The Effect of Kinetin, Kinetin Ribofuranoside and Gibberellic Acid upon Cultures of Skin and Mammary Carcinoma and Cystic Disease

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SUMMARY

Kinetin and kinetin ribofuranoside have been tested on tissue cultures of adult human skin, breast carcinomas, and cystic disease of the breast. Outgrowths of epithelium from skin cultures were retarded by kinetin and completely inhibited by kinetin ribofuranoside when treated with 1.0 mg. per cent. A concentration of 0.1 mg. per cent of kinetin had no effect on the extent or population of cells in the zone of epithelial outgrowth from skin. There was no effect from kinetin at either 0.1 or 1.0 mg. per cent on outgrowths of fibroblasts and epithelial cells from carcinomas and cystic disease. Kinetin ribofuranoside equally reduced the outgrowth of fibroblasts and epithelial cells at a concentration of 1.0 mg. per cent but had no effect at 0.1 mg. per cent on cultures of carcinomas and cystic disease. Gibberellic acid (0.1-2.5 mg. per cent) had no effect upon the outgrowths of fibroblasts or epithelium from tissue cultures of adult human skin, breast carcinomas, or cystic disease of the breast.

An earlier report (7) from this laboratory described the effects of kinetin (6-furfurylamino-purine) upon tissue cultures of skin and fibroblasts. Kinetin (1.0-0.25 mg/100 ml nutrient fluid) retarded the outgrowth of epithelium from skin cultures. The outgrowths equaled controls in concentrations of 0.05-0.012 mg per cent; 0.006-0.0015 mg per cent produced epithelial sheets greater than controls. A strain of fibroblasts after 20 days' treatment with 0.006 mg. per cent had outgrowth that exceeded the controls.

Kinetin ribofuranoside (4) (6-furfurylamino-9-β-D-ribofuranosylpurine) has been prepared and tested for differential toxicity to mammalian cells. Hampton, Biesele, Moore, and Brown (4) have reported that "1 X 10^-5" solution killed 99 per cent of the cells of a strain of adult human fibroblasts in 24 hours but was almost without effect on the rate of cell division or proportion of dead cells in three strains (HeLa, H. Ep. #1 and Ep. #2) of human carcinoma cells." This substance was found to be much more toxic than kinetin.

The fungus Gibberella fujikuroi (2) elaborates gibberellin A1, gibberellin A3, and gibberellic acid, which are substances capable of promoting shoot growth by cell elongation and inducing flower formation in plants. Gibberellic acid is the most accessible and can be obtained in pure form. It appears (2) to be a tetracyclic dihydroxylactonic acid (C_{19}H_{22}O_{6}).

This is a report on the results obtained with kinetin ribofuranoside, kinetin, and gibberellic acid upon tissue cultures of adult human skin, carcinomas of the breast, and cystic disease of the breast.

MATERIALS AND METHODS

Skin specimens were taken from amputated breasts. Carcinoma and cystic disease tissue came from biopsies and amputated breasts. The tissues were cut into explants approximately 2 mm. square and washed in Hanks balanced salt solution. Three explants were embedded in a clot composed of equal parts chicken plasma and fresh 10-day-old chick embryo extract on No. 1, 12 X 50-mm. coverglasses. These preparations were placed in 16 X 250-mm. test tubes; 2.5 ml. of nutrient fluid, composed of 50 per cent Hanks balanced salt solution, 47 per cent ascitic fluid, and 3 per cent embryo extract, was used. Cultures were

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incubated at 36°C in a slanted stationary position.

Kinetin and kinetin ribofuranoside were dissolved in Hanks balanced salt solution (minus bicarbonate). Kinetin was sterilized by autoclaving and kinetin ribofuranoside by Seitz filtration. Preliminary studies with the use of various concentrations revealed that 1.0 and 0.1 mg/100 ml nutrient fluid would show the effects of these substances.

