RELATIONSHIP BETWEEN MITOTIC ACTIVITY IN THE CELLS OF RAT ASCITIC HEPATOMA AND SIZE OF THE CELL COMPLEX

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One of the most interesting problems of hitophysiology is that of the regulation of mitotic activity in simple multicellular systems. Certain ascitic hepatomas can serve as very suitable material for the study of this particular problem, as the growth of such tumors give rise to cellular complexes—"islets"—consisting of varying numbers of cells. The distribution of cancer cells among the complexes, i.e., the percentage of such cells in complexes of various sizes, is characterized by its known constancy for each strain of ascitic hepatoma [3, 5]. It is possible to suppose that one of the factors determining this constancy is the particular degree of mitotic activity among cells associated with complexes of various sizes. The aim of this particular research was to compare the mitotic rates in isolated tumor cells and in cells contained within complexes of various sizes.

EXPERIMENTAL

The object of our investigation was rat ascitic hepatoma (Zaidel strain, C variety). Such hepatomas exist in the form of complexes of various sizes, consisting of 2-100 cells or more. In the ascitic fluid of the hepatoma are a certain number of isolated cancer cells. This type of tumor does not form macroscopically identifiable solid nodules. The average survival time of animals after receiving grafts of Zaidel's ascitic hepatoma in doses of $5 \cdot 10^7$ $7 \cdot 10^7$ (as may be attained by the injection of 0.5 ml of undiluted ascitic fluid) is from 7-10 days [4].

The hepatoma was established in impure-strain white male rats weighing 150-170 g. Two series of experiments were set up involving 10 consecutive generations of hepatoma. In the first series of experiments, 0.3 ml of 0.01% colchicine solution was injected into the animals (2 injections with a half hour interval between them) 5-6 days after implantation of the cancer cells. In the second series of experiments no colchicine was given. Smears were prepared from freshly obtained ascitic fluid and these were stained using the May—Grunwald—Giemsa technique of Pappenheim. The smears were examined under an MBI-3 miscroscope with binocular attachment (objective 40 ×, ocular 10 ×), tube magnification 1.5 ×), by moving the preparation 1 division of the verier scale at each examination. In doubtful cases a 90 × immersion objective was used. The number of mitoses in the isolated hepatoma cells of the ascitic fluid and in the cells of the "islet" complexes were counted. Only those complexes consisting of from 2 to 20 cells had their mitotic activity recorded. Complexes consisting of more than 20 cells were left out because it proved difficult to determine the precise number of cells contained in them. On the average from 3000 to 5000 cells were counted in each smear.

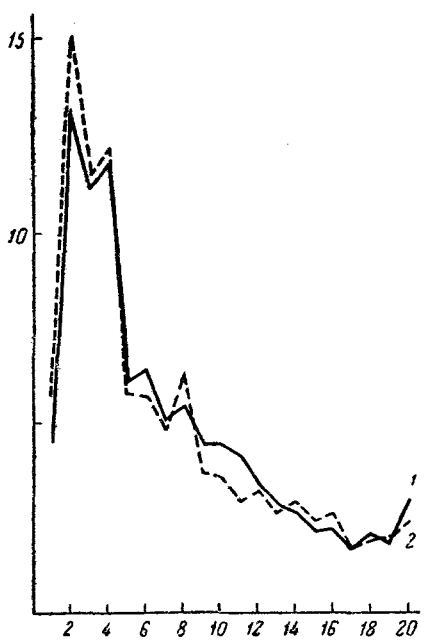
RESULTS

From counts of mitoses in smear preparations it was possible to calculate values for the following mitotic coefficients: the relationship between number of mitoses and the total number of cells counted (as a %); and mitotic coefficients for complexes of different sizes, i.e., consisting of various numbers of cells.

TABLE 1. Distribution of Mitoses in Zaidel Ascitic Hepatoma Cells and the Relationship of These Values to the Size of the Cell Complex

No. of cells in complex	No. of cells in complexes of that particular type	No. of cells undergoing mitotic division in complexes of that particular type	Mitotic coefficient (as %) for cells belonging to a complex of that particular type
First series of experiments			
1	3 176	384	12.09
2	9 406	411	4.37
3	8 007	342	4.27
4	8 380	267	3.18
5	4 395	210	4.77
6	4 488	167	3.72
7	3 626	128	3.53
8	3 856	129	3.37
9	3 186	115	3.61
10	3 180	95	2.98
11	2 981	100	3.35
12	2 424	76	3.13
13	2 067	68	3.29
14	1 890	60	3.17
15	1 530	50	3.27
16	1 632	51	3.12
17	1 275	30	2.35
18	1 530	42	2.74
19	1 444	33	2.28
20	2 100	40	1.90
Second series of experiments			
1	1 446	79	5.46
2	3 748	89	2.37
3	2 901	75	2.58
4	3 052	61	1.99
5	1 460	34	2.33
6	1 416	21	1.48
7	1 197	23	1.92
8	1 560	20	1.28
9	918	12	1.31
10	900	15	1.67
11	759	8	1.05
12	828	8	0.96
13	689	6	0.87
14	756	11	1.45
15	615	9	1.46
16	672	10	1.49
17	442	7	1.58
18	506	4	0.79
19	532	5	0.94
20	640	4	0.63

