Effect of Sulfomucopolysaccharides on Growth of Tumor Tissue.* (17356)

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Fischer[1] found that injury regeneration in tissue cultures is partially or completely suppressed by heparin. By treating transplants of Flexner-Jobling rat carcinoma with heparin, Goerner[2] could suppress its growth. Zakerzewski[3–6] made a systematic investigation of the influence of heparin on the growth of different tissues *in vitro*. He found that the growth of normal embryonic tissue was suppressed by heparin and that the same thing was true with Jensen sarcoma. He assumes that the growth-suppressing effect of heparin depends on an antiprothrombin action. Zakerzewski has also investigated the growth-suppressing effect of heparin on Jensen sarcoma in rats and on polymorphocellular sarcoma in mice. He could show that less tumor growth and less necrosis resulted from intravenous treatment. Fischer,[7] using pure heparin preparations, later showed that the suppressing effect of heparin on blood-coagulation and tissue growth disappeared after boiling with hydrochloric acid for a short time.

Balazs[8] has shown that other sulfonated mucopolysaccharides (the sodium salt of agar acid and the calcium salt of chondroitinsulfuric acid) as well as heparin suppress the growth of embryonic tissue *in vitro*. This suppressing effect depends on negative groups that are part of the polysaccharide. He could also show that there was a parallelism between the growth-suppressing effect and the number of negative groups per molecule. Only the undifferentiated tissues were found to be sensitive to the substances in question.

It is not only the growth of proliferated tissues from higher animal species that is suppressed by heparin. Fischer and Nyström[9] showed that the growth of yeast cells was also suppressed by heparin. Heilbrunn and Wilson[10] were recently able to demonstrate that heparin suppressed the mitosis of Chaetopterus eggs after fertilization. This action is reversible and has no degenerating effect on the eggs. They assume that heparin prevents the mitotic gelation.

Experimental. In vitro. Our *in vitro* experiments were performed on tumor cells from methyl cholanthrene tumors of the rat. All of the experiments were performed on cultures in Carrel flasks using a 2 phase system. (D 5). The cultures were placed in chick plasma without embryonic extract. In all the experiments the fluid phase of the control cultures has consisted of embryonic extract and physiological saline. Instead of the physiological saline, equivalent amounts of sodium heparinate (0.4 mg per flask) were added to the tissue cultures in one series, and an equivalent amount of the sodium salt of agar acid in another series. In preparing the sodium heparinate we used the powdered form having 13.35% moisture: S, 11.47% and N, 2.00%. This preparation we have gratefully received from Professor E. Jorpes. The sodium salt of agar acid preparation was made according to Hoffman and Gortner from the commercial agar. The preparations were dissolved in physiological saline solution (Ringer), and the pH was controlled between 6.7 and 7.2. In both cases the substances in question were added to cultures 24 hours old.

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2 Goerner, A., *J. Lab. Clin. Med.*, 1930, **16**, 369.

3 Zakerzewski, Z., *Z. f. Krebsforsch.*, 1932, **36**, 513.

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7 Fischer, A., *Protoplasma*, 1936, **26**, 344.

8 Balazs, A., in press.

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10 Heilbrunn, L. V., and Wilson, W. L., Proc. Soc. Exp. Biol. and Med., 1949, **69**, 179.

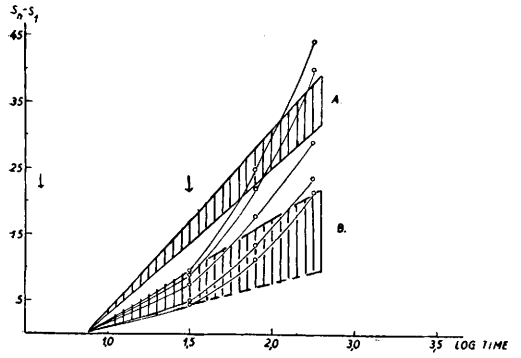

FIG. 1.

Growth of the methyl cholanthrene tumor of rats in a Carrel culture flask. Growth index: S_n–S_1. S_n is the measured growth at different times and S_1 is the growth of the first day (before addition of the fluid phase). The shaded area A represents the control culture (embryonic extract plus Ringer's solution in fluid phase). The shaded area B shows the cultures where the liquid phase has been added twice (embryonic extract plus heparin 25 mg%). The solid lines show the cultures where the fluid phase has been added once (embryonic extract plus heparin 25 mg%). The time of growth is given on a logarithmic scale. Arrows show the changes of the fluid phases.

