The Effect of D-Glucosamine Hydrochloride and Related Compounds on Tissue Cultures of the Solid Form of Mouse Sarcoma 37*

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Filtrates of cultures of bacteria, such as Escherichia coli (3), Neisseria meningitidis (11), and Bacillus prodigiosus (Serratia marcescens) (4, 10) have been shown in laboratory animals to have inhibitory effects on cancer growth. Relatively pure preparations from Serratia marcescens filtrates have been used with equivocal results in patients with malignancies (8). In general, the effect of these preparations upon cancer appears to be associated with carbohydrate moieties containing hexosamine in the form of N-acetyl-D-glucosamine (4, 6). In addition, hypotension frequently follows their administration to animals or man. Quastel and Cantero (9) recently reported that the hexosamine D-glucosamine HCI, itself, inhibited the growth of Sarcoma 37 in Carworth Farms (C.W.F.) mice. They found that these sarcoma-bearing mice developed hemorrhagic, necrotic lesions within their tumors following repeated intraperitoneal injections of D-glucosamine HCl in concentrations which produced no adverse effects on normal animals. However, Lettré (7) in a short paper indicated that he could not confirm the results obtained by Quastel and Cantero. The strain of mice he used was not mentioned. To obtain more information as to the nature of a possible tumor-inhibiting mechanism of D-glucosamine HCl, we have studied its effect, as well as that of several related substances, on Sarcoma 37 grown in tissue culture where hemorrhage within the tumor or a host reaction such as hypotension cannot take place.

MATERIALS AND METHODS

The solid form of Sarcoma 37 was implanted with a trocar in the right axillary region of adult male albino C.W.F. mice (Webster strain). The mice bearing Sarcoma 37 were originally obtained from the Roscoe B. Jackson Memorial Laboratory. The tumor was maintained by weekly transplant in C.W.F. mice. Six to 10 days following inoculation with the tumor, the mice were killed with ether. Aseptic technic was employed throughout the experiments. The tumors were removed and washed in Gey’s solution (2). Material as free as possible from blood vessels was used.

The tumor was next submerged in a nutrient fluid consisting of 6 ml of Gey’s solution and 6 ml of chick embryo extract (1 part of 10-day-old chick embryo and 2 parts of Gey’s solution). To this mixture were added 6-9 drops of human placental serum and approximately 0.001 gm. of potassium penicillin G. In this medium the tumor was cut into 2 mm. pieces. Each piece was then placed in a drop of nutrient medium on a cover slip. One drop of chicken plasma was added. This medium coagulated. The cultures were inverted over depression slides, sealed with a paraffin-vaseline mixture, and incubated at 37° C.

Cultures were prepared on twelve different occasions over a 7-month period, with tumors obtained from 28 mice.

The number of cultures made in each experiment varied with the size and condition of the tumors. A total of 794 cultures was prepared, but only 504 had grown well enough after 24 hours to be used for experimentation. At that time relatively few cells had begun to degenerate. The cultures were examined under the microscope, and the extent of growth and percentage of dead cells estimated. The cultures were graded according to the following scheme:

Good: Good growth; sarcoma cells migrating from 2 or more of explant; few cells degenerating (Fig. 1).

Fair: Fair growth; not so many sarcoma cells, and migration from smaller area of ex-
The pH ranges for the final mixtures with chicken plasma were: nutrient medium, 7.4–8.0; D-glucose with nutrient medium, 7.6–8.1; D-glucosamine HCl with nutrient medium, 7.1–7.8; N-acetyl-D-glucosamine with nutrient medium, 7.25–7.6; and D-glucose and NH₄Cl (equimolecular mixture) with nutrient medium, 7.1–7.5. All solutions were used immediately after preparation.

A drop of the material to be tested was added to 24-hour-old cultures exhibiting good growth. To diminish dilution of the test material, the drop was withdrawn and a second drop of the same material added. The cultures were resealed and incubated. In all experiments, about 25 per cent of the cultures were grown in nutrient medium alone. These served as controls.

