gives the mean and standard error (vertical bar) derived from at least four culture dishes.

HR₁ (MEM + horse serum + 8 day-chick embryo retina extract).

HR₂ (MEM + horse serum + 12 day-chick embryo retina extract).

HR₃ (MEM + horse serum + 18 day-chick embryo retina extract).

H control (MEM + 5% horse serum).

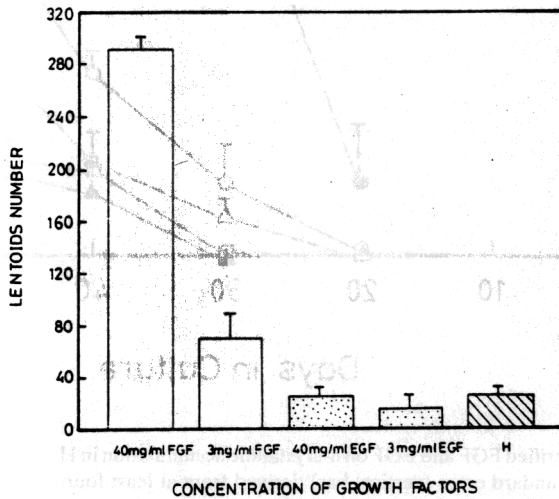


FIG. 4. Effects of purified FGF and EGF on lentoids number in H cultures (45 day cultures). Each point gives the mean and standard error derived from at least four culture dishes.

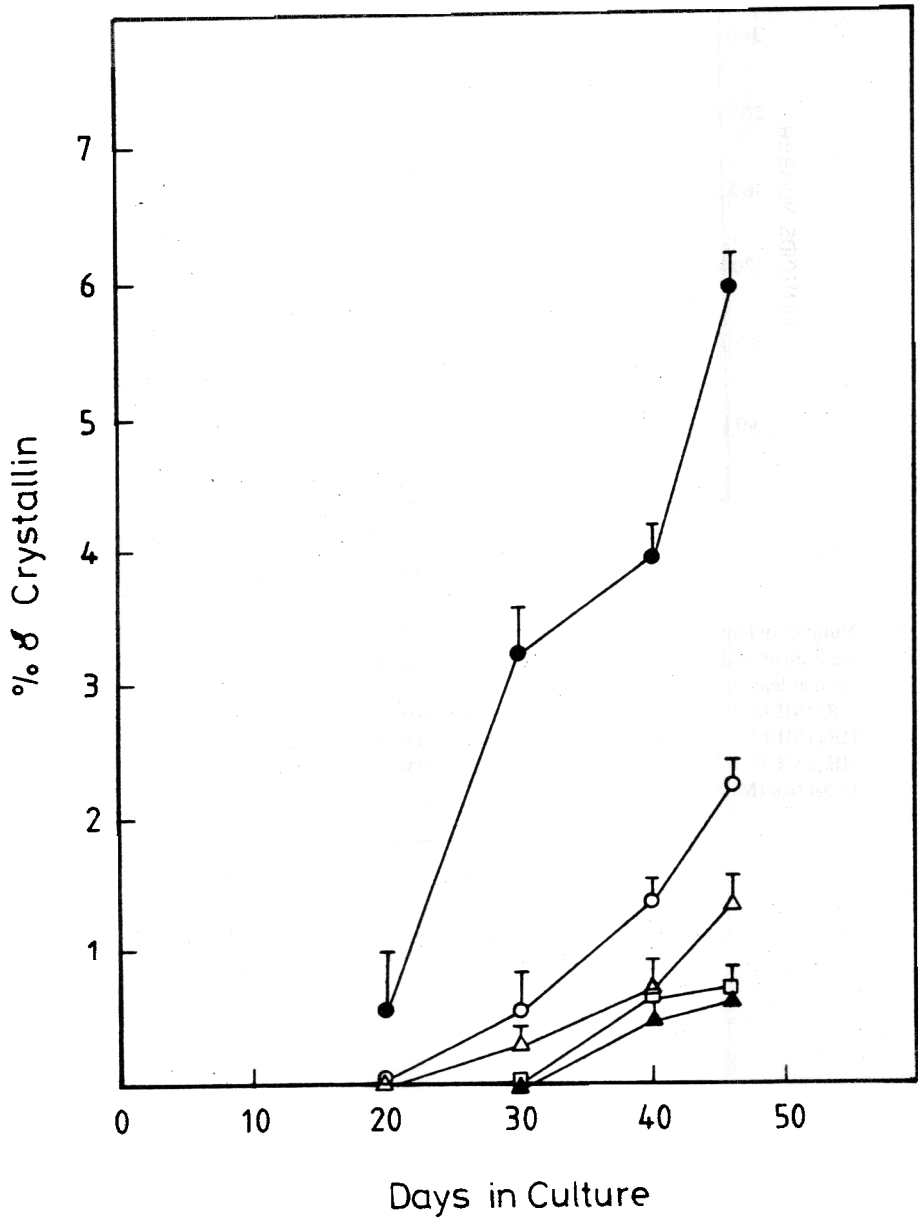


FIG. 5. Effects of purified FGF and EGF on δ -crystallin accumulation in H cultures. Each point gives the mean and standard error (vertical bar) derived from at least four culture dishes. ●—●, H medium plus 40 mg/ml FGF; ○—○, H medium plus 3 mg/ml FGF; △—△, H medium plus 40 mg/ml EGF, ▲—▲, H medium plus 3 mg/ml EGF; (H control is shown as ■—■).

by chick embryo extract, whereas adult chicken serum support much less extensive production of lens cells (Depomerai and Gali)^[2]. It is particularly striking that the low molecular weight dialysis fraction prepared from embryonic extract is able to promote neural retina transdifferentiation into lens, whereas that from adult is not. These effects might be related to the rapid differentiation of many different cell types during embryonic development as compared with the relative stability of the differentiated state during adult stages (Depomerai and Gali)^[2].

