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Inhibitory Effect of D-Glucosamine and Other Sugar Analogs on the Viability and Transplantability of Ascites Tumor Cells[1]

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SUMMARY

D-Glucosamine has been shown to have a powerful cytotoxic effect on various ascites tumor lines, resulting in a decrease in viability and transplantability of the neoplastic cells. The toxic effect of glucosamine was not significantly altered by addition of either glucose or pyruvate. The effects of D-glucosamine and other sugar analogs were evident histologically even before the cells became stainable by trypan blue.

D-Galactosamine and D-mannosamine also reduced the viability and transplantability of ascites tumor cells similar to that of D-glucosamine. However, the *N*-acetylhexosamines caused only a minor decrease in cell viability, and these cells developed tumors when inoculated into mice.

D-Mannose was the only neutral sugar showing a cytotoxic effect on ascites tumor cells.

INTRODUCTION

In 1953 Quastel and Cantero (25) reported that daily administration of D-glucosamine inhibited the growth of Sarcoma 37 in mice. Since this original observation, D-glucosamine has been tested as an antitumor agent in a variety of experimental tumors with inconclusive or negative results (1, 5, 18, 20, 28, 31). Inhibitory effects of D-glucosamine on the growth of neoplastic tumors was reexamined in our laboratory, and it was found, that under specific conditions, this amino sugar caused the regression of experimental tumors in mice and rats (J. G. Bekesi, Z. Molnar, and R. J. Winzler, unpublished experiment).

In vivo treatment of neoplastic tissues has limitations for study of the biochemical bases of the effects of any agent, since concentrations of the test compound as well as other parameters at any given time is unknown and cannot readily be regulated. We have, therefore, studied the effects of incubation *in vitro* with D-glucosamine and other sugar analogs on the ultrastructure (Z. Molnar, J. G. Bekesi, and R. J. Winzler, Cytotoxic Effect of D-Glucosamine and 2-Deoxy-D-Glucose on the Ultrastructure of Ascites Tumor Cells, submitted to Cancer Research), the synthesis of protein, RNA, and DNA (2), and on the viability and transplantability of Ehrlich ascites carcinoma, Sarcoma 37, and Sarcoma 180 ascites tumor cells. This paper deals with the effects of glucosamine and other sugar analogs on viability and transplantability.

Woodward (32) has shown that D-glucosamine inhibits the growth of yeast cells. Ely *et al.* (8) reported that D-glucosamine and 2-deoxy-D-glucose inhibited the growth of embryonic chicken heart in tissue culture without altering the cell viability. Rubin *et al.* (27) showed that D-glucosamine, but not *N*-acetylglucosamine, caused degeneration of Sarcoma 37 ascites tumor cells in tissue culture.

Cytotoxic effects of glucosamine on human epidermoid carcinoma cells have been noted by Fjelde *et al.* (10) and on Yoshida ascites tumor cells by Lindner *et al.* (17). The effect of various monosaccharides on the pattern of growth and metabolism of several mammalian cell lines in tissue cultures were described by Cox and Gesner (4). It was found that only D-mannose, not any other simple sugar, had a striking effect on the morphologic appearance of human skin fibroblast and BS-C-1 monkey kidney cell lines, while D-mannose was ineffective on 3T3 and CT6 cell lines derived from mouse embryo.

In the present work Ehrlich ascites carcinoma, Sarcoma 37 and Sarcoma 180 ascites tumor cells were incubated in various media in the presence of D-glucosamine or other test compounds, and the transplantability and viability of the incubated cells were examined.

MATERIALS AND METHODS

Animals and Transplantation of Tumors. Male Swiss-Webster mice weighing 30–35 gm were used. The animals were maintained in a thermostatically controlled room at 20°C with a 12-hour light cycle. They were fed with Purina Chow and tap water *ad libitum.*

The Ehrlich ascites carcinoma, Sarcoma 37, and Sarcoma 180 ascites tumors, originally obtained in 1965 from Dr. L. Weiss, Roswell Park Memorial Institute, were maintained by weekly intraperitoneal transplantation of 0.2 ml of diluted ascites fluid (one part of ascites fluid to 10 parts of 0.9% sterile sodium chloride solution, containing 10,000 units of penicillin) into the recipient mice.

