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Effect of Extrinsic Factors on Lentoidogenesis in Chick Embryo Retinal Glial Cell Cultures

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ABSTRACT. The effect of some factors like serum, medium and glucose on lentoidogenesis were studied in chick embryo retinal glial cell cultures, each one alone. Foeteal calf serum had great ability to support lentoidogenesis during RG culture especially in high concentrations, while using horse serum during RG culture supported only little lentoids production. Using the MEM medium with 10% foetal calf serum (F) in the same cultures produced good lentoids. On the other hand, using the 199 medium with 10% foetal calf serum (F) produced small number of small lentoids, but not as much as those formed in the MEM media culture. As well as we found that glucose block lentoidogenesis in these cultures.

Introduction

The transdifferentiation of NR cells into lentoids is highly affected by the type of medium and its constituents used during culturing. Using MEM medium (minimal essential medium) with 10% foetal calf serum permitted lentoidogenesis within 28 days of culturing, Okada *et al.*[1] While using MEM medium with 10% horse serum does not support lentoidogenesis, Depomeral and Gali[2]. On the other hand using 199 medium with 10% foetal calf serum blocks lentoidogenesis from NR cells, Gali and Depomeria[3].

Lens specific Delta crystalline is accumulated when culturing chick embryo neural retina cells in MEM medium containing 5% foetal calf serum and 5% horse serum (FH) medium, but these cells stop their transdifferentiation to lentoids if an excess of

glucose is added to their culture medium which is now called (FHG) medium, Depomerai and Gali[4].

The present study reports the effect of extrinsic factors on lentoidogenesis in chick embryo retina glial cell cultures.

Material and Methods

Material

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

Methods

Cell Culture

Nine-day chick embryo NR cells were cultured for up to 50 days[4]. 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 and 100 μg/ml streptomycin. 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.*[5] 5% foetal calf serum, and 5% Horse serum (FH medium) F medium comprised FH medium but with 10% foetal calf serum and without horse serum. H medium comprised FH medium, but with 10% horse serum instead of 5% horse serum and 5% foetal calf serum.

FHG medium comprised FH medium containing extra glucose 18 mM final (instead of 6 mM). F199 medium like FH medium, but with 10% FCS in place of 5% each of FCS and HS, and with medium 199 replacing MEM.

Results

As shown in (Fig. 1a), both small and large lentoids are appeared by foetal calf serum concentrations, but when adding different concentrations of the horse serum to the media, neither small lentoids nor large lentoids appeared in early stage (Fig. 1b). As shown in Fig. 2 high concentrations of foetal calf serum had great ability to support lentoidogenesis during glial cell culture, while low concentrations of FCS delays the appearance and amount of lentoids. Also from the figure, it can be seen that in case of horse serum, there is also a clear inverse dose-response relationship between the maximal levels of lentoids number and the concentrations of serum in the medium used.

During chick embryo retina glial cell cultures in the MEM media with 10% foetal calf serum (F), well developed lentoids (L) were formed as seen in Fig. 3a. Also in the same figure one bottle cell (bc) near the lentoid is seen, but having the same kind of culture in 199 media, only small number of small lentoids were formed (Fig. 3b). Figure 4 compares the number of lentoids formed when using each of 199 and MEM media during the chick embryo retina glial cell cultures.