Our data presents a consistent pattern whereby the stimulator effects of retinal extract on transdifferentiating RG cultures are most clearly seen when supplementary H medium, which itself supports little lentoids production. The simplest interpretation is that components of the retinal extract act as growth factors to stimulate mitosis among retinal glial cells. It will be interesting to determine whether the active factors in RG transdifferentiation are the same as those which promote new lens regeneration in organ culture (i.e. EDGE II and III; Cuny *et al.*)^[9].

The reason why the retinal extract is most active in H cultures is probably due lack of this medium to serum growth factors (Depomerai and Gali)^[2]. Recently, Karim and Depomerai (1990)^[3] found that cell numbers decline slowly throughout the culturing period in H medium, but increase markedly after two weeks in HR medium (with retinal extract). Going back to the effects of retinal extracts on the extent of lentoidogenesis (monitored as lentoids number), two features are eminent. First, there is a clear dose-dependence on the concentration of retinal extract added (Karim and Depomerai)^[3]. Second, extracts prepared from early embryonic retina show higher activity than in those prepared from later stages. The clear implication of this is that the active factors are present in higher levels during early development and declining as the tissue architecture becomes established and cell proliferation slows down. If the eye-derived growth factors are themselves of retinal origin, then the survival of factor-secreting cells during *in vitro* culturing might, in part, account for the fact that early embryonic neural retina cells transdifferentiate much faster and more extensively than do their counterparts from later stages^[3,10].

The effect of retinal extract or purified FGF which mediated via glial cell division is supported indirectly by studies of CAT activity^[3]. Our data (using RG cultures) suggest that Muller glial (precursor) cells probably carry receptors for growth factors present in the retinal extract, such as FGF/EDGFII^[11]. Some evidence in support of this view is provided by the data in Fig. 4 and 5, which show that purified FGF (but not EGF) can exert similar stimulatory effects on lentoids number and accumulation when added as a supplement to H-medium cultures.

References

- [1] Okada, T., Ioh, Y., Watanabe, K. and Eguchi, G., Differentiation of lens in cultures of neural retina cells of chick embryos, *Dev. Biol.* **45**: 318-329 (1975).
- [2] Depomerai, D.I. and Gali, M., Embryonic serum factors required for transdifferentiation of chick embryo neural retina cells in culture, *Dev. Growth Diff.* **24**: 233-243 (1982).
- [3] Karim, S. and Depomerai, D.I., Stimulatory effects of retinal extract on lentoidogenesis in culture of chick embryo neuroretinal cells, *Cell Diff. Dev.* **31**: 176-196 (1990).

- [4] Arruti, C. and Courtois, G., Morphological changes and growth stimulation of bovine epithelial lens cells by a retinal extract *in vitro*, *Exp. Cell Res.* **117**: 282-292 (1978).
- [5] McAvoy, J.W. and Chamberlain, C.G., Fibroblast growth factor (FgF) induces different responses in lens epithelial cells depending on its concentration, *Development* **107**: 221-228 (1989).
- [6] Depomerai, 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).
- [7] Ohtsuka, K., Ohishi, N., Eguchi, G. and Yagi, K., Degeneration of cultured neural retina cells chionoform ferric, *Chelata Experientia* **38**: 121-122 (1982).
- [8] Depomerai, D.I. and Ellis, D.K. and Carr, A., Alamprey lens protein related to avian and reptilian &-crystalline, *Curr. Eye Res.* **3**: 729-735 (1984).
- [9] Cuny, R., Jeanny, J. and Courtois, Y., Lens regeneration from cultured newt irises stimulated by retina-derived growth factors (EDGFS), *Differentiation* **32**: 221-229 (1986).
- [10] Depomerai, D.I. and Clayton, R.M., Influence of embryonic stage on the transdifferentiation of chick neural retina cells in culture, *J. Embryol. Exp. Morph.* **62**: 291-308 (1978).
- [11] Courty, J., Loret, C., Moenner, M., Chevalier, B., Lagente, O., Courtois, Y. and Barritault, D., Bovine retina contains three growth factor activities with different affinity to heparin: eye-derived growth factor, I, II, III, *Biochimie*, **67**: 265-269 (1985).

تأثير مستخلصات الشبكية وعامل نمو الخلايا الليفية على تكوين العدسيات في زراعات الخلايا الغروية لشبكية أجنة الدجاج

صالح عبد العزيز كريم* ، فاطمة محمد سعد القدسي* 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 . المستخلص المحضر من شبكية أجنة الدجاج يحفز نمو وتكوين العدسيات عند إضافته إلى زراعات خلايا الشبكية الغروية . ويلاحظ هذا التأثير جيداً عند استعمال بيئة (H) المحتوية على 5٪ من مصّل الحصان ، حيث تكون عوامل النمو محدودة . يزداد عدد العدسيات في مثل هذه الزراعات بزيادة تركيز المستخلص . باستعمال مستخلصات الشبكية لأطوار جنينية مبكرة أو متأخرة يتكون لدينا انحناء متوقف على العمر في مدى حث هذه المستخلصات لتكوين العدسيات . وتتوسط هذه التأثيرات عوامل النمو الموجودة في مستخلصات الشبكية ، حيث تؤثر هذه على خلايا مولر Muller cells الغروية أو على أسلافها في زراعات الغروية الشبكية (RG) . وبدعم من هذا الاقتراح ، نظهر أن عامل نمو الخلايا الليفية (ليس عامل نمو ابيديرمي epidermal) يؤثر بنفس الطريقة على تراكم الكريستالين (&-crystallin) وأعداد العدسيات .