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Transplantability and Viability of Ascites Tumor Cells. Freshly harvested 7- to 10-day-old Ehrlich ascites carcinoma, Sarcoma 37, and Sarcoma 180 ascites tumor cells were utilized. To each reaction flask 5 ml of one of the following incubation media were added: (*a*) 0.9% sodium chloride, pH 7.4; (*b*) Krebs-Ringer phosphate buffer, pH 7.4; (*c*) Krebs-Ringer phosphate buffer + 50 μmoles of D-glucose, pH 7.4; or (*d*) Krebs-Ringer phosphate buffer + 100 μmoles of pyruvic acid, pH 7.4. Two ml of Krebs-Ringer solution, containing 0.5 mmole per ml of various carbohydrates under study, were added (the final pH of the solution containing the test compound was always adjusted to 7.4). The flasks were placed in a Dubnoff metabolic shaker (80–85 oscillations per minute) at 37°C for 2 minutes to attain temperature equilibrium, and to each flask was added 1 ml (1.1 to 1.5 X 10^8 cells) of either Ehrlich ascites carcinoma, Sarcoma 37, or Sarcoma 180 ascites tumor cells. The final concentration of the test compounds was 125 μmoles per ml. At selected time intervals, from 0 to 5 hours, the reaction flasks were removed, brought to room temperature and thoroughly mixed; then 0.25 ml of the incubated tumor cells was transplanted intraperitoneally into each mouse. At least seven mice were used to establish each survival time.

Estimation of cell viability of the treated and untreated ascites tumor cells was carried out using trypan blue as vital stain (11, 12, 29). To 0.5 ml of cell suspension, 0.2 ml of 0.5% trypan blue was added. After incubation for 5 minutes at 37°C, 10 to 15 ml of the corresponding incubation medium was added to the cell suspension, which was then mixed, and one to two drops of cell suspension was placed on a standard blood counting chamber. The percentage of viable (unstained) cells was determined by counting three to five hundred ascites cells in each of four counting chambers.

RESULTS

Data presented in Table 1 show the difference in transplantability of unwashed Sarcoma 180 ascites tumor cells and of cells washed with Krebs-Ringer phosphate buffer. There was no significant difference in results with washed or unwashed ascites tumor cells. Therefore, unwashed cells were utilized for all subsequent experiments. Table 2 shows the relation between glucosamine concentration and loss of transplantability and viability of Sarcoma 180 ascites tumor cells. At a glucosamine concentration of 125 μmoles per ml, less than 20% of tumor cells were viable after 4 hours incubation; when inoculated into mice, these cells did not produce tumors. Eighty-seven percent of control Sarcoma 180 ascites cells incubated for 4 hours in the absence of glucosamine remained viable and produced tumors in all inoculated animals.

Studies of the influence of the incubation medium as well as the time of incubation on the cytotoxic effect of D-glucosamine on the neoplastic cells were made. For these experiments the following incubation media were used: (*a*) 0.9% sodium chloride, pH 7.4; (*b*) Krebs-Ringer phosphate buffer, pH 7.4; (*c*) Krebs-Ringer phosphate buffer containing D-glucose (50 μmoles per reaction flask); or (*d*) Krebs-Ringer phosphate buffer containing pyruvic acid (100 μmoles per reaction flask). Such experiments were carried out on both Ehrlich ascites carcinoma and Sarcoma 180 ascites tumor cells; results are presented in Table 3 and 4. It is apparent that the transplantability of both Ehrlich ascites carcinoma and Sarcoma 180 ascites tumors was lost as early as 2 hours, when incubation was carried out in physiologic saline in the presence of 125 μmoles per ml D-glucosamine. The cytotoxic effect of D-glucosamine on these ascites tumors was slightly decreased when the incubation was carried out in the presence of Krebs-Ringer

Table 1

	Unwashed cells	T/I[a]	Washed cells	T/I[a]
Control, unincubated				
Viability (% unstained cells)	93.4 ± 1.8		89.1 ± 2.1	
Transplantability (survival in days)	15.6 ± 1.6	7/7	15.9 ± 1.2	7/7
Control, incubated				
Viability (% unstained cells)	82.2 ± 1.1		76.4 ± 1.4	
Transplantability (survival in days)	22.5 ± 1.5	7/7	21.8 ± 2.2	7/7
Glucosamine, treated				
Viability (% unstained cells)	20.5 ± 2.9		18.1 ± 2.5	
Transplantability (survival in days)	No tumor	7/0	No tumor	7/7

Effect of glucosamine (125 μmoles/ml) on the viability and transplantability of washed and unwashed Sarcoma 180 ascites tumor cells. Freshly harvested ascites fluid was divided into two portions. One portion was centrifuged at 2°C for 3 to 4 minutes at 900 rpm; the sedimented cells were washed twice with 15 ml of ice-cold Krebs-Ringer phosphate buffer and resuspended in the original volume of Krebs-Ringer phosphate buffer containing 50 μmoles of glucose. Incubation of washed and unwashed ascites cells was carried out as described in text in the presence and absence of 125 μmoles of glucosamine per ml.

