Short Communications

Changes in Lectin-Induced Deoxyribonucleic Acid Synthesis in Cultures of Chick-Embryo Fibroblasts at Various Stages of Development

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Concanavalin A and *Robinia pseudoacacia* lectin decreased [^3H]thymidine incorporation into acid-insoluble material of fibroblasts cultured from 6–10-day chick embryos. In contrast, these lectins stimulated [^3H]thymidine incorporation in cells from 16-day embryos. These effects are due to neither [^3H]thymidine permeability modification nor toxicity of the lectins. The specificity of lectin action was proved by blocking experiments with α -methyl mannopyranoside and with anti-(*Robinia* lectin) serum.

Stimulation of DNA synthesis by concanavalin A has been reported in cultures of neural retina cells from 9–12-day chick embryos (Kaplowitz & Moscona, 1973). Various lectins have different effects on embryo cell growth (Aubery & Bourrillon, 1973) and agglutinability (Moscona, 1971; Kleinschuster & Moscona, 1972; Lallier, 1972; Weiser, 1972), depending on embryonic age, suggesting that developmental changes occur on the cell surface during embryo differentiation. Lectins decrease the growth of rapidly proliferating cells (8–10-day chick embryos) and stimulate the growth of slowly proliferating cells (16-day chick embryos) (Aubery & Bourrillon, 1973).

It seemed of interest therefore to make a comparative study of changes in cell growth induced by lectins and DNA synthesis by these cells. We have thus undertaken an investigation of the effects of concanavalin A and *Robinia* lectin on [^3H]thymidine incorporation in fibroblasts during embryo differentiation.

Materials and methods

Robinia pseudoacacia lectin and anti-(*Robinia* lectin) serum were prepared by methods previously described (Bourrillon & Font, 1968). Concanavalin A, α -methyl mannopyranoside, galactose and *N*-acetylgalactosamine were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A., and [^3H]thymidine (20 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Suspensions of chick embryonic fibroblasts were obtained by the technique of Rein & Rubin (1968), but using mechanical dispersion. After aseptic dissociation of the dorsal muscle in Eagle's medium (Pasteur Institut, France), tissues were gently ground with a pestle through a cheesecloth (600 μm),

filtered through a second cheesecloth (60 μm) and washed three times with Eagle's medium. A sample was counted in a haemocytometer. The erythrocyte contamination was less than 5%. Cells were more than 97% viable as assayed by Trypan Blue exclusion.

The cultures were primary-explant monolayers in 50 mm Petri dishes in Eagle's medium (3.25 ml) supplemented with 10% (w/v) foetal calf serum (Gibco, U.K.). They were sown at an initial concentration of 5×10^5 cells/ml and incubated at 37°C in humidified air with 5% CO_2 . The cell cultures consisted entirely of fibroblasts as determined by phase-contrast microscopy. Lectin was added at the beginning of the culture at a final concentration varying from 1 to 100 $\mu\text{g}/\text{ml}$.

Thymidine incorporation was assayed by adding [^3H]thymidine to each dish at various times of the culture (6–72 h). After 2 h, the cells were mechanically harvested and washed twice with ice-cold phosphate-buffered saline (0.15 M-NaCl–0.05 M-sodium phosphate, pH 7). Once the cells were in suspension in phosphate-buffered saline (5 ml), a portion was counted in a haemocytometer and the viability tested by Trypan Blue exclusion. The resulting suspension was sonicated, collected on Millipore filters and washed twice with ice-cold 10% (w/v) trichloroacetic acid and once with methanol. Radioactivity was determined in a Packard liquid-scintillation spectrometer [with 0.5% 1,5-diphenyloxazole–0.04% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in toluene].

For inhibition assays, 0.05 ml of a monosaccharide solution (0.2 M) or 0.025 ml of a 1:10 dilution of anti-lectin serum was mixed with an equal volume of lectin solution. After incubation for 30 min at 37°C, the mixture was added to the cell culture. Cultures were incubated for 24 h, and [^3H]thymidine incorporation was determined as above.

Fig. 1. Time-course of [^3H]thymidine incorporation into cultures of embryo fibroblasts in the absence of lectins

For experimental details see the text. Fibroblasts from 6-day embryo (Δ), 8-day embryo ($\blacktriangle$), 10-day embryo ($\bullet$), 12-day embryo ($\circ$) and 16-day embryo ($\blacksquare$) were used. The error bars show the range of values obtained from four determinations.

Results and discussion

The range of values obtained for each point is given on the graphs. Maximum variation of the mean values obtained for a given point was 5% in an experiment and 8% in repeated experiments.