All cultures were studied 24 hours after treatment with the test material. They were usually re-examined at the end of 48 hours. The cultures were graded according to the scheme described above. Some were observed 3, 5, and 6 days after treatment. Since all cultures older than 48 hours began to show considerable degeneration unless fed, and since their refeeding as well as the accumulating metabolic products introduce further variables, findings more than 48 hours after inoculation with the test material are not reported.

Neutral red was added to the cultures if there was any question as to the viability of the cells. The absorption of the neutral red into the cytoplasm of the cells was considered indicative of their viability.

**RESULTS**

Table 1 summarizes the results. All the substances tested, except N-acetyl-D-glucosamine, had highly significant adverse effects on the tissue cultures when compared with the controls.¹ At the end of 24 hours, in 14 per cent of the cultures treated with 1 per cent D-glucosamine HCl, all cells were dead. In 71 per cent, one-third or more of the cells were degenerating (Fig. 3). All the controls were alive, and only 3 per cent showed some degeneration (Fig. 1). After 48 hours only 16 per cent of the controls were degenerating or dead, whereas 88 per cent of the specimens with 1 per cent D-glucosamine HCl were either damaged or dead. With a 1 per cent equimolecular mixture of glucose and ammonium chloride, 100 per cent of cultures were dead or degenerating after 24 hours.

The addition of 1 per cent D-glucose to the sarcoma cultures was also detrimental to their growth (Fig. 2). This became especially evident after 48 hours. Degeneration or death was found in 50 per cent of the cultures after 24 hours; 71 per cent of the controls were degenerating or dead after 48 hours.

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¹ The probability of these differences being due to chance is less than one in one thousand (P < .001). For the purposes of analysis, all cultures less than “good” were grouped together. An analysis of variance indicated no significant differences between the control groups; therefore, they were considered as a single population.

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**TABLE 1**

<table>
<thead>
<tr>
<th>HEXOSE IN NUTRIENT MEDIUM</th>
<th>No. EXPERIMENTS</th>
<th>No. CULTURES</th>
<th>GOOD* 48 hrs.</th>
<th>FAIR 48 hrs.</th>
<th>POOR 48 hrs.</th>
<th>NONE 48 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucosamine HCl (1.0 per cent)</td>
<td>8</td>
<td>120</td>
<td>29†</td>
<td>12†</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>D-glucosamine HCl (0.5 per cent)</td>
<td>2</td>
<td>51</td>
<td>53†</td>
<td>53†</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>D-glucose and NH₄Cl (equimolecular) (1.0 per cent)</td>
<td>2</td>
<td>54</td>
<td>0†</td>
<td>0†</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>D-glucose (1.0 per cent)</td>
<td>8</td>
<td>66</td>
<td>50†</td>
<td>29†</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>D-glucose (0.5 per cent)</td>
<td>2</td>
<td>49</td>
<td>94</td>
<td>37†</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>N-acetyl-D-glucosamine (1.0 per cent)</td>
<td>2</td>
<td>55</td>
<td>23</td>
<td>93</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Nutrient medium alone</td>
<td>12</td>
<td>109</td>
<td>97</td>
<td>84</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

* Definitions of good, fair, poor, and none are given under "Methods."

² Highly significant (P < .001) difference from controls (nutrient medium alone) by χ² analysis.

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the cultures fell into this category after 48 hours. After 24 hours, 0.5 per cent D-glucosamine HCl was comparable in toxicity to 1 per cent D-glucose. However, after 48 hours, even 0.5 per cent glucose was significantly more toxic (P > 0.02 < 0.05) than 0.5 per cent D-glucosamine HCl. On the other hand, 1 per cent N-acetyl-D-glucosamine had no significant effect on cultures after 24 or 48 hours. Only 7 per cent of the cultures treated with this compound showed extensive degeneration even after 48 hours. This figure compared favorably with that obtained for the controls.