[a] Number of mice developing tumors/number inoculated.

Table 2

Concentration of glucosamine in incubation media (μmoles per ml)	Viable cells after incubation (% ± S.D.)	T/I[a]
0	87.1 ± 1.9	7/7
31.7	73.0 ± 0.9	7/7
62.5	59.1 ± 1.2	5/7
94.2	29.8 ± 1.3	1/7
125	19.5 ± 1.8	0/7

Effect of D-glucosamine concentration on the viability and transplantability of Sarcoma 180 ascites tumor cells. Each reaction flask contained 5 ml of Krebs-Ringer phosphate buffer, pH 7.4, + 50 μmoles of D-glucose. To this, 2 ml of Krebs-Ringer solution, containing D-glucosamine (adjusted to pH 7.4) to give the indicated concentrations, was added. After 2 minutes preincubation at 37°C, 1 ml (1.4 × 10^8 cells) of Sarcoma 180 ascites tumor cells was added to each flask. Incubation was for 4 hours at 37°C in a Dubnoff incubator cell. Viability was measured by the trypan blue method, and transplantability was determined by intraperitoneal administration of 0.25 ml of the incubated tumor cells into mice. Observation was maintained on these animals for 8 weeks after tumor inoculation. Control experiments were carried out under the same conditions except that glucosamine was omitted.

[a] Number of mice developing tumors/number inoculated.

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Table 3

Incubation time (hours)	Survival time (days)			
	Physiologic saline + glucosamine	Krebs-Ringer phosphate buffer + glucosamine	Krebs-Ringer phosphate buffer + 50 μmoles glucose + glucosamine	Krebs-Ringer phosphate buffer + 100 μmoles pyruvic acid + glucosamine
1	21.2 ± 3.7	11.8 ± 1.1	16.7 ± 0.94	16.0 ± 1.3
2	23.1 ± 3.2	16.0 ± 1.3	17.5 ± 1.38	17.3 ± 0.7
3	No tumor	17.5 ± 1.4	18.7 ± 1.11	16.5 ± 2.3
4	No tumor	No tumor	22.5 ± 1.98	20.5 ± 3.9
5	No tumor	No tumor	No tumor	No tumor
5	No glucosamine	No glucosamine	No glucosamine	No glucosamine
Control	19.2 ± 1.5	17.0 ± 2.0	16.2 ± 1.10	17.2 ± 2.1
Control (no incubation)		15.4 ± 2.6		

Effect of time of exposure to D-glucosamine in different media on the transplantability of Ehrlich ascites carcinoma. To each reaction flask was added 7 ml of the indicated medium, containing 1 mmole of D-glucosamine. Final concentration of glucosamine in the incubation media was 125 μmoles per ml. After 2 minutes equilibrium at 37°C, 1 ml of (1.1 to 1.5 × 10^8 cells) Ehrlich ascites carcinoma cells were added to each flask. Incubation time ranged from 1 to 5 hours in a Dubnoff incubator at 37°C. At indicated times 0.25 ml, representing about 2.5 × 10^6 ascites cells, was transplanted intraperitoneally into each mouse. Each survival time value was obtained from at least seven mice. Observation of these animals was maintained for 8 weeks after tumor inoculation. The control (incubated) experiment was carried out under the same conditions except glucosamine was omitted from the incubation media. The second control ascites tumor was implanted without incubation.