Fig. 1



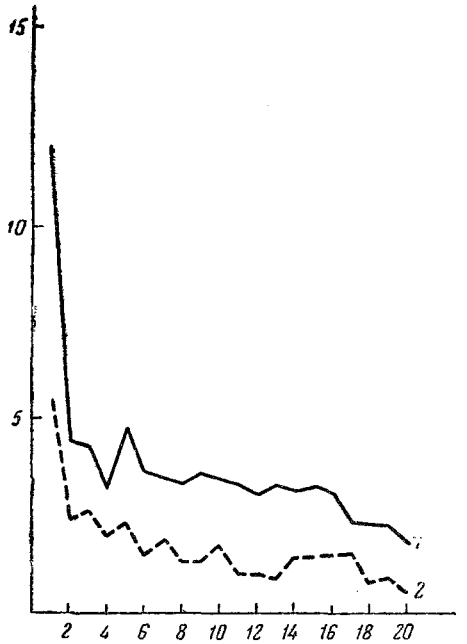


Fig. 2

Fig. 1. Distribution of Zaidel ascitic heptoma cells according to the size of the complex 1) Experiments with colchicine; 2) without colchicine. Abscissa) number of cells in complex; ordinate number of cells in complexes of that particular type (% of total number of cells).

Fig. 2. Mitotic coefficients for complexes consisting of different numbers of cells. 1) Experiments with colchicine; 2) without colchicine. Abscissa) number of cells in complex; ordinate) mitotic coefficient (as %).

In the experimental series involving treatment with colchicine 70,537 cells from 7 rats were counted and the mitotic coefficient was to be 3.96. In the second series (without colchicine treatment) we counted 25,037 cells from 3 rats; the mitotic coefficient was 2.0. Thus, in the first series the mitotic coefficient was found to fall within the limits of 2.08-5.61% and in the second series 1.65-2.69%. The values for the mitotic coefficient in smears derived from the same animal were found to lie near together.

Table 1 and Figs. 1 and 2 show the distribution of cells according to complex and size, and the distribution of mitoses in relation to number of cells in the complex. As is evident from Table 1, the mitotic activity in complexes of different sizes is itself different: high activity is associated with single and isolated hepatoma cells (in the first series single cells produce 13.7% of the total mitoses). The mitotic coefficient of single cells, as determined from smears in the first experimental series, varies within rather broad limits (from 5.64 to 24.8%); nevertheless, it greatly exceeds that of any other of the complexes in this series and is from 2-4 times greater than that for 2-cell complexes (on average 2.74 times). The mitotic coefficient of 2-cell complexes varies from 2.15 to 7.52%, with a mean of 4.37%. It is found that as the number of cells in the complex increases, that mitotic coefficient falls (from 4.27 for 3-cell complexes to 1.9% for 20-cell complexes). This tendency is exhibited, with some degree of variation, by all the smears in the first series of experiments. However, no very clear difference is observable between adjacent classes of complexes (for example, the mean mitotic coefficient for 8-cell complexes is 3.37% and that for 15-cell complexes 3.27% i.e., they are almost equal). In addition, statistical analysis shows that the differences in size of the mitotic coefficients between complexes of from 10-20 cells is not at all significant ($P<0.01$).

The same regularity of change in the mitotic coefficient with change in the complex size was found in the second series of experiments where colchicine treatment was not given. Although the second series of experiments involved considerably less material and the mitotic coefficients for particular groups of smears were several times less than in the first series, it is still quite apparent that the number of mitoses among single cells are on the average 2.3 times the number of mitoses among 2-cell complexes. As the number of cells in the complexes increases beyond 2, there is a further reduction in the mitotic coefficient (for cells of 20-cell complexes it is only 0.63%). However, this reduction in the case of complexes containing more than 8 cells is not expressed at all clearly (cf. Fig. 2). On the whole, it should be said that the curve for mitotic activity among cells belonging to complexes of different sizes,

as obtained from the second series of experiments, is very near to the curve embodying the results from the first series; it is also like (indeed almost identical with) the curve showing the distribution of hepatoma cells according to complex size in both the first and second of experiments (cf. Fig. 1). It is interesting to observe that the difference between the heights of the curves for colchicine treated and untreated material is most clearly expressed in the case of single cells (cf. Fig. 2.).

The observed differences in the mitotic rate of isolated cells in rat ascitic hepatomas as compared with that of cells forming complexes ("islets") may be explained in two ways. On the one hand, it is known that in cells undergoing mitosis the links with neighboring cells and (in tissue culture) with the substrate are weakened. Certain procedures used to synchronize cell-division in vitro are, in part, based on this phenomenon [2]. Moreover, if we accept the possibility of this weakening of the connection between dividing cell and other adjacent cells it follows that the former is adapted to escape from the complex either by active movement or passively (by the "washing out" of the cell from the complex by currents in the ascitic fluid). From a study of the distribution of different phases of mitosis in the cell complexes of ascitic hepatomas in mice [5] it has been established that there is a considerable predominance of single cells in metaphase as compared with other phases of mitosis; this leads the author to suppose that "escape" of cells from the complex must take place at the prophase stage of division.

On the other hand, it is possible to suggest that cell division in the "islet" depends to a large extent on that amount of cell surface which is not in contact with other cells of the complex but is directed towards the environmental medium, i.e., in this case, the ascitic fluid with its dissolved nutrient material [1]. In this case also, the isolated cells with a maximum surface area in contact with the ascitic fluid must undergo the greatest amount of mitotic activity. As the number of cells in the "islet" increases from 2-6 their mitotic activity will decrease substantially and approach very near to that of the large "islets".

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