**DISCUSSION**

Under the experimental conditions described, D-glucosamine HCl was toxic to the Sarcoma 37 cells grown in tissue cultures. This effect was not due to an altered hydrogen ion concentration, since the pH of all final mixtures was approximately the same (slightly above 7.0), owing to the buffering capacity of the nutrient medium. It is known that D-glucosamine HCl under such circumstances exists as the free base, D-glucosamine, and chloride ion (12). Free D-glucosamine decomposes above pH 7 (1, 5) and liberates ammonia. The chloride ion and ammonia may account for the toxic effect of the D-glucosamine HCl. This view is supported by the finding that an equimolecular mixture of D-glucose and ammonium chloride in 1 per cent concentration was extremely injurious to the tissue cultures. However, it must be remembered that 1 per cent D-glucose alone had a markedly toxic effect on Sarcoma 37.

The toxicity of the test solutions also should be considered. However, this factor does not seem to have been of importance, as 1 per cent D-glucose, which is approximately equal in toxicity to 0.5 per cent D-glucosamine HCl, was more toxic than the latter. Also, 1 per cent N-acetyl-D-glucosamine, with the same toxicity as 1 per cent D-glucose, had no adverse effect on the tissue cultures. Indeed, it appeared that in several cultures the growth was unusually prolific after the addition of N-acetyl-D-glucosamine.

**SUMMARY**

The effect of various hexoses upon mouse Sarcoma 37 growing in tissue culture was investigated. The hydrogen ion concentrations of all the hexose preparations were similar. A 1 per cent equimolecular mixture of D-glucose and ammonium chloride, when added to the nutrient medium, proved the most toxic of the substances tested. One per cent D-glucosamine HCl was next most toxic. One per cent D-glucose was less injurious. D-Glucose and D-glucosamine HCl in 0.5 per cent concentration still had an unfavorable influence. All these results were statistically highly significant (P < 0.001) when compared with those obtained with the controls. One per cent N-acetyl-D-glucosamine exhibited no harmful effects. It is suggested that the deleterious action of D-glucosamine HCl on tissue cultures of Sarcoma 37 may be due to the chloride ions and decomposition of the glucosamine molecule.

**ACKNOWLEDGMENTS**

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**REFERENCES**

tumors (7). The relatively slight difference between these two series for mice with AK4 leukemia early massive blood-borne invasion of organs. For transition from peritoneal exudate was overshadowed by organ invasion from various sites (charts) was paralleled by their trend to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs is the essential lethal factor.

In the flank indicated the role of primary growth as tissue either at the site of inoculation or from the opposite tendencies to spread extensively into the wall and to grow subcutaneously in the scalp and eventually being the direct lethal factor.

Leukemic cells into organs was the essential lethal factor. However shorter survival of all leukemic strains the difference in the amount of tissue growth 'from benign and malignant tumors with regard to their two portions was found for the tendency of tumor cells on the 6th day) was higher for leukemias reported above and those on other tumors (6) that both tendencies vary only quantitatively for all the mouse tumors investigated (leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to implantation into abdominal organs from peritoneal exudate. It appears from the results on xenografts that organs were invaded from intraperitoneal growth earlier and to a greater extent than from subcutaneous growth. This was attributed to their autonomy and, perhaps, more precisely to their interdependence, while the ability to grow as free cells in the blood, and to infiltrate into organs is based on their interdependence, specificity characteristic of malignant cells due to their interdependence, specificity characteristic of malignant cells due to...
All photomicrographs were made of living tissue cultures from mouse Sarcoma 37.

Fig. 1.—A 48-hour-old culture treated with nutrient medium when it was 24 hours old. The culture is growing well, and the cells appear normal.

Fig. 2.—A 48-hour-old culture treated with 1 per cent D-glucose in nutrient medium when it was 24 hours old. The growth is fair. Some of the cells have contracted and are abnormal.

Fig. 3.—A 48-hour-old culture treated with 1 per cent D-glucosamine HCl when it was 24 hours old. Many of the cells have contracted and are abnormal or dead.
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