Table 4

Incubation time (hours)	Survival time (days)		
	Physiologic saline + glucosamine	Krebs-Ringer phosphate buffer + glucosamine	Krebs-Ringer phosphate buffer + 50 μmoles glucose + glucosamine
1	19.0 ± 0.94	18.0 ± 0.56	19.3 ± 0.4
2	22.3 ± 0.47	20.2 ± 1.07	23.6 ± 0.5
3	No tumor	19.8 ± 0.69	25.0 ± 0.5
4	No tumor	No tumor	No tumor
5	No tumor	No tumor	No tumor
5	No glucosamine	No glucosamine	No glucosamine
Control	20.5 ± 0.5	17.8 ± 0.69	22.0 ± 0.6
Control (no incubation)		16.2 ± 1.3	

Effect of D-glucosamine on the transplantability of Sarcoma 180 ascites tumor. Incubation of Sarcoma 180 was carried out as described in Table 3. Incubation time ranged from 1 to 5 hours in a Dubnoff incubator at 37°C. At indicated times 0.25 ml, representing about 3.2 × 10^6 ascites cells, was transplanted intraperitoneally into each mouse. Each survival time value was obtained from at least seven mice.

phosphate buffer. Both D-glucose and pyruvate slightly reduced the inhibitory effect of glucosamine on Ehrlich ascites carcinoma cells (Table 3). Addition of glucose provided no further protection against glucosamine in Sarcoma 180 ascites cells (Table 4).

The powerful toxic effect of exogenous glucosamine was not due to the change of extracellular pH during the incubation, since the decrease of pH was maximal (pH 6.8) when the incubation was carried out in unbuffered physiologic saline for a period of 5 hours. When Krebs-Ringer phosphate buffer was utilized, the pH change was less than 0.4 pH units. Comparable pH changes were noted in the absence of glucosamine. This is not surprising since administered D-glucosamine is almost exclusively utilized in the metabolic pathway leading to the formation of uridine diphosphate (UDP)-*N*-acetylhexosamine and only 1 to 3% of the administered dose is oxidized *in vivo* to CO_2 (3, 13).

The effect of incubation time in several media with and without glucosamine on the viability of Ehrlich ascites tumor cells was examined by the trypan blue staining method. Results are presented in Chart 1. Control Ehrlich ascites cells incubated in Krebs-Ringer phosphate buffer, containing glucose or pyruvic acid but no glucosamine, maintained their viability quite well. This was less true when incubation of control ascites cells was carried out only in Krebs-Ringer phosphate buffer. However, incubation of cells in the same buffer systems in the presence of 125 μmoles per ml glucosamine resulted in rapid loss of viability, and after 5 hours less than 5% of the cell population was unstained.

In order to examine whether the effect of D-glucosamine is similar with different experimental tumors, Sarcoma 37, Sarcoma 180, and Ehrlich ascites carcinoma cells were incubated in the presence of D-glucosamine, and their viability was examined by the trypan blue staining method. From the results

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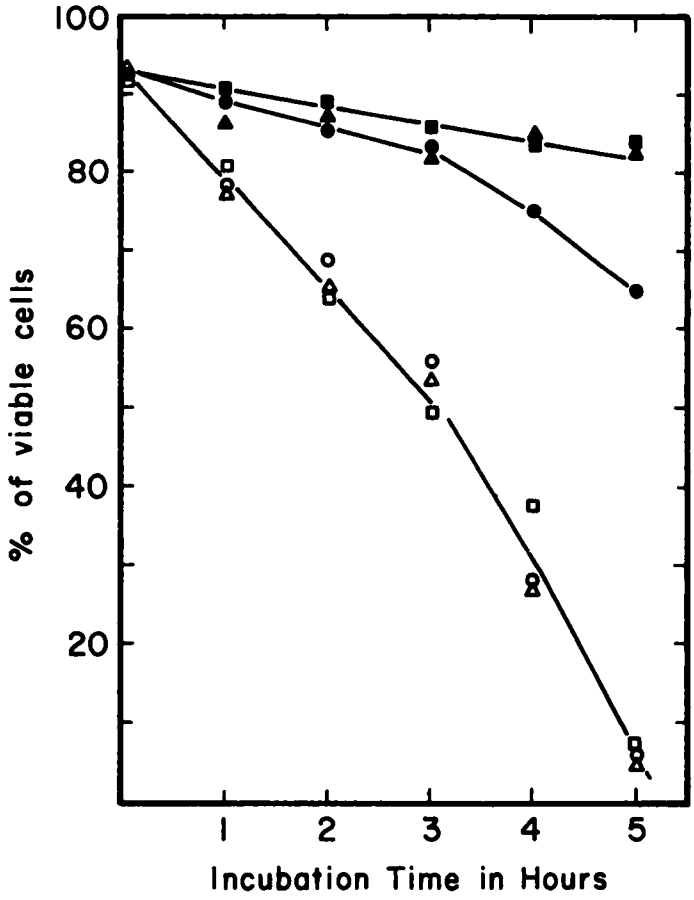