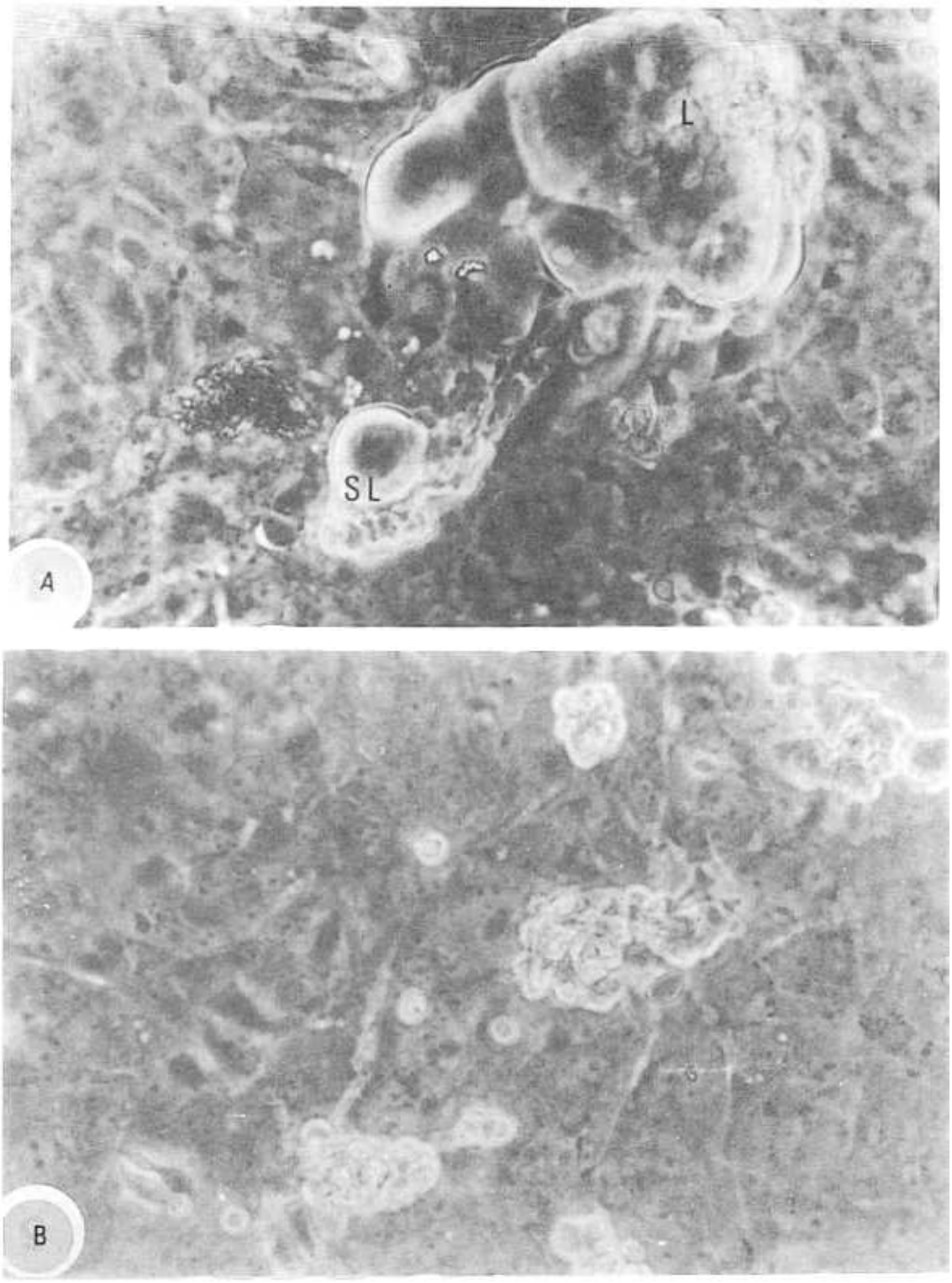

FIG. 1. (a) Lentoidogenesis during embryonic glial cells culture of retina in media containing 10% foetal calf serum (× 200).
SL : small lentoid
L : lentoid
(b) culture of 9-day embryonic glial cells of retina in media containing 10% horse serum (× 200).

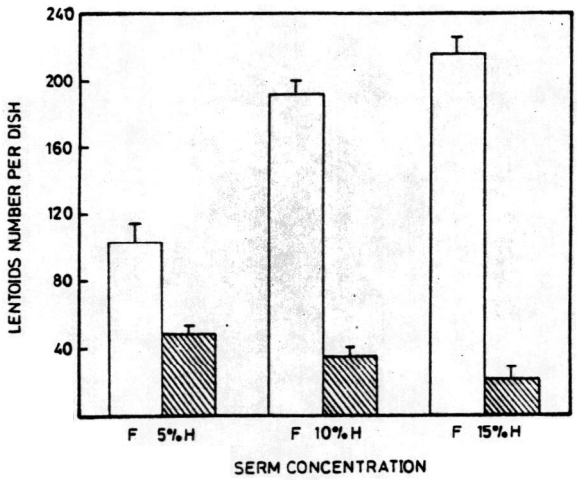

FIG. 2. Number of lentoids formed during embryonic glial cell culture of retina in different concentrations of foetal calf serum and horse serum of 45 days of cultures. Each point gives the mean and standard error (vertical bar) derived from at least four culture dishes.

