embryos exposed to the transplacental action of OAAT may perhaps be the result of regeneration of the hepatic epithelium. Such phenomena are observed, for example, in liver cultures from adult animals (mice, rabbits): after preliminary administration of CCl_4 to the animals their liver survived much better in culture than did the liver of intact animals [1,2].

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EFFECT OF HEPARIN ON INCORPORATION OF THYMIDINE-^{3}H INTO A-1 CELLS IN CONTINUOUS CULTURE

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The effect of heparin on incorporation of thymidine-^{3}H into A-1 cells in continuous culture was studied. Incubation of the cells in medium No. 199 containing heparin (1-200 units/ml) did not lead to any decrease in the percentage of labeled nuclei. The duration of the G_2-and S-periods likewise was unchanged compared with the control. However, the intensity of incorporation of thymidine-^{3}H (mean number of grains above one labeled nucleus) was significantly reduced. The results indicate that heparin affects the permeability of the plasmalemma but has no effect on DNA synthesis. The antimitotic action of heparin is probably connected with its effect on the cell surface.

KEY WORDS: heparin; autoradiography; cell cultures.

The writers previously [4] described quantitative and qualitative changes in the mitotic regime of monolayer continuous cultures of A-1 cells under the influence of heparin. Heparin reduced mitotic activity and caused a phase shift such that the relative proportion of telophases among the dividing cells rose considerably. High doses of heparin (500 units/ml) blocked the transition from telophase to the subsequent interphase and many of the cells blocked in telophase underwent pycnosis and died without completing cytotomy.

To study the extent to which the changes observed in mitosis are linked with a disturbance of DNA synthesis in cells in the S period, an autoradiographic study (using thymidine-^{3}H) was made of continuous cultures of A-1 cells incubated in the presence of heparin.

EXPERIMENTAL METHOD

A suspension of a continuous culture of A-1 cells ($1 \cdot 10^5$ cells in 1 ml medium No. 199 with 10% bovine

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TABLE 1. Intensity of Incorporation of Thymidine-^{3}H into Nuclei of A-1 Cells of Continuous Culture Incubated with Heparin (nuclei of young daughter cells labeled after telophase not included in the count), M±m

Heparin concentration in medium, units/ml	Mean number of grains over nucleus. Time of reincubation after rinsing out label, h				
	1	2	12	18	24
0					
Control	44,0±1,4	86,4±1,4	96,0±1,2	>100	50,7±1,3
1	24,0±0,8	15,6±0,2	18,1±0,2	35,4±1,7	25,7±1,3
P	<0,001	<0,001	<0,001	<0,001	<0,001
10	32,0±1,8	18,1±0,3	23,4±0,2	18,8±0,3	9,4±0,14
P	<0,001	<0,001	<0,001	<0,001	<0,001
100	37,0±1,9	13,1±1,0	12,6±0,2	9,6±1,1	13,3±0,3
P	<0,001	<0,001	<0,001	<0,001	<0,001
200	25,0±1,1	17,0±1,0	9,0±0,3	14,8±0,7	20,5±1,2
P	<0,001	<0,001	<0,001	<0,001	<0,001

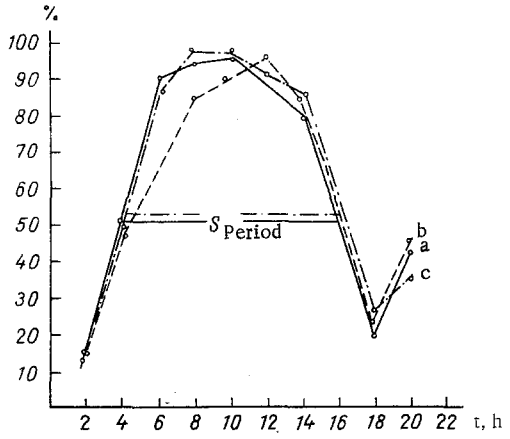

Fig. 1. Dynamics of labeled mitoses of A-1 culture: a) control culture; b) culture incubated with 10 units/ml heparin; c) with 100 units/ml heparin.