Chart 1. The effect of D-glucosamine on the viability of Ehrlich ascites carcinoma as a function of time and media. Incubation of Ehrlich ascites tumor cells was as described for Table 3. After incubation the cell viability was determined by the trypan blue staining method, where only unstained cells were considered viable. Krebs-Ringer phosphate buffer + glucosamine, —o—o—; Krebs-Ringer phosphate buffer, —●—●—; Krebs-Ringer phosphate buffer + 50 μmoles glucose + D-glucosamine, —△—△—; Krebs-Ringer phosphate buffer + 50 μmoles glucose, —▲—▲—; Krebs-Ringer phosphate buffer + 100 μmoles pyruvic acid + glucosamine, —□—□—; Krebs-Ringer phosphate buffer + 100 μmoles pyruvic acid, —■—■—.

presented in Chart 2, it is apparent that the three ascites tumor lines were equally susceptible to glucosamine treatment.

It has been noted before that the inhibitory effects of sugars on tumor growth was limited to the nonacetylated amino sugars. Table 5 shows the effects of 19 different sugars, all at a concentration of 125 μmoles per ml, on the viability and transplantability of Sarcoma 180 ascites tumor cells. It is apparent that only the nonacetylated amino sugars and D-mannose caused the loss of viability and destroyed the transplantability of Sarcoma 180 tumor cells. It is noteworthy that D-mannose was the only neutral sugar showing a powerful cytotoxic effect. This was an unexpected finding, since it is generally assumed that exogenous D-mannose, like D-glucose and D-fructose, can be utilized by the cells (9). Since D-mannose showed toxic effect on tumor cells in the pilot study, the effect of various concentrations of D-mannose on the viability and transplantability of Sarcoma 180 ascites cells was examined. It can be seen in Table 6 that the loss of viability and transplantability of this ascites tumor cells is progressively decreased with increasing concentration of D-mannose in the incubation media. The effect of different incubation medium as a function of incubation time on the cytotoxic effect of D-mannose on the Sarcoma 180 ascites tumor cells was studied. Table 7 and Chart 3 show that the cytotoxic effect of D-mannose was slightly decreased when the incubation was performed in the presence of Krebs-Ringer phosphate buffer containing either glucose or pyruvate. Thus the effect of D-mannose in every way tested paralleled the cytotoxic effect of D-glucosamine. While D-mannose caused the death of Sarcoma 180 ascites cells, the same concentration of L-mannose showed only a minor change in cell viability: the cells produced tumors when inoculated into mice (Table 5).

DISCUSSION

In the present work various lines of ascites tumor cells were exposed to D-glucosamine *in vitro*, and the transplantability and viability of the incubated neoplastic cells were examined. It is clear from the data presented that the decrease in cell viability and transplantability was proportional to the increasing concentration of glucosamine and dependent on the composition of incubation media. The cytotoxic effect of glucosamine was not prevented by addition of either glucose or pyruvate. This substantiates the observations of Kono and Quastel (14) and Martin (21) that the inhibitory effect of D-glucosamine is not due to the impairment of glucose phosphorylation, since this metabolic step is apparently not involved in the utilization of exogenous pyruvic acid.

The effect of glucosamine on the viability of Sarcoma 37, Sarcoma 180, and Ehrlich ascites carcinoma cells was examined by the trypan blue staining method. The data thus obtained were in good agreement with those obtained from the transplantability study. However, cytotoxicity measured by the trypan blue method appeared to be less sensitive.

The cytotoxic effects of glucosamine on the neoplastic cells were evident histologically even before the cells became stainable by trypan blue. The glucosamine-treated ascites cells first became swollen, and this was followed by the formation of cytoplasmic vacuoles after exposure to glucosamine for about 2 hours. After three hours incubation, the cytoplasm and nucleus of ascites cells became filled with fine granules. These findings support the suggestion by King *et al.* (12), Holmberg (11), and Eaton *et al.* (7) that there must be a complete breakdown in the cell metabolism before the cell becomes stainable by trypan blue.

A detailed study of the effects of glucosamine on the ultrastructure of Ehrlich ascites carcinoma and Sarcoma 180 is presented separately (Z. Molnar, J. G. Bekesi, and R. J. Winzler, Cytotoxic Effect of D-Glucosamine and 2-Deoxy-D-Glucose on the Ultrastructure of Ascites Tumor Cells, submitted to Cancer Research).

