The Lack of Effect of Heparin on Mitosis in Strain L Cells*

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There has been considerable controversy about the action of heparin on mitosis. Early workers in tissue culture routinely used heparinized plasma without apparent inhibition of growth (2). Later, however, several investigators reported that heparin inhibited mitosis either in vitro or in vivo (1, 4, 5, 15), although others found it was not inhibitory (8, 10, 14). A search is being made in our laboratory for a mitotic inhibitor which will not interfere with other cellular functions. Heparin was accordingly tested for its effect on mitosis on strain L cells.

MATERIALS AND METHODS

Strain L cells were received through the kindness of Dr. Gabriel C. Godman of the College of Physicians and Surgeons, Columbia University.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>EFFECT OF HEPARIN (0.1 PER CENT) ON STRAIN L FIBROBLASTS</strong></td>
</tr>
<tr>
<td>Mitotic index per 1000 cells.</td>
</tr>
<tr>
<td><strong>EXPERIMENT 1</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Day 0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

They were grown as free cells in a suspension culture in Erlenmeyer flasks as described by Kuchler (9). Each culture initially contained between 200,000 and 300,000 cells/cc in 70 cc. Eagle’s medium (3) supplemented with 8 cc. of horse serum. Penicillin (100 units/ml) and streptomycin (10 units/ml) were added to all cultures, and all procedures were carried out in a hood sterilized with ultraviolet light. A culture was usually divided equally into two flasks; one received varying concentrations of heparin, and the other was kept as a control.

Cell counts were done in standard blood counting hemocytometers by counting ten squares. Mitotic indices were determined by counting 1000 cells on smears stained with aceto-carmine after fixation in ether-alcohol. Heparin concentration was determined daily by the azure dye method of Jaques (7) on duplicate 2-cc. samples of noncellular media after the cells had been removed by centrifugation. Samples were read in the Beckman D.U. Spectrophotometer at 490 m. Heparin standards were prepared with equal quantities of the culture medium from control flasks.

When intracellular heparin was to be determined, the cells were carefully washed 3 times in Krebs-Ringer phosphate solution, ruptured by sonic disintegration, and centrifuged at 8000 × g for 30 minutes in a Servall centrifuge. The heparin in the soluble protein cell supernatant fraction was assayed. A control culture of cells prepared in an identical manner without the addition of heparin was used to prepare the blank and standard curve. Cell protein was determined by modifying the Lowry method (11) as proposed by Oyama and Eagle (12). Bovine serum albumin was used as a standard.

The heparin used in these experiments was obtained from the California Foundation for Biochemical Research and Heparin, Inc.; the products assayed at 100 units/mg and 126 units/mg, respectively.

RESULTS

Strain L cells treated with 0.1 per cent heparin showed no change in mitotic index as compared with control cultures (Table 1). Quantities of heparin ranging from .0001 or 0.1 per cent did not affect the growth curves of cells. Both control and experimental cultures showed the usual 1-day lag period before commencing logarithmic division. Table 2 includes the growth curve when colchicine, a known mitotic inhibitor, was added to the culture. Although control flasks were run with each concentration of heparin, only the controls for the highest concentrations of heparin are included in this figure.

Since heparin had little effect on cell division, it appeared of interest to determine whether it could permeate into the cell or was degraded in
any manner. From the balance study in Table 3 it appears that small amounts of heparin do pass across the cell membrane and somewhat larger amounts are degraded either extracellularly. Control cultures containing media and heparin, but lacking cells, showed spontaneous degradation of assayable heparin, owing perhaps to the rapid shaking at 37.5° C. However, the amount of heparin lost in addition to this appears significant.

**DISCUSSION**

A. Fischer (4) first claimed inhibition of mitosis, using heart fibroblasts and concentrations of heparin varying from 0.002 to 0.05 per cent. Zakrzewski (15) claimed that the Jensen sarcoma in tissue culture was inhibited by heparin but emphasized that this drug was much less effective on normal embryonic tissue. In both instances growth was measured qualitatively, and the preparations of different species, different routes of administration, and perhaps differences in permeability of different cells. Other cells in tissue culture may also behave differently. However, our experiments with L strain cells grown in vitro in a well defined environment, using two sources of heparin at widely different concentrations, showed no effect on mitosis.

Heparin does pass across the cell wall of L strain cells. The method may be by diffusion, active transport, or phagocytosis. We have shown in other experiments that these cells readily ingest carmine and carbon particles. Heparin has been reported to have been retained for as long as 2 weeks, particularly in the kidney, spleen, liver, and lung (6). However, a comparatively small amount of heparin was found intracellularly, and the cell may liberate an extracellular heparinase. Payza and Korn (18) have reported on an adapted bacterial culture which was able to degrade heparin and heparin used by the early workers were admittedly impure.