Glial cells in the experimental cultures in FH media showed a precocious appearance of pigmentation and small lentoids, which became pigmented cells and large lentoids as seen in Fig. 5a. On the contrary, it can be seen from Fig. 5b that FHG medium blocked lentoidogenesis completely during chick embryo retina glial cell cultures.

As it is clear from Fig. 6, the number of lentoids formed during the culture of chick embryo retina glial cell in the FH medium is very high in comparison with the number of lentoids formed during the culture of the same kind of cells in the FHG medium.

Discussion

Extensive lentoidogenesis is strongly promoted by 10% foetal calf serum (FCS), while 10% horse medium (HS) do not support this process (Figs. 1a,b) (Depomerai and Gali).[2] In media 10% horse serum, neuronal cell processes develop better than in media supplemented with 10% foetal calf serum (Depomerai and Gali).[2,4,6,7] The results obtained (Fig. 2) indicate that the lentoid production varies markedly according to the serum used and its concentrations (Depomerai and Gali).[6] The present study shows that high foetal calf serum concentrations support better lentoidogenesis while supplementing the medium with high horse serum concentrations leads to opposite results (Fig. 2). These preliminary results indicate that high foetal calf serum concentration may stimulate glial cell proliferation. Increasing the foetal calf serum concentration enhances lentoidogenesis. However, this relationship between serum concentration and lentoidogenesis is not clear-cut with horse serum as