Glucosamine has been found to be a major constituent of plasma glycoproteins as well as mucopolysaccharides and

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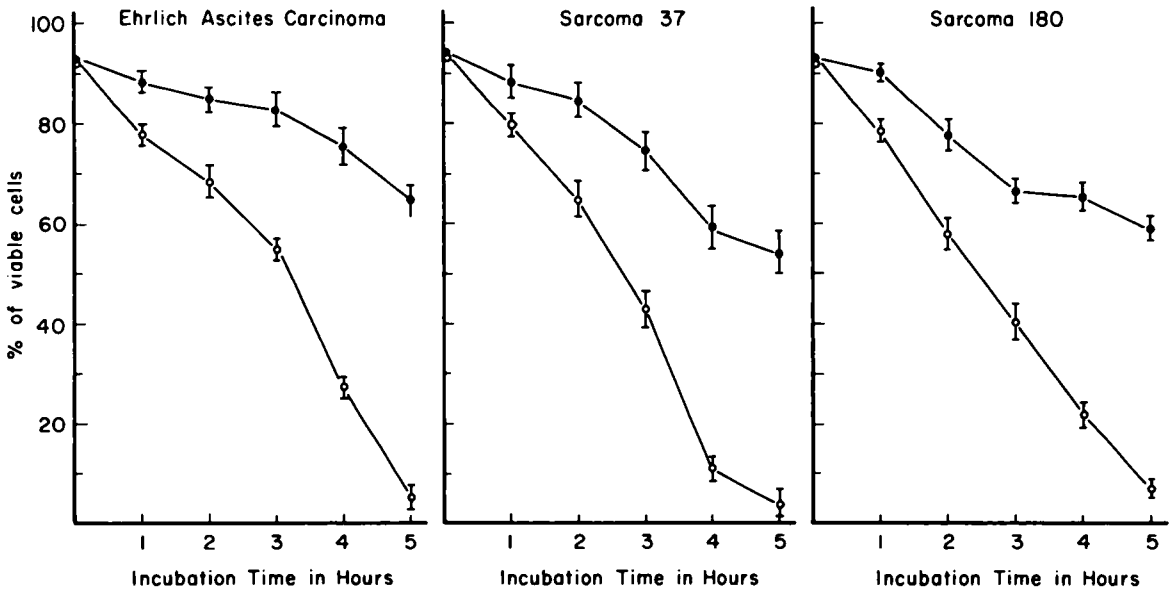

Chart 2. The effect of D-glucosamine on the viability of Ehrlich ascites carcinoma, Sarcoma 37 and Sarcoma 180 ascites tumors. Each reaction flask contained 5 ml of Krebs-Ringer phosphate buffer, pH 7.4, and 2 ml of 0.5 mmole per ml glucosamine dissolved in Krebs-Ringer solution (adjusted to pH 7.4). After 2 minutes equilibrium at 37°C, 1 ml (1.1 to 1.5 × 10^8 cells) of Ehrlich ascites carcinoma, or Sarcoma 37, or Sarcoma 180 ascites tumor cells were added to each flask. Incubation times ranged from 0 to 5 hours in a Dubnoff incubator at 37°C. Control experiments were carried out under the same conditions except that glucosamine was omitted. Cell viability was measured by the trypan blue staining method. Krebs-Ringer phosphate buffer + D-glucosamine, —○—○—; Krebs-Ringer phosphate buffer, —●—●—.

Table 5

Compounds	Viable cells after incubation, (% ± S.D.)	% of animals with tumors	Survival time, animals dying with tumor, days ± S.D.
No incubation	92.7 ± 1.2	100	15.8 ± 1.5
Incubated control	83.3 ± 1.9	100	21.5 ± 1.9
D-Glucosamine	24.1 ± 3.2	0	No tumor
N-Acetylglucosamine	70.5 ± 1.8	100	22.4 ± 2.0
D-Mannosamine	32.6 ± 4.6	0	No tumor
N-Acetylmannosamine	75.3 ± 2.5	100	21.9 ± 3.2
D-Galactosamine	30.5 ± 4.9	100	No tumor
N-Acetylgalactosamine	84.8 ± 2.3	100	19.3 ± 1.6
D-Glucose	81.3 ± 1.1	100	22.8 ± 2.7
α-Methyl-D-glucoside	79.9 ± 4.0	100	16.8 ± 1.9
2-Deoxy-D-glucose	82.4 ± 2.4	100	22.6 ± 1.5
D-Galactose	68.0 ± 3.4	100	19.0 ± 2.1
D-Fructose	77.7 ± 1.2	100	20.4 ± 2.9
D-Mannose	32.1 ± 2.8	0	No tumor
L-Mannose	82.4 ± 0.9	100	20.7 ± 2.3
D-Fucose	71.0 ± 1.9	100	18.2 ± 1.5
L-Fucose	61.2 ± 1.6	100	20.0 ± 1.0
L-Rhamnose	82.6 ± 2.4	100	21.0 ± 1.6
D-Ribose	88.5 ± 0.5	100	22.0 ± 1.9
D-Xylose	82.8 ± 1.5	100	19.8 ± 1.7
L-Arabinose	89.2 ± 4.1	100	18.8 ± 1.1
Pyruvate	68.7 ± 4.0	100	19.4 ± 2.3

