Some Effects of Polysaccharide Preparations from Serratia marcescens and Aerobacter aerogenes on Cells in Tissue Culture*  

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When certain polysaccharide fractions isolated from cultures of Serratia marcescens (8, 2), Aerobacter aerogenes, or other Gram-negative bacteria (8) are injected in large doses into mice, symptoms very similar to those of shock occur. The animals are prostrated, their muscular response is much lower than normal, they breathe with difficulty, and the body temperature drops. Similar symptoms are observed when these polysaccharides are injected into sarcomatous mice, but, in addition, zones of hemorrhage and necrosis appear in the tumor itself, sometimes resulting in the destruction of the neoplasm. Whether this necrosis occurs as a result of the direct action of the polysaccharide on the tumor cell or on the action of a complex formed in vivo after the polysaccharide injection, or results indirectly from a systemic disturbance, is not yet settled. Tissue culture technics may be helpful in solving it. It appears that the only observations along these lines are those of McConnell (4), who reported no damaging effect by a polysaccharide from S. marcescens (obtained from Dr. M. J. Shear) on cultures of Sarcoma 87 cells. Our own experiments bring out some facts which may be of interest.

MATERIALS AND METHODS

The cultures were carried out according to the hanging drop technic. The basic culture medium consisted of one drop of chicken embryo extract diluted 1:1 in Tyrode's solution and one drop of chicken plasma. The effect of the polysaccharide preparations was studied in two ways: (a) by direct inclusion in the nutrient at concentrations ranging from 8.8 to 800 µg/ml and (b) by permitting tissues to stand for 4 hours in solutions containing polysaccharide material, after which interval the cultures were transferred to the control nutrient. The cultured cells were tissue explants taken from 4-day chick embryo hearts (myocardial fibroblasts) or from Sarcoma 37 tumors in Swiss mice.

Most of the experiments were conducted with preparation A-2Y from S. marcescens, the remainder with preparation A-2 from A. aerogenes. The polysaccharides were obtained by the phenol-trichloroacetic acid method of Perrault and Shear (5) and after tryptic digestion were nondialysable and protein negative. The N content of A-2Y was 2.2 per cent, the P content 1.4 per cent, and reducing sugars (calculated as glucose) 54.2 per cent. The corresponding values for A-2 were 2.8, 1.9, and 57.9. A dose of 100 µg. of A-2Y was lethal in 96 hours for about 15 per cent of 8-week-old mice injected intraperitoneally and produced pronounced hemorrhage and necrosis of tumors in mice; 380 µg. of A-2 had a somewhat similar lethal effect in normal mice, and 100 µg. was highly lethal, hemorrhage-inducing, and tumor-necrotizing in tumor-bearing mice.

RESULTS

Action on normal chicken fibroblasts and on Sarcoma 37 cells.—A saline solution of A-2Y, introduced into the medium of cultures of normal chicken fibroblasts, at a final concentration of 20 µg/ml, had no effect on the cellular migration and areal increment of the tissue fragments. After 48 hours, the area in the controls with Tyrode's solution (50 cultures) was approximately the same as in the presence of polysaccharide (50 cultures). These experiments, repeated 3 times (500 cultures in all), confirmed the results of McConnell (4). When fragments of myocardium from a 4-day-old chick embryo were placed for 4 hours in contact with the polysaccharide solution at a concentration of 80 µg/ml in saline and were then grown in normal medium, they showed after 24 hours a much greater cell migration than the controls.
After 48 hours the relative increase in diameter was greater by about one-half than in the controls where the tissue fragments had been in contact with the Tyrode’s solution only (Table 1).

**TABLE 1**

**RELATIVE INCREASE IN DIAMETER OF EXPLANTS OF NORMAL CHICKEN FIBROBLASTS IN SOLUTIONS OF POLYSACCHARIDE A-2Y**

<table>
<thead>
<tr>
<th>Hours</th>
<th>Control sol.</th>
<th>Tyrode nutrient</th>
<th>Tyrode basic med.</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

When Sarcoma 37 tissue had been in contact for 4 hours with a concentration of 80 µg., or even 45 µg., of A-2Y per milliliter in saline, and was then transferred to the basic culture medium (75 cultures per solution), it showed a pronounced increase in diameter as compared with tissue previously immersed in saline for the same length of time (Table 2). This increase, regularly observed, was of the same order as for the chicken fibroblasts. No increase was noted after contact with 450 µg. of A-2Y. This was likewise true when the tissue was immersed in concentrations of 80 µg. and 800 µg. of A-2, 80 µg. resulting in increased diameter, 800 µg. being without effect (Table 2).

When A-2Y was introduced into the medium in concentrations of 3.3, 20, or 35 µg/ml, it failed to modify the increase of Sarcoma 37. Slight increase resulted when the concentration was held at 350 µg/ml (Table 3).

**DISCUSSION**

In view of the fact that certain bacterial polysaccharides, when injected into mice, induce symptoms of shock followed, in tumor-bearing animals, by hemorrhage and necrosis of the tumor, it might be expected that they would have a damaging effect on isolated tissue. However, in agreement with McConnell’s observation (4), we could see no evidence of damage, as indicated by fatty degeneration, bleb formation, or excessive cell death, when such polysaccharides were applied to tissue cultures of chick heart fibroblasts or of mouse Sarcoma 37 cells. On the contrary, the striking fact brought out by these experiments was the ability of appropriate concentrations of the polysaccharide preparations to enhance the areal increase of chick heart fibroblasts and Sarcoma 37 cells in culture.

There is sufficient variation in the tumornecrotizing properties known to exist between different polysaccharide preparations to make it dangerous to assume without direct evidence that two preparations known to possess like properties with regard to their effects on tissue cultures will also possess like necrotizing effects. However, the results of these experiments nevertheless make it appear doubtful that the toxic effects such as hemorrhage and tumor necrosis, which follow the administration of polysaccharide to mice or human patients, can be attributed to any direct toxic action of the polysaccharide on the tumor. Algire (1) considers, rather, that the damage done to tumors is secondary to vascular damage and the resulting anoxia of shock. It is possible that there may also be intermediate products of metabolism responsible for the toxic effects observed.

It is not clear how the observed enhancement of areal increase following treatment with bacterial preparations is brought about. It is possible that there is an enzymatic breakdown of polysaccharide to simple sugars which supply energy to the tissue. Such complexes sometimes provide detoxifying mechanisms for the removal of products of tissue metabolism.

**SUMMARY**

The effect of bacterial polysaccharide fractions isolated from *Serratia marcescens* and *Aerobacter aerogenes* has been studied on tissue cultures of normal chicken fibroblasts and of cells of Sarcoma 37 from mice. These fractions are not directly toxic for the tissues but may rather enhance the areal increase of both types of tissue.

**REFERENCES**

1. Algire, G. H. The Transparent Chamber Technique as a Tool in Experimental Tumor Therapy. Approaches to


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