EFFECT OF HEPARIN ON MALIGNANT TUMOR CELLS IN TISSUE CULTURE

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Numerous recent investigations have shown that high polymers of mucopolysaccharide type play an active part in intermediate metabolism, in the processes of interaction between viruses and bacteria and the host organism, and in immunogenesis and carcinogenesis. Because of its high negative electrical charge, the acid mucopolysaccharide heparin inhibits the action of trypsin [3, 6], alkaline and acid ribonuclease [7, 8], pepsin [4], hyaluronidase [2, 5], desoxyribonuclease, cathepsin, and lysozyme [9].

We have studied the action of heparin on the enzymic activity of the cells of malignant tumors in tissue culture. To determine the specificity of the action of heparin on the enzymic activity of the cell, protectors (glutathione, cysteine) were used to remove the enzymes from the mitochondrial membranes into the cytoplasm, after which heparin was allowed to act on the tissue.

Experimental and clinical data indicate that peroxide compounds, giving off atomic oxygen on decomposition, are capable of activating the aerobic phase of tissue respiration. In particular, sodium persulfate (Sulnat) is effective in overcoming tissue hypoxin [1]. We were interested in studying the combined action of Sulnat and heparin intracellular enzymes.

EXPERIMENTAL METHODS

The following transplantable strains of malignant tumors were studied: 1) epithelial – HeLa and KB; 2) connective-tissue – strain No. 709 (angiosarcoma). The tissue was cultuvated for 3 days on glass slides. The culture medium consisted of medium 199 with 10% ox serum. Purified crystalline heparin was used. For treatment of the tissue, heparin was added to the culture medium in a concentration of 13 units/ml (0.1 mg) or a solution of the same concentration was made in buffered physiological saline at pH 5.8. The presence of heparin, or of heparin activity, after addition to the culture medium was judged by the anticoagulant properties of the medium (prevention of clotting of a drop of fresh rabbit's blood).

Glutathione or free cysteine was dissolved in a concentration of 10 mg/100 ml physiological saline and Sulnat in a concentration of 10-20 mg/100 ml physiological saline. The solutions were prepared just before use. The cells of the culture were treated for 5, 10, 30, 60, and 120 min, after which the tissue was examined histochemically. The order of the experiment was as follows: 1) treatment with heparin, 2) with glutathione or cysteine, 3) with glutathione or cysteine, followed by heparin, 4) with Sulnat, 5) with heparin followed by Sulnat, 6) with Sulnat and heparin at the same time, 7) with Sulnat followed by heparin.

Mucopolysaccharides were determined by staining with alcian blue, RNA by Brachet's method, lipids with Sudan black B, and mitochondria were demonstrated by vital staining with Janus green. Tests were also made for the following enzymes: cytochrome oxidase, succinate dehydrogenase, acid and alkaline phosphatase by Gomori's method, nonspecific esterases, and lipases. To study the effect of heparin on mitotic activity the substance was added to the culture medium in a concentration of 13 units/ml. At the end of 6 h, preparations of the tissue culture were fixed with acetone, stained by Brachet's method, and the mitotic index was then calculated. Cells treated with heparin were also investigated by phase contrast. Altogether 1500 preparations of tissue cultures were studied in the experiments.

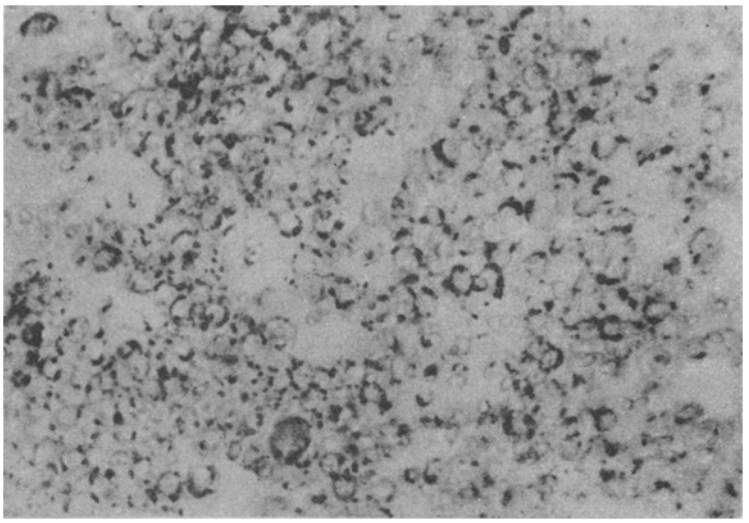

Fig. 1. Culture of KB tissue (control). NADI-reaction. Distribution of diformazan granules in the cytoplasm of the cells. Photomicrograph. Ocular 10, oxjective 20.