Effect of various carbohydrates on the viability and transplantability of Sarcoma 180 ascites tumor. Each reaction flask contained 5 ml of Krebs-Ringer phosphate buffer, pH 7.4, + 50 μmoles of glucose and 2 ml of 0.5 mmole per ml of various carbohydrates (dissolved in Krebs-Ringer solution adjusted to pH 7.4). The final concentration of the test compound was 125 μmoles per ml. This incubation mixture was equilibrated at 37°C for 2 minutes, then 1 ml (1.4 × 10^8 cells) of Sarcoma 180 ascites tumor cells was added to each flask and further incubated for 4 hours at 37°C in a Dubnoff incubator. After incubation 0.25 ml of the incubated tumor cells, representing about 3.2 × 10^6 cells, was transplanted intraperitoneally into each mouse. For each compound at least 6 mice were used.

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Table 6

Concentration of D-mannose in incubation media (μmoles per ml)	Viable cells after incubation (% ± S.D.)	T/I[a]
0	84.1 ± 1.8	7/7
31.7	69.5 ± 2.3	7/7
62.5	58.8 ± 2.1	4/7
94.2	31.7 ± 1.4	1/7
125	22.4 ± 1.7	0/7

Effect of D-mannose concentration on the viability and transplantability of Sarcoma 180 ascites tumor cells. Incubation of Sarcoma 180 was carried out as described in Table 2.

[a]Number of mice developing tumors/number inoculated.

glycoproteins of various animal tissues. Administered glucosamine-^{14}C serves as a precursor for both protein-bound hexosamine and *N*-acetylneuraminic acid in normal and tumor-bearing animals (3, 13, 19, 23, 26, 30). The metabolism of exogenous glucosamine by Ehrlich ascites tumor cells (23, 24) and by HeLa S_3 cells in tissue culture (15) has been investigated. The data obtained from these experiments suggest that glucosamine-^{14}C was also extensively utilized by both cell lines in the biosynthetic pathway of protein-bound hexosamine and *N*-acetylneuraminic acid.

Kornfeld *et al.* (16) have shown that UDP-*N*-acetylhexosamine regulates the *de novo* synthesis of glucosamine through its action as a feedback inhibitor of glutamine-fructose-6-phosphate transamidase, which catylizes the conversion of fructose-6-phosphate to glucosamine-6-phosphate. The entry of exogenous glucosamine into the metabolic pathway bypasses this control step, and thus its metabolism is not regulated by this feedback inhibition. It was demonstrated by the authors that the administration of a high concentration of glucosamine can result in significant accumulation of the intracellular pool of UDP-*N*-acetylhexosamine in both liver (16) and HeLa cells (15). This increase in sugar nucleotide pool may result in an impairment in the formation of other nucleotides (16).

Studies on the biochemical basis for the cytotoxic effect of D-glucosamine have been initiated in our laboratory. At the concentrations used for the present viability and transplantability study, glucosamine inhibits by 70 to 85% the synthesis of protein, RNA, and DNA by several experimental tumors