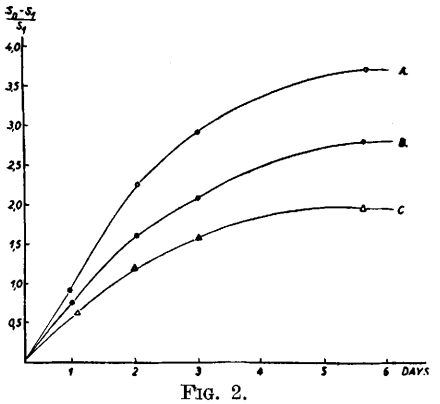

FIG. 2.

The growth of the methyl cholanthrene tumor of rats in Carrel culture flasks. Growth index: $\frac{S_n-S_1}{S_1}$ (S_n is the measured growth at different times, and S_1 is the growth of the first day).

A. Embryonic extract plus Ringer's sol.

B. Embryonic extract plus sodium heparinate (40 mg%).

C. Embryonic extract plus sodium salt of agar acid (40 mg%).

Before the fluid phase was added, the entire culture with the growth area was measured (S_1). The growth area of the cultures (S_n) were then determined every day. Data can be obtained from Fig. 1 and 2.

Fig. 1 illustrates the growth of those cultures which were treated with sodium heparinate (0.2 mg per flask). In the experiments, controls and experimental cultures are included. The fluid phase always contains the same concentration of growth promoting, water soluble, thermolabil substances of embryonic (12 days old chicken embryos) tissue-extract. To this fluid medium was added the test substances. The time is given logarithmically and indicates that the growth of the control cultures can be represented by a straight line from which the growth of the experimental cultures deviates markedly. When heparin is added once to the fluid phase an obvious suppression of the growth is observed; but this suppression is later compensated. This compensation shows itself by a marked increase in growth, in some cases even greater than that of the controls.

The heparin fluid phase in another series of cultures was changed twice. Under these conditions the growth was uniformly suppressed during the entire course of the experiment as shown by Fig. 1. Similar to the control cultures, the cultures repeatedly treated with heparin show a linear growth.

In an additional experiment, the growth of the cultures during continuous addition of sodium heparinate (40 mg % in every flask) and the sodium salt of agar acid was studied. Fig. 2 is a graphic representation of the relative growth of the cultures. In this case, the time scale is not logarithmical. It can be seen from the figure that the growth of the experimental cultures is considerably less than that of the controls. The most marked suppression of the growth was obtained when the agar salt was used, but the sodium heparinate also gives a similar suppression of growth. Using standard methods we have not been able to observe any difference in the morphology of the cells in the control and the cells in the experimental cultures.

In vivo. As our experiments have shown that the growth of tumor cells is suppressed by sodium heparinate and the sodium salt of agar acid, it seemed interesting to us to investigate whether the cellular growth could also be suppressed *in vivo*. Attempting to answer this question we have inoculated intraperi-

toneally suspensions of cells from a solid Ehrlich's carcinoma into 300 white male mice (20-25 g) in 3 experiments.

In the first experiment the animals were given sodium heparinate together with the tumor cells intraperitoneally. In another series the animals were treated intraperitoneally with sodium heparinate for a few days after the tumor had been inoculated. In both these experiments it was found that the experimental animals started to die a few days later than the control animals. However, this difference was not persistent and the mortality curves gradually coincide. In a third experiment we have given a more consistent treatment. One hundred fifty mice were divided into 3 groups of 50 each. All were inoculated with an equal amount of tumor cell suspension (1.5 million cells in 0.1 ml physiological saline). At the same time 50 animals were given 0.3 mg sodium heparinate and 50 animals 0.3 mg of the sodium salt of agar acid subcutaneously. The third group served as a control. The experimental animals were treated daily with the same quantity of sodium heparinate and sodium salt of agar acid. The results obtained are seen from Fig. 3. The mortality of each of the series is calculated. It can be seen from the figure that the lines which represent the mortality of the 3 groups run parallel, which suggests that the difference between the groups is constant during the time of the experiment. At the end of the experiment, 11 days after the inoculation, 82% of the control animals, 72% of the heparinate treated animals, and 66% of the animals treated with agar extract had died. The experiment, it seems to us, indicates that there is a real difference in mortality between the control and experimental animals. As the groups are relatively large this difference could hardly be due to mere chance but is by all probability due to the treatment.

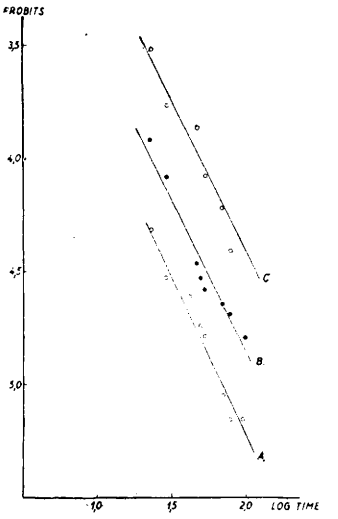

FIG. 3.

Dosage-mortality curve of ascites tumor of mice. Probits units are plotted against the logarithm of the time.