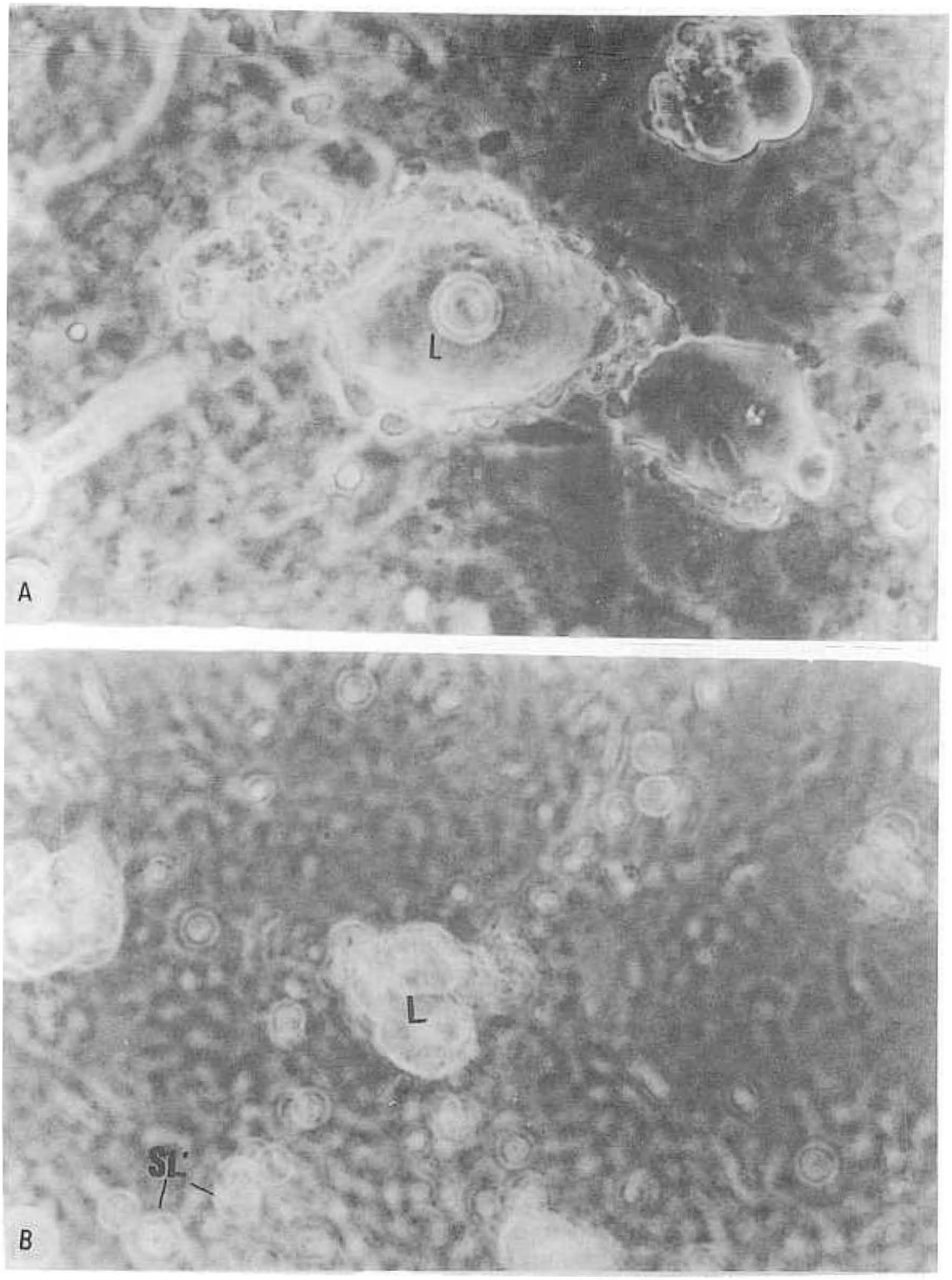

FIG. 3. (a) Effect of using the MEM medium during chick embryo retina glial cell cultures on lentoidogenesis (× 120). Notice the lentoids (L) and bottle cell (BC).
(b) Effect of using the 199 medium during chick embryo retina glial cell cultures on lentoidogenesis (× 120). Notice the small lentoids formations (SL) with some lentoids (L).

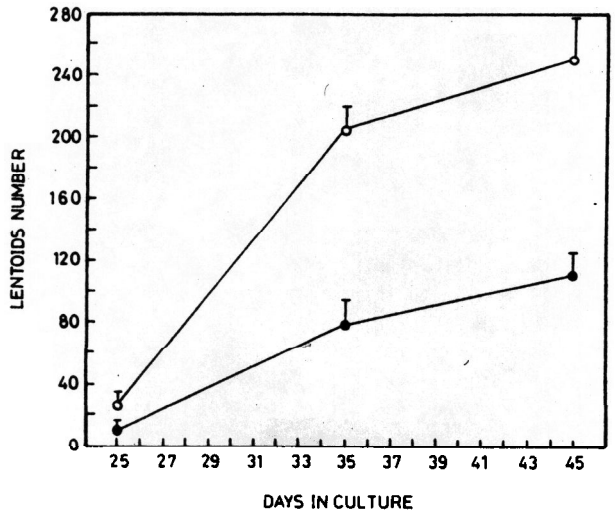

FIG. 4. Effect of medium on lentoidogenesis and number of lentoids formed during chick embryo retina glial cell cultures in each of MEM and 199 media. Each point gives the mean and standard error (vertical bar) derived from at least four culture dishes.
o———o, MEM media
●———●, 199 media.

with foetal calf serum. Depomerai and Gali[7] suggested the presence of stimulating factor(s) in foetal calf serum (but not horse serum) for the transdifferentation of NR to lens during neural retina cultures. The identity of the transdifferentiation-promoting (TP) factor(s) in embryonic sera remains obscure. However, the evidence presented by Depomerai and Gali[2] suggests that the major TP activities in foetal calf serum are not macromolecular, but rather of low molecular weight. Their results also suggest that horse serum contains macromolecule factors inhibitory to transdifferentiation (TI factors).

Minimal essential medium permits more extensive transdifferentiation into lens than does F199 (Fig. 4). The results of the present work were different from those of Gali and Depomerai[3], who claimed that F199 completely inhibits lentoidogenesis. It should be noted that Gali and Depomerai[3] used neural retina cultures and not retina glial cultures. Enhanced transdifferentiation in the absence of neuroblasts suggest a putative "inhibitory influence" and also that transdifferentiation appears to occur in this system from retina glial cells.[8,10] It is already known that minimal essential medium promotes extensively lentoidogenesis (Agata *et al.*)[9], (Gali and Depomerai[3], during neural culturing. The results of using retina glial cultures show (Fig. 4) that lentoids appear early and give rise to lens cells up to 50 days of cultures.