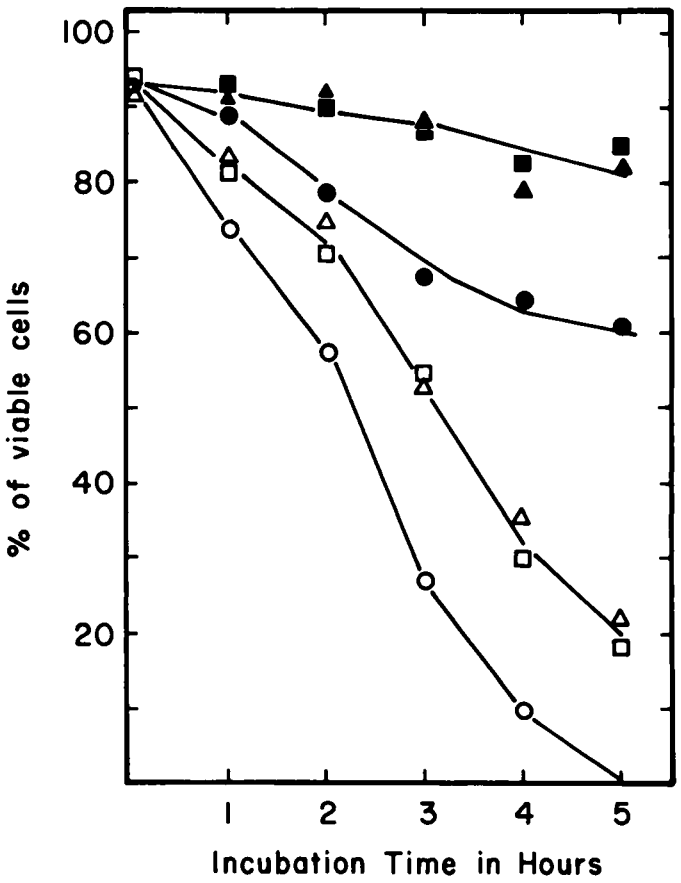

Chart 3. The effect of D-mannose on the viability of Sarcoma 180 ascites tumor in the presence of either glucose or pyruvic acid. Incubation of ascites cells was carried out as described in Table 3 except glucosamine was replaced with D-mannose. Krebs-Ringer phosphate buffer + D-mannose, —○—○—; Krebs-Ringer phosphate buffer, —●—●—; Krebs-Ringer phosphate buffer + 50 μmoles or glucose + D-mannose, —△—△—; Krebs-Ringer phosphate buffer + 50 μmoles glucose, —▲—▲—; Krebs-Ringer phosphate buffer + 100 μmoles of pyruvate + D-mannose, —□—□—; Krebs-Ringer phosphate buffer + 100 μmoles pyruvic acid, —■—■—.

over a 30-minute, *in vitro* incubation period (2). This effect of glucosamine appears to be related to an extensive intracellular

Table 7

Incubation time (hours)	Survival time (days)		
	Krebs-Ringer phosphate buffer + D-mannose	Krebs-Ringer phosphate buffer + 50 μmoles glucose + D-mannose	Krebs-Ringer phosphate buffer + 100 μmoles pyruvic acid + D-mannose
1	18.5 ± 1.6	18.0 ± 1.0	17.3 ± 1.7
2	21.5 ± 1.1	20.8 ± 1.0	20.5 ± 1.3
3	No tumor	29.5 ± 3.1	28.1 ± 2.5
4	No tumor	No tumor	No tumor
4	No D-mannose added	No D-mannose added	No D-mannose added
Control	17.5 ± 1.4	19.5 ± 0.5	18.5 ± 0.8
Control (no incubation)		15.8 ± 1.5	

The effect of D-mannose on the transplantability of Sarcoma 180 ascites tumor cells. Incubation of ascites cells was carried out as described in Table 3 except that glucosamine was replaced by D-mannose.

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accumulation of glucosamine derivatives, including UDP-*N*-acetylhexosamine (2). At the same time there was a significant reduction of the other uridine nucleotide pools, namely, the mono-, di-, and triphosphates, UMP, UDP, and UTP (2). Evidence is not yet available to indicate whether any of these changes is the primary cause of the inhibitory effects of glucosamine on the tumors.

It is interesting and significant that *N*-acetylglucosamine did not alter the viability and transplantability of Sarcoma 180 ascites tumor cells. The most reasonable explanation is a difference in the permeability of neoplastic cells of glucosamine and *N*-acetylglucosamine. Differences between the metabolism of these two amino sugars administered in trace amounts has already been reported (13, 22). In addition, other amino sugars (D-galactosamine and D-mannosamine), but not their *N*-acetyl derivatives, showed carcinostatic activities.

Another surprising observation is that D-mannose, of all the neutral sugars tested, was the only one having cytotoxic activity. L-mannose at the same concentration showed no inhibition of growth of neoplastic tissues. Studies on the antitumor activity of D-mannose are under way and will be reported separately.

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