serum) was poured into penicillin flasks with coverslips and incubated at 37°C. After 48 h, when a continuous monolayer had formed, the growth medium was replaced by the same medium containing heparin (Polfa; 1-200 units/ml) or without heparin (control). The cultures were then incubated in the presence of heparin for 24 h, then transferred for 15 min into medium with thymidine-^{3}H (1 μCi/ml, specific activity 12.2 Ci/mmole), washed in medium without the labeled precursor, and then transferred into medium containing heparin in the same concentrations as before, incubated again at 37°C, and fixed in 96° ethanol after intervals of 1 and 2 h and every 2 h thereafter until 24 h. The fixed preparations were coated with R nuclear emulsion and exposed for 5 days. The percentage of labeled interphase nuclei was dtermined (in 1000 cells) in autoradiographs developed and stained with Carazzi's hematoxylin. The number of grains of silver per nucleus was counted in 50 labeled cells. The rate at which the cells of the control and haparin-treated cultures passed through the mitotic cycle was determined from the change in the percentage of labeled mitoses after incubation with thymidine-^{3}H [3].

EXPERIMENTAL RESULTS

At all times of the investigation no statistically significant decrease or increase in the fraction of labeled nuclei was found in cultures incubated with heparin (variation from 47.8 to 60.0%).

The mean number of grains of silver per labeled nucleus was then determined in the experimental and control cultures. The results of counting showed that when small concentrations of heparin were used (1 and 10 units/ml) there was a statistically significant decrease in this index at all times of the experiment (Table 1). No strict correlation was found between the heparin concentration in the medium and the decrease in the in-

tensity of label per nucleus.

The dynamics of labeled mitoses in the control and heparin-treated cultures was similar (Fig. 1). The mean duration of the $G_2 + \frac{1}{2}M$ period in both cases was about 4 h and of the S-period about 12 h. The number of grains of silver above the metaphase chromosomes of cells of the experimental cultures was only one-third to one-fourth of the number above the chromosomes of cells of the control cultures.

There is ample evidence in the literature of the animitotic action of heparin. It is manifested on different objects: monolayer cell cultures, transplantable tumors, cleaving oocytes [2,5,7,8,9,10,11]. The view is held [9] that heparin and other polyanions can perform the role of unique tissue regulators of mitosis through their action on membranes. The results presented above confirm the validity of this hypothesis. The fact that the percentage of nuclei labeled with thymidine-^{3}H in the control A-1 cultures and in cultures incubated in the presence of heparin was the same is evidence against any direct effect of heparin on DNA synthesis in the cells. This conclusion is also supported by the absence of change in the rate at which cells treated with heparin pass through the G_2 and S periods. Meanwhile the highly significant decrease in the number of grains of silver above labeled interphase nuclei of cells incubated in the presence of heparin suggests that heparin evidently affects the permeability of the plasma membrane, as a result of which the quantity of thymidine-^{3}H which penetrates into the cell during pulse labeling is much smaller than in the control. To obtain this blocking effect, only small concentrations of heparin are sufficient (1-10 units/ml). Higher doses of heparin do not give any additional effect, as is shown by the absence of strict correlation between the decrease in the number of grains over the nuclei and the heparin concentration in the medium. The reason for this phenomenon may be that the block cell receptors only small concentrations of heparin in the medium are required, and a further increase in the dose of heparin thus has no additional effect on the permeability of the plasma membrane.

These results can be compared with data for the effect of cytochalasin B on the cell surface [6]. Under the influence of this substance the transport of thymidine-^{3}H into the cell also is disturbed, so that the intensity of label above the nuclei is reduced. Meanwhile cytochalasin B does not change the rate of DNA synthesis.

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