Other investigators have determined the effect of heparin on transplantable tumors. Goerner (5) found that fragments of Flexner-Jobling carcinoma treated in vitro with 0.1 per cent heparin did not grow when transplanted into the rat. However, Kreisler (8) found that 15 units of heparin given every 12 hours intravenously had no effect on lymphosarcoma transplanted to mice. Baluzs et al. (1) found a slightly greater mortality in mice with Ehrlich ascites tumor which were treated with heparin, but Lippmann (10) in a very similar experiment found no effect on mortality at all. Although the latter author found a decrease in the mitotic index after one injection of the drug, curiously no decrease in mitotic index was noted after several injections.

The previous evidence is certainly equivocal, owing no doubt in part to different dosages, different species, and perhaps differences in permeability of different cells. Other cells in tissue culture may also behave differently. However, our experiments with L strain cells grown in vitro in a well defined environment, using two sources of heparin at widely different concentrations, showed no effect on mitosis.

**TABLE 2**

**EFFECT OF HEPARIN ON THE GROWTH OF L STRAIN FIBROBLASTS**

<table>
<thead>
<tr>
<th>Heparin</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001 per cent</td>
<td>3.26</td>
<td>4.0</td>
<td>8.60</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>0.001 per cent</td>
<td>2.70</td>
<td>2.85</td>
<td>5.60</td>
<td>11.50</td>
<td></td>
</tr>
<tr>
<td>0.01 per cent</td>
<td>5.60</td>
<td>5.70</td>
<td>5.70</td>
<td>11.57</td>
<td></td>
</tr>
<tr>
<td>0.1 per cent</td>
<td>1.80</td>
<td>1.98</td>
<td>3.60</td>
<td>6.60</td>
<td>11.00</td>
</tr>
<tr>
<td>Control culture</td>
<td>1.80</td>
<td>1.90</td>
<td>3.50</td>
<td>6.00</td>
<td>9.10</td>
</tr>
<tr>
<td>Control culture</td>
<td>2.04</td>
<td>2.70</td>
<td>4.80</td>
<td>9.60</td>
<td>12.00</td>
</tr>
<tr>
<td>Colchicine $10^{-8}$ M.</td>
<td>2.04</td>
<td>2.68</td>
<td>4.80</td>
<td>9.00</td>
<td>10.00</td>
</tr>
<tr>
<td>(Heparin, Inc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.000004 per cent</td>
<td>3.44</td>
<td>2.90</td>
<td>1.48</td>
<td>1.10</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3**

**BALANCE CHART OF HEPARIN LOST FROM 4-DAY-OLD CULTURES OF L STRAIN FIBROBLASTS**

<table>
<thead>
<tr>
<th></th>
<th>Experiment I</th>
<th>Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total heparin in culture initially</td>
<td>970</td>
<td>1165</td>
</tr>
<tr>
<td>Total heparin lost in 4 days</td>
<td>221.6</td>
<td>172.0</td>
</tr>
<tr>
<td>Spontaneous breakdown of heparin *</td>
<td>89.7</td>
<td>107.5</td>
</tr>
<tr>
<td>Heparin breakdown per total culture of cells</td>
<td>151.9</td>
<td>64.5</td>
</tr>
<tr>
<td>Heparin breakdown per Ehrlich cells</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Heparin found within cells (total culture)</td>
<td>15.4</td>
<td>12.6</td>
</tr>
<tr>
<td>Heparin found within cells per $1 \times 10^6$ cells</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Heparin degraded within or outside cells per $1 \times 10^6$ cells</td>
<td>1.15</td>
<td>0.65</td>
</tr>
<tr>
<td>Heparin lost per mg. cell protein</td>
<td>2.94</td>
<td></td>
</tr>
</tbody>
</table>

*Calculated from spontaneous breakdown of heparin from control flasks lacking only cells. All figures relating to heparin lost from cultures of L strain fibroblasts have been corrected for these values.
use it as its sole carbon, nitrogen, and sulfur source for growth. Whether the L strain cells are able to utilize the breakdown products of heparin remains unknown.

**SUMMARY**

Strain L cells grown in suspension cultures containing 0.0001–0.1 per cent heparin showed no inhibition of mitosis or cell growth. Heparin was found intracellularly in strain L cells and was degraded by these cells.

**REFERENCES**

The Lack of Effect of Heparin on Mitosis in Strain L Cells

Donald W. King, Klaus G. Bensch and Stanley Simbonis