[^3H]Thymidine incorporation is more important in cells from 6–10-day embryos than in cells from older (16-day) embryos, in agreement with fibroblast proliferation (Aubery & Bourrillon, 1973). Incorporation of [^3H]thymidine rapidly increased to reach a maximum after 12 h of culture for 6–10-day embryo cells and after 48 h of culture for 16-day embryo cells. After longer culture periods, [^3H]thymidine incorporation decreased (Fig. 1). The rapid decline in incorporation after the plateau could be due to a decrease in the capacity of the cells to divide *in vitro*.

[^3H]Thymidine incorporation into embryo fibroblasts is modified by adding lectins. These modifications were critically dependent on embryo age, lectin concentration and culture time. In 6-day embryo cells, a $3\text{ }\mu\text{g/ml}$ concentration of concanavalin A or *Robinia* lectin elicited a 60 or 40% decrease respectively in [^3H]thymidine incorporation, compared with the control. In contrast, [^3H]thymidine incorporation is stimulated (10–20%) in 16-day embryo cells by both lectins. No significant effect of lectins was observed in [^3H]thymidine incorporation in 12-day embryo cells regardless of the concentration tested (Fig. 2).

Fig. 2. Effects of *Robinia* lectin and concanavalin A on [^3H]thymidine incorporation in embryo fibroblasts at different stages of embryo development

Cultures were incubated for 24 h continuously in the presence of either lectin as described in the text. $\blacksquare$, *Robinia* lectin; $\square$, concanavalin A. Values are given as percentages of [^3H]thymidine incorporation in the control. The error bars show the range of values obtained from four determinations.

A similar observation has been reported for chick embryonic neural retina cells treated with concanavalin A (Kaplowitz & Moscona, 1973), but with some differences. The decrease in [^3H]thymidine incorporation induced by concanavalin A in young cells was less important than in young fibroblasts. In contrast, stimulation of [^3H]thymidine incorporation was higher in 10-day embryo retina cells than in 16-day embryo fibroblasts. The two systems also differ in their age response to lectins; stimulation of [^3H]thymidine incorporation into embryo retina cells occurred at an earlier age (at 12 days) than in fibroblasts (at 16 days). These differences could be related to the differentiation stage peculiar to each cell system.

The effect of both lectins on [^3H]thymidine incorporation depends on lectin concentration. Maximal inhibition (6–10-day embryo cells) or stimulation (16-day embryo cells) of [^3H]thymidine incorporation was obtained with $3\text{ }\mu\text{g/ml}$ solutions of both lectins, and increasing the concentration up to $100\text{ }\mu\text{g/ml}$ did not significantly change these effects. In 12-day embryo cells thymidine incorporation is not affected by lectin concentration at all.

A time-course study of [^3H]thymidine incorporation showed that both lectins had a maximal effect, inhibition or stimulation, after 24 h of culture at each cell age tested. After this time, the effect of concanavalin A was constant whereas the inhibition of [^3H]thymidine incorporation caused by *Robinia* lectin slowly increased.

The study of [^3H]thymidine uptake at low temperatures at which metabolic events are inhibited allows us to distinguish between transport processes and the rate of DNA synthesis. The rate of [^3H]thymidine uptake at 2°C under conditions in which DNA synthesis is inhibited (Hauschka *et al.*, 1972) is not significantly affected by concanavalin A or *Robinia* lectin. If the relative rate of thymidine transport at 2°C is an indication of transport at 37°C, the inhibition of [^3H]thymidine incorporation caused by both lectins cannot be explained by a modification of [^3H]thymidine permeability, but reflects a decrease in the rate of DNA synthesis.

The effects of concanavalin A and *Robinia* lectin were specifically inhibited by α -methyl mannoside and by a monospecific anti-(*Robinia* lectin) serum respectively. *Robinia* lectin is not inhibited by any monosaccharide of Makela's classification (Makela, 1957).

A toxic effect of lectins has been proposed to explain growth inhibition of transformed cells by lectin (Shoham *et al.*, 1970). However, a decrease in [^3H]thymidine incorporation in rapidly proliferating fibroblasts (6–10-day embryo cells) was obtained with low concentrations of lectins (3 $\mu\text{g}/\text{ml}$), and this effect was not changed by raising the lectin concentration. These results are at variance with an hypothesis that lectin has a toxic effect.

Stimulation of [^3H]thymidine incorporation in 16-day embryo cells clearly differs from that caused by lectins in lymphocytes (Powell & Leon, 1970). In lymphocytes, DNA synthesis is greater and occurs later (72h culture) than in fibroblasts (24h culture).

Probably, the explanation for these differences lies in the fact that in the absence of lectin lymphocytes are in a quiescent state and do not synthesize DNA, whereas the embryo fibroblasts are dividing and incorporating [^3H]thymidine.

These results underline once more the similarity between young embryo cells and tumour cells (Pierce & Johnson, 1971).

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