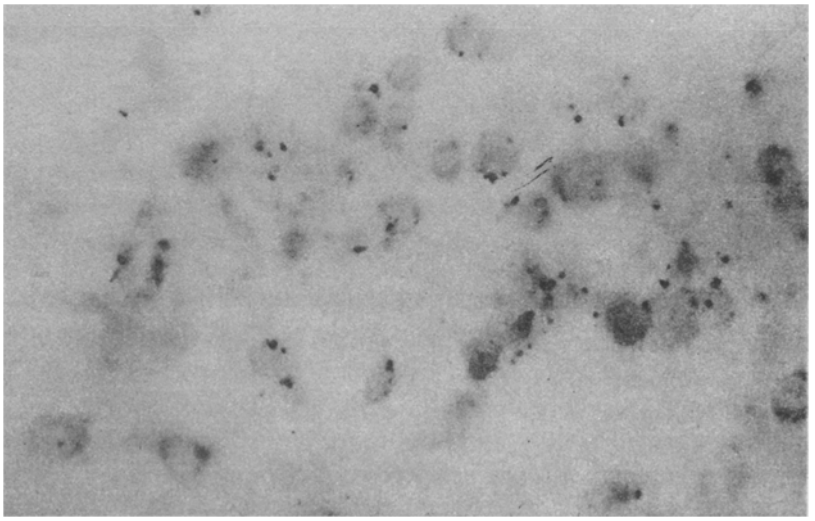

Fig. 2. Culture of KB tissue, treated for 30 min with heparin. NADI-reaction. Depression of enzyme activity. Photomicrograph. Ocular 10, objective 20.

EXPERIMENTAL RESULTS

The results for both sarcoma and carcinoma cells were similar. After exposure to the action of heparin for 30 min morphological changes were observed in the malignant cells in tissue culture, characterized by distintegration of the monolayer, rounding of individual cells, and pycnosis. Some cells had thrown off a large number of pseudopodia, whereas their ameboid movement had ceased. Some cells had become detached from the glass slide.

On staining by Brachet's method large quantities of RNA were found in nearly all the cells of the layer, especially in the rounded and pycnotic cells. Accordingly the mitotic index fell sharply (from 70-150 to 20-30 cells per 1000). Abnormal forms of mitosis appeared, with giant hyperchromic chromosomes. Substances of mucopolysaccharide nature were found in the form of a thin border around the cells and also as inclusions within the cytoplasm.

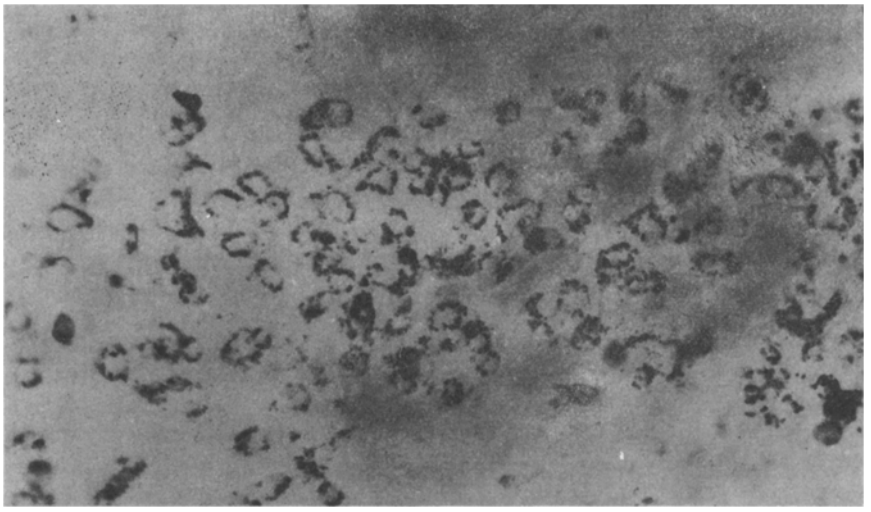

Fig. 3. Culture of KB tissue, treated with heparin (60 min) followed by Sulnat (60 min). NADI-reaction. Increase in enzyme activity. Photomicrograph. Ocular 10, objective 20.

TABLE 1. Effect of Heparin on Enzymic Activity of Malignant Cells in Tissue Culture

Enzyme	Control	Period of action of heparin (in min)				
		5	10	30	60	120
Cytochrome oxidase	++++	++	++	+	0	+++
Succinate dehydrogenase	++++	++	+	+	0	+++
Alkaline phosphatase	++++	+++	+++	++	+	+
Acid phosphatase	++++	+++	+++	++	+	++
Esterase	++++	++++	++++	+++	++	++
Lipase	++++	++++	++++	+++	+++	++

Legend: ±, +, ++, +++, ++++ etc. denote different degrees of activity of enzymes. 0) absence of activity.