Gibberellic acid was dissolved in Hanks balanced salt solution and sterilized by Seitz filtration. Concentrations tested were 0.1, 0.25, 0.5, 1.0, and 2.5 mg/100 ml of nutrient fluid; 2.5 mg per cent will not dissolve completely.

A complete experiment was done on at least three different specimens of each tissue. Five roller tube cultures of each specimen (fifteen explants) were used for each test condition and controls. Cultures received test solutions at the time of explantation. Skin cultures were fixed and stained on the 10th day. Cultures of carcinomas and cystic disease were usually kept 3-4 weeks, depending upon the rate of outgrowth.

A staining method employing Giemsa and May-Greenwald stains was used.

RESULTS

Skin cultures (Fig. 1) most frequently produce outgrowths of sheets of epithelial cells that liquefy the clot and adhere to the glass. As in previous experiments with kinetin (7) these cultures were judged on the basis of consistency of cell population and extent of outgrowth. All experiments were analyzed separately and together.

Kinetin ribofuranoside is more toxic to skin epithelium at a concentration of 1.0 mg per cent than kinetin which retards the outgrowth but does not completely inhibit it. This effect is not evident at 0.1 mg per cent. Epithelial outgrowths began by the 2d or 3d day and had reached their maximum by the 5th day in both treated and control cultures. There was no difference in the time at which outgrowth began to appear. Figures 2-5 illustrate the results with skin cultures.

The culture characteristics of carcinomas of the breast have been described in another publication (8). Fibroblasts are typical of a connective tissue culture. One of the frequent outgrowths of epithelium is the sheet of cells (Fig. 6) which liquefies the clot and adheres to the glass. The results with this tissue are shown in Figures 7-10.

1.0 mg. per cent of kinetin ribofuranoside is equally inhibitory to the outgrowth of fibroblasts or epithelium at a concentration of 1.0 or 0.1 mg. per cent.

Gibberellic acid, regardless of the concentration, had no effect on the outgrowths from skin cultures, carcinomas, or cystic disease.

The results for all tissues with kinetin and kinetin ribofuranoside are summarized in Table 1.

The outgrowth of fibroblasts or epithelium at a concentration of 1.0 or 0.1 mg. per cent.

Cultures from cystic disease of the breast produce many outgrowths, both fibroblastic and epithelial (Fig. 11), similar to carcinoma. The reaction of this tissue to kinetin and kinetin ribofuranoside is the same as that for carcinoma. This is illustrated in photomicrographs (Figs. 12-15).

<table>
<thead>
<tr>
<th>Mg. of test substance per 100 ml. of nutrient fluid</th>
<th>Kinetin</th>
<th>Kinetin Ribofuranoside</th>
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<tr>
<td>Skin</td>
<td></td>
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<tr>
<td>1.0 Extent and cell population less than controls</td>
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<td>0.1 Extent and cell population equals controls</td>
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<td>0.1 Extent and cell population equals controls</td>
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Breast Carcinomas

1.0 Outgrowth of fibroblasts and epithelium equals controls

0.1 Outgrowth of fibroblasts and epithelium equals controls

Cystic Disease

1.0 Outgrowth of fibroblasts and epithelium equals controls

0.1 Outgrowth of fibroblasts and epithelium equals controls

The results for all tissues with kinetin and kinetin ribofuranoside are summarized in Table 1.

Gibberellic acid, regardless of the concentration, had no effect on the outgrowths from skin cultures, carcinomas, or cystic disease.

COMMENTS

Kinetin (5) is prepared from yeast deoxyribonucleic acid (DNA). It was found to be a cell division hormone (3) which increases cell division in cultures of tobacco "wound" callus tissue (6). In this study the results obtained with kinetin on epithelial outgrowths from skin were similar to those that have been reported (7). No effect on
either epithelial or fibroblastic outgrowth from carcinomas or cystic disease could be demonstrated.