A. Control animals.

B. A total of 3 mg sodium heparinate injected subcutaneously during 9 days.

C. A total of 3 mg of the sodium salt of the agar acid injected subcutaneously during 9 days.

All the animals in the three groups have been given 1,5 million Ehrlich's carcinoma ascites cells intraperitoneally. The dosage-mortality curve has been calculated according to Bliss, C. L., *Quart. J. Pharmacol.*, 1938, **11**, 192.

Discussion. Our results seem to confirm completely the statement of Zakerzewski that heparin suppresses the growth of tumor tissues *in vitro*. In addition to that we have shown that the sodium salt of agar acid has the same effect. The question arises how the effect of these sulfomucopolysaccharides is brought about *in vitro*. From this point of view there are two possibilities: either the sulfomucopolysaccharides used have a neutralizing effect on the growth promoting substance present in the embryonic extract and the original tissue, or they act directly on the growing cell. Whether or not the sulfomucopolysaccharides in the latter case suppress mitosis and migration, we cannot decide. The effect seems to be specific as shown by Balazs.[8] He found that the heart tissue from chick embryos pulsates faster and longer in a substrate containing sodium heparinate than in the control cultures containing embryonic extract. Using a suitable concentration of sodium heparinate, the growth could be completely suppressed in the former case.

Concerning the experiments *in vivo* it seems clear to us that death was delayed by treatment with sodium heparinate and the sodium salt of agar acid. We have not been able to prevent the death of the animals; but one must consider the fact that we are dealing

with a form of tumor which under these experimental conditions in 12 to 15 days produces a 100% mortality in mice. How the treatment has influenced the tumor cells is, of course, hard to tell. Three possibilities seem acceptable:

1. The sulfomucopolysaccharides used have a neutralizing effect on the toxic products derived from the tumor cells.

2. The sulfomucopolysaccharides exercise a general resistance-increasing effect on the tissues of the organism.

3. The sulfomucopolysaccharides in question exercise a direct growth-suppressing effect on the tumor cells.

Naturally, we have not been able to decide how the substances in question act; we only wanted to suggest these possibilities. It is of interest to note that Cramer and Simpson[11] and Holmgren and Wohlfart[12] have shown that in methyl cholanthrene tumors in mice and rats, great masses of mast cells can appear. Cramer and Simpson point out that the mast cells in the skin increase in number before the tumors are developed, and that resistant mice show an ample number of mast cells in the skin. They further point out that the reaction of the mast cell is involved in a defensive process directed against the development of skin cancer. They also point out that this is of interest as the mast cells contain heparin (Holmgren and Wilander[13]), Jorpes, Holmgren, and Wilander.[14] It is also of interest that hyaluronic acid and other mucopolysaccharides are widely distributed in different tissues. Balazs and Holmgren[15] have shown that sulfomucopolysaccharides appear in injured tissue where they with all probability have a suppressing effect on the growth of the regenerating tissue.

Summary. Sulfomucopolysaccharides (sodium heparinate and the sodium salt of agar acid) suppress the growth of cells from methyl cholanthrene tumors in the rat *in vitro*. To ascertain whether the substances in question had a similar effect on growing tissues *in vivo* mice were inoculated intraperitoneally with the suspension of cells from Ehrlich's carcinoma, after which they were treated in various ways with sodium heparinate and with sodium salt of agar acid. It was found that the mortality of the control animals was greater than that of the treated animals. The results and various possible explanations are discussed.

[11] Cramer, W., and Simpson, W. L., *Cancer Res.*, 1944, **4**, 601.

[12] Holmgren, Hj., and Wohlfart, G., *Cancer Res.*, 1947, **7**, 686.

[13] Holmgren, Hj. and Wilander, O., *Ztschr. f. mikr. anat. Forsch.*, 1937, **42**, 242.

[14] Jorpes, E., Holmgren, Hj., and Wilander, O., *Ztschr. f. mikr. anat. Forsch.*, 1937, **42**, 279.

[15] Balazs, A., and Holmgren, Hj., *Cell. Res.*, 1949, in press.

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Detection of Mumps Virus in Mice Sacrificed at Different Periods of Time after Injection. (17357)

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During the past 2 years we have experimented with the cultivation of mumps virus in embryonated chicken eggs, and have prepared inactivated virus vaccine for laboratory use and trial in human subjects.[1]

In the course of a portion of this work, we have recently attempted to infect (looking for both apparent and inapparent infections) several of the smaller laboratory animals. To date, we have not been able to bring about any such infections or produce definite gross or microscopic lesions. We have used "raw" periembryonic egg fluid virus, and also such virus after concentration by the alcohol

[1] Muntz, H. M., Powell, H. M., and Culbertson, C. G., *J. Lab. and Clin. Med.*, 1949, **34**, 199.