Chick embryo neuro-retinal (NR) cells transdifferentiate into both lens and pigment cells after 4-5 weeks *in vitro* when cultured in MEM medium containing 10% foetal calf serum (F), but lentoid appearance and δ-crystalline accumulations are in-

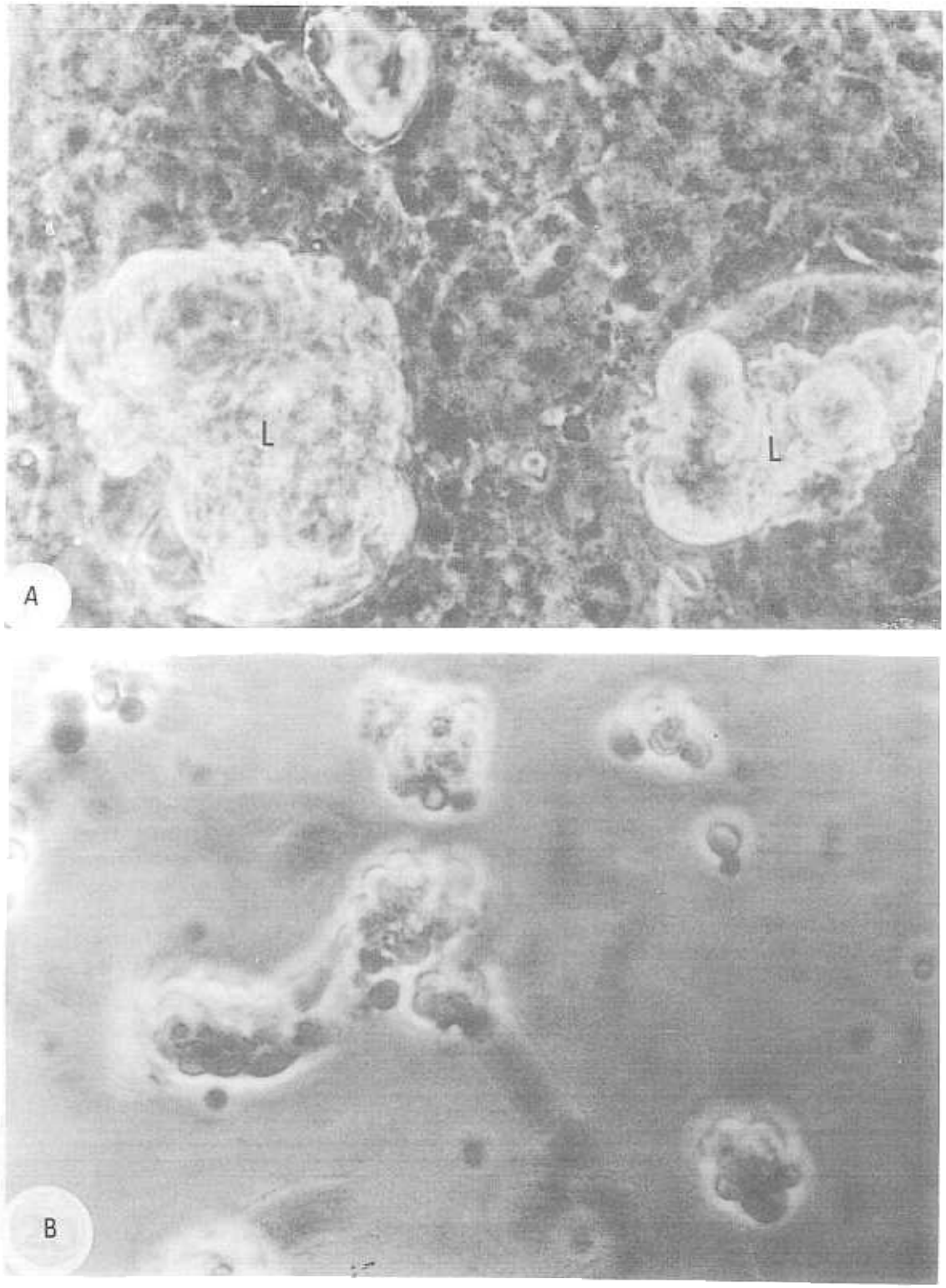

FIG. 5. (a) Culture of 9 day embryonic glial cells of retina in FH media after 50 days *in vitro* (× 200).
L : Lentoids
(b) Culture of 9 day embryonic glial cells of retina in FHG media after 50 days *in vitro* (× 200).

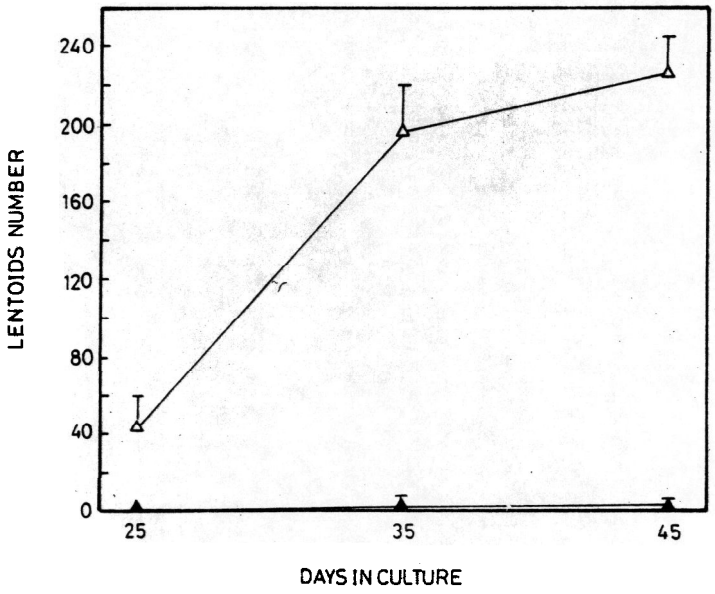

FIG. 6. Number of lentoids formed during chick embryo retina glial cell cultures in each of FH and FHG media. Each point gives the mean and standard error (vertical bar) derived from at least four culture dishes.
▼———▼, FH media
▲———▲, FHG media.

hibited if supplementary glucose is present (FG) (Depomerai and Gali).[2] FHG medium prevents lentoid appearance and almost completely blocks δ-crystalline accumulation. If the supplementary glucose is omitted from this medium, then extensive formation of lentoids is observed and high levels of δ-crystalline accumulate (Depomerai and Gali).