Hampton, Bieseie, Moore, and Brown (4), and Bieseie (1) have reported the effect of kinetin ribofuranoside (6-furfurylamino-9-β-D-ribofuranosylpurine) on strains of adult human cells and on cultures of mouse embryonic skin. They have found kinetin ribofuranoside to be more toxic to fibroblasts than epithelial cells.

A differential effect between fibroblasts and epithelial outgrowths from primary tissue cultures of adult skin, breast carcinoma, and cystic disease treated with kinetin ribofuranoside could not be demonstrated. When the outgrowth of one cell type was reduced the other was equally affected. Kinetin ribofuranoside was found to be more toxic than kinetin on all the tissues at a concentration of 1.0 mg. per cent but was not at 0.1 mg. per cent. Both substances acted very much alike at this concentration.

The response (2) to gibberellic acid by plants can be produced by spraying, putting drops on the leaves, applying to the roots, or by incorporating in the soil or culture medium. An example of this response is the effect on dwarf pea plants. Weekly doses (2) of 1 mg. of gibberellic acid increased the height by 50 per cent. The length was almost entirely due to increased cell extension. There is little evidence of increased cell multiplication. The effects of gibberellic acid on plants such as stem extension and flowering are normal physiological processes (2) and can be produced by “appropriate light and temperature conditions in the plant’s environment.”

In this study the concentrations of gibberellic acid (0.1–2.5 mg. per cent) tested did not show a stimulating or toxic effect upon tissue cultures of adult human skin, breast carcinomas, or cystic disease.

ACKNOWLEDGMENTS

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Samples of gibberellic acid were kindly supplied by the Lilly Research Laboratories, Indianapolis, Ind.

REFERENCES


Fig. 1.—Epithelial outgrowth from a control skin culture.

Fig. 2.—Epithelial outgrowth from a skin culture treated with 1.0 mg. kinetin per 100 ml. of nutrient fluid.

Fig. 3.—Epithelial outgrowth from a skin culture treated with 0.1 mg. kinetin per 100 ml. of nutrient fluid.

Fig. 4.—Outgrowth completely inhibited in a skin culture treated with 1.0 mg. kinetin ribofuranoside per 100 ml. of nutrient fluid.

Fig. 5.—Epithelial outgrowth from a skin culture treated with 0.1 mg. kinetin ribofuranoside per 100 ml. of nutrient fluid.
Fig. 6.—Epithelial outgrowth from a control culture of carcinoma of the breast.

Fig. 7.—Epithelial outgrowth from a culture of carcinoma of the breast treated with 1.0 mg. kinetin per 100 ml. of nutrient fluid.

Fig. 8.—Epithelial outgrowth from a culture of carcinoma of the breast treated with 0.1 mg. kinetin per 100 ml. of nutrient fluid.

Fig. 9.—Outgrowth completely inhibited in a culture of carcinoma of the breast treated with 1.0 mg. kinetin ribofuranoside per 100 ml. of nutrient fluid.

Fig. 10.—Outgrowth of fibroblasts from a culture of carcinoma of the breast treated with 0.1 mg. kinetin ribofuranoside per 100 ml. of nutrient fluid.
FIG. 11.—Epithelial outgrowth from a control culture of cystic disease of the breast.

FIG. 12.—Epithelial outgrowth from a culture of cystic disease of the breast treated with 1.0 mg. of kinetin per 100 ml. of nutrient fluid.

FIG. 13.—Epithelial outgrowth from a culture of cystic disease of the breast treated with 0.1 mg. of kinetin per 100 ml. of nutrient fluid.

FIG. 14.—Outgrowth greatly retarded in a culture of cystic disease of the breast treated with 1.0 mg. of kinetin ribofuranoside per 100 ml. of nutrient fluid.

FIG. 15.—Outgrowth of fibroblasts from a culture of cystic disease of the breast treated with 0.1 mg. of kinetin ribofuranoside per 100 ml. of nutrient fluid.
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