The cytoplasm of many cells contained a mass of lipoid granules. In the cells retaining their processes and in those with pseudopodia these granules were very tiny and numerous; in the rounded cells the lipoid granules were merged to form large droplets. The ability of the mitochondria to fix Janus green fell sharply 5-10 min after addition of heparin. The lowering of the activity of the mitochondria was reversible in character: their normal activity was restored 2 h after addition of heparin to the culture.

The results given in Table 1 show that heparin depresses the activity of different categories of enzymes. The respiratory enzymes cytochrome oxidase and succinate dehydrogenase were inhibited particularly rapidly and completely, and their inactivation was complete after treatment with heparin for 1 h. The activity of these enzymes was restored after exposure to heparin for 2 h, although active heparin, retaining its anticoagulant properties, continued to be detectable in the incubation medium. The activity of the acid and alkaline phosphatases also was inhibited quickly, but less completely. During the period of observation (2 h) we were able to observe any marked reversion of phosphatase activity in only two experiments; usually this was slight. Lipases and esterases proved more resistant to the inhibitory action of heparin.

To determine the mechanism of the inhibitory action of heparin experiments were carried out with the use of protectors – cysteine and glutathione. It may be seen from Table 2 that as a result of exposure of a tissue culture of malignant cells the cysteine or glutathione for 1 h the activity of the intracellular enzymes, apart from esterases and lipases, fell slightly. This was probably due to release of the enzymes into the nutrient medium.

TABLE 2. Effect of Heparin on Cells of a Tissue Culture Treated with Protectors

Enzyme	Control	Cysteine or glutathione	Cysteine or glutathione (50 min) followed by heparin			Heparin			
		period of action (in min)							
		60	5	10	30	60	5	10	30	60
Cytochrome oxidase	++++	+++	+	0	0	0	++	++	+	0
Succinate dehydrogenase	++++	+++	+	0	0	0	++	+	+	0
Alkaline phosphatase	++++	+++	++	+	0	0	+++	+++	++	+
Acid phosphatase	++++	+++	++	±	0	0	+++	+++	++	+
Esterase	++++	++++	++	±	0	0	++++	+++	+++	++
Lipase	++++	++++	++	++	+	0	++++	++++	+++	+++

Legend as in Table 1.

TABLE 3. Effect of Sulnate on Cells of a Tissue Culture Treated with Heparin

Enzyme	Control	Sulnat (60 min)	Heparin (60 min)	Heparin (60 min) followed by Sulnat (60 min)	Sulnat (60 min) followed by heparin (60 min)
Cytochrome oxidase	++++	++++++	±	++++++++	+
Succinate dehydrogenase	++++	++++++	+	++++++++++	+
Alkaline phosphatase	++++	++++	++	++	++
Acid phosphatase	++++	++++	++	++	+
Esterase	++++	++++	+++	+++	+++
Lipase	++++	++++	+++	+++	+++

Legend as in Table 1.

Successive treatment of the cells with protectors followed by heparin led to rapid and almost complete depression of enzymic activity. After exposure to heparin for 30 min after cysteine or glutathione the enzymes were almost completely blocked, whereas pure heparin lowered the enzymic activity considerably later (60 min), and in this respect was less effective. It must be noted that in this variant of the experimental procedure the respiratory enzymes were inhibited before the others.

It may be concluded from the fact that the inhibition of the intracellular enzymes by heparin after treatment with protectors was most rapid and complete, that the interaction between heparin and enzyme is based on the property of this mucopolysaccharide to form a salt-like complex with proteins in general, and with enzyme protein in particular.

The results given inTable 3 show that exposure of cells for 1 h to a freshly prepared solution of Sulnat led to an appreciable increase in the activity of cytochrome oxidase and succinate dehydrogenase. Meanwhile the activity of the remaining enzymes was unchanged. Treatment of the tissue with heparin for 1 h as a rule caused a marked depression of all the enzymes, especially the respiratory. If the tissue culture was treated successively for 1 h with heparin followed by Sulnat, the activity of the respiratory enzymes rose to 2-3 times their initial level. The activity of the remaining enzymes remained depressed to the same degree as before treatment with Sulnat (Figs. 1, 2 and 3). Meanwhile successive treatment of the tissue culture with Sulnat followed by heparin lowered the activity of all the enzymes, including the respiratory (Table 3).

Incubation of heparin with Sulnat for 2 h at 37° in vitro did not lead to loss of the anticoagulant properties of heparin or of its properties related to enzyme inhibition. These results demonstrate that heparin, in vivo, may play the role of a mediator in intermediate metabolism, influencing the character of tumor growth.

SUMMARY

In experiments on the KB, HeLa and 709 tissue culture cells heparin was shown to inhibit the activity of the enzymes, especially of the cytochromoxidase and succindehydrogenase. The highest degree of inactivation was attained by a consecutive protector and heparin action upon the cells. Respiratory enzymes of the cells inhibited with heparin may be selectively reactivated with sodium persulfate (sulnat).

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