[4] Fig. 5b and 6 showed that FHG medium blocks lentoidogenesis during retina glial cultures, but in the absence of supplementary glucose (FH). Lentoids appear in considerable amounts by 30-40 days (Fig. 5 and 6). This result shows that glucose can exert a controlling influence on lentoidogenesis during RG cultures.

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تأثير العوامل الخارجية على تكوين العديسات في زراعات الخلايا الغروية الشبكية لأجنة الدجاج

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المستخلص . تمت دراسة تأثير بعض العوامل ، مثل المصل والبيئة والجلوكوز على تكوين العديسات في زراعات الخلايا الغروية الشبكية لأجنة الدجاج (RG) ، كلٌ على حدة . وجد أن مصل جنين العجل له قدرة كبيرة على تشجيع تكوين العديسات خلال زراعات الخلايا الغروية الشبكية (RG) خاصة عند وجوده بتركيزات عالية ، في حين وجد عند استعمال مصل الحصان خلال زراعات الخلايا الغروية الشبكية (RG) أنها تسمح بتكوين القليل فقط من العديسات .

استعمال بيئة MEM مع ١٠٪ من مصل جنين العجل (F) في نفس نوع الزراعات (RG) أنتجت عديسات جيدة . في حين أن استعمال بيئة 199 مع ١٠٪ من مصل جنين العجل (F) أنتج عددًا أقل بكثير من العديسات التي تكونت في بيئة MEM . كذلك وُجد أن الجلوكوز يمنع تكوين العديسات في هذه الزراعات .