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Transmission of Mouse Sarcoma with Small Numbers of Counted Cells.*†

MORTON C. KAHN AND JACOB FURTH.

From the Departments of Public Health and Preventive Medicine and Pathology, Cornell University Medical College, New York.

Several of the current theories of the etiology of cancer[1] postulate the presence of malignant cells in apparently normal individuals. It is assumed that such isolated cells are incapable of producing a neoplasm unless they attain, in a small region, a large enough number to overcome resistance. This opinion is based in part on transmission experiments with carcinoma and sarcoma of laboratory animals. The smallest number of cells required for the transplantation of these neoplasms is estimated by different workers as between 12,500 and 1,000,000.[2]

Successful inoculations with tissue cultures containing a small, though uncounted, number of malignant cells led Dr. Warren H. Lewis[3] to believe that a few malignant cells might under favorable conditions suffice for the transmission of sarcoma. Our quantitative studies with transmissible leukemia indicate that a single malignant cell isolated in a microdroplet with the micropipette, prior to animal inoculation, may transmit the disease.[4] We have found it possible to use essentially the same technic in transmitting mouse sarcoma with counted numbers of cells. The tumor is derived from a sarcoma produced by subcutaneous injections of 1-2 benzpyrene. The following experiments indicate that this strain of sarcoma can be readily transmitted with from 50 to 100 cells.

Technic. The suspension of tumor cells was prepared in a manner similar to that of malignant lymphoid cells with the difference that the tumor fragments were washed with Tyrode solution to remove most of the blood that they contained. The washed particles were then cut up in a 10% solution of normal mouse serum in Tyrode solution. The tumor particles were allowed to settle out

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1 *Cf.* Fischer, A., *Am. J. Cancer*, 1937, **31**, 1.

2 De Gaëtani, G. F., and Blottner, E., *Z. f. Krebsf.*, 1936, **44**, 108.

3 Lewis, Warren H., personal communication to J. Furth.

4 Furth, J., and Kahn, M. C., *Am. J. Cancer*, 1937, **31**, 276.

and the supernatant fluid containing free cells and small clumps of cells was pipetted off. These were subsequently isolated in microdroplets and counted prior to injection. Of the 12 successfully inoculated mice, 7 received a counted number of cells in each site injected; but in each of 2 mice the suspension containing a counted number of cells was divided equally and injected into 2 sites; and in each of 3 mice the suspension was divided equally and injected into 4 sites. The cell suspension used for inoculation contained mostly single cells and occasional small clumps of cells. Clumps of cells were injected only when the number of cells in them could be determined. The number of cells in an injected clump never exceeded 12. All cells that might possibly have been tumor cells were counted. Neutral red and trypan blue preparations indicated that most cells were alive.

The results of inoculation are shown in Table I.

TABLE I.
Number of Mice Successfully Injected with Counted Sarcoma Cells.*

Lot No.	No. of cells injected	No. of mice injected	No. of sites injected	No. of successful injections	No. of mice successfully injected
1	50 to 100	7	28	5	4
2	60 to 290	6	24	0	0
3	50 to 100	14	26	4	4
4	50 to 100	7	28	9	4
5	39 to 62	7	28	1	1
6	10 to 43	6	24	0	0
	Total	47	158	19	13

*All mice were injected with an exact number of cells. For instance, mouse No. 1 received 64 cells; mouse 2, 73 cells, etc.

Most of the unsuccessfully injected mice are still alive and it is possible that some of them will develop sarcoma at the site of injection, but these retarded tumors would in no way alter our conclusions. In one experiment 4 mice that failed to develop sarcoma received 450 r of X-rays, both groins where the inoculations had been made 3 months previously being shielded, but the mice remained free from growths. In another experiment, 3 of the 6 mice injected received 400 r of X-rays before inoculation, but none of these mice developed tumors. These experiments were undertaken to promote the growth of malignant cells should they remain dormant as suggested by Fischer,[1] but there is no evidence in our experiments in favor of this possibility.

Summary. Sarcoma of mice can be transmitted with approximately 50 cells.