Growth Characteristics of Free Tumor Cells Transferred Serially in the Peritoneal Fluid of the Mouse*

HORACE GOLDIE AND MARIE DINGMAN FELIX

(Cancer Research Laboratories, Meharry Medical College, Nashville, Tenn., and National Cancer Institute, National Institutes of Health, Bethesda, Md.)

It is common knowledge that the presence of abdominal tumors in cancer patients and the growth of peritoneal implants in laboratory animals may be associated with the appearance of free tumor cells in the peritoneal fluid (2-30). However, the specific biological characteristics of these free cells—their growth cycle, growth potential, and relationship to peritoneal implants in the same animal—have not been experimentally investigated. In the present paper, the number of cells and their growth rate in the peritoneal fluid, as well as their regressive changes, were estimated repeatedly in the same mouse, which received intraperitoneal inoculation of a predetermined number of tumor cells. By carrying out serial intraperitoneal transfers of Sarcoma 37 cells and of malignant lymphoma cells from mouse to mouse, at intervals of 3-7 days, it was possible to maintain continuous multiplication of these cells in the peritoneal fluid for more than 10 months. Our data indicate the possibility of using mouse tumor cells in vivo culture for the quantitative study of various problems in the field of research.

MATERIAL AND METHODS

Two tumors were chosen for this study: (a) S-37; this tumor is not strain specific; it grows readily in different strains of mice, with varying percentages of regression, and elicits a marked leukocytic reaction in the peritoneal fluid; (b) a malignant lymphoma arising spontaneously in the thymus of a strain dba (212) mouse. The tumor was found to be practically strain specific, growing poorly if at all in strains other than dba (212). In strain dba (212) it grows steadily as a localized tumor with almost no regression. It was found in preliminary experiments to elicit a relatively mild leukocytic reaction. The mice used in quantitative experiments with S-37 were all males of the Carworth Farms W Strain (CFW), but dba, Swiss, and C3H mice also were found convenient in preliminary studies. Male dba (212) mice were used in the work with malignant lymphoma.

Subcutaneous implants 6 or 7 days old were ground in a tissue press and suspended in a convenient volume of 0.85 per cent NaCl solution. Before inoculation of the requisite number of tumor mash cells, the number of viable cells in 0.1 cc. of the mash suspension was determined. The suspension was separated from tissue fragments and cell clumps by sedimentation and decantation. A sample of suspension was placed in a hemocytometer previously coated with a 0.01 per cent alcoholic solution of neutral red. Five minutes later a count was made to compute the number of viable tumor cells per cubic centimeter. Tumor cell counts in the peritoneal exudate were made in the same way, except that the total number of viable tumor cells per 0.1 cubic centimeter was determined by combined methods of total cell counts in the hemocytometer and differential tumor cell counts in stained smears. One smear was stained with Wright's stain and the other by the aceto-orcein method. The results of counts in both
smears usually agreed within 5 per cent. Information from these counts was used to determine the number of viable tumor cells, the percentage of mitoses, and the percentage of necrotic tumor cells per 0.1 cubic centimeter of the original exudate. Peritoneal exudate was removed by exploratory puncture with glass capillary pipettes. Preliminary experiments have demonstrated that the cellular composition of the peritoneal fluid does not vary significantly in samples withdrawn from different pockets of the peritoneal cavity in the same mouse.

RESULTS

TUMOR CELLS IN PERITONEAL FLUID

Counts on specimens of the peritoneal fluid obtained from twelve normal CFW mice indicated a wide range in the number of cells—from 63,000 to 160,000 per cubic millimeter. In the majority the lymphoid cells were most numerous. In differential counts the average percentages found were as follows: lymphocytes, 60.4—80.5 per cent; polymorphonuclears, 0—0.7 per cent; large monocytes, 7.5—29.2 per cent; macrophages, 4.0—23 per cent; and mast cells, 1.0—4.6 per cent (Fig. 1). Similar counts were found in the peritoneal fluid of fifteen dba mice. The fate of normal mouse tissue cells in the peritoneal fluid was investigated in four groups each of fifteen mice of various strains (5 C3H, 5 dba, and 5 CFW) that received intraperitoneal injections of various doses (1 cc. of 1:10 to 1:500 dilution of tissue mash) of mashed liver, lung, spleen, and brain from normal mice of the same strain. Only detritus of these cells was found in all specimens of peritoneal fluid withdrawn 4 or 5 days after injection. No mitotic figures were found at any time in the injected cells withdrawn with the peritoneal fluid. Thus, the cells of the normal tissues tested failed to multiply or even to survive in the peritoneal fluid. These preliminary experiments served as control experiments for the following studies on tumor cells.

A suspension of mashed S-37 tumor cells (Fig. 2) obtained from a 6-day-old subcutaneous transplant was prepared and diluted to the concentration of 10,000 cells per 0.1 cubic centimeter. Doses of 10,000 cells were injected intraperitoneally into each of 27 mice, and samples of the peritoneal fluid were taken at various intervals for total and differential cell counts. In fourteen mice the exploratory punctures of the abdomen were repeated at short intervals (after 6, 24, 48 hours, etc.) over a period of 7 days, and in thirteen mice at longer intervals (every fourth or fifth day) over a period of 12 days. The results are presented graphically in Chart 1. These results indicated that the number of sarcoma cells and the percentage of mitotic figures decreased during the first 24 hours after inoculation. This decrease (lag period) was followed by a slow increase during the next 24 hours (24—48 hours after inoculation), and in the 48—72-hour period there was a sharp increase both in the number of cells and in the percentage of their mitotic figures for a period of 4—5 days. Finally, a sharp rise in the relative number of necrotic tumor cells was noted and at the same time a steady decrease in the total number of sarcoma cells per 0.1 cubic centimeter. Chart 1 also shows that there is an early rise in the curve for the number of polymorphonuclear leukocytes in the peritoneal fluid which reaches a peak on the third day after inoculation. It is of interest that on the tenth day after inoculation, when the percentage of necrotic cells has increased significantly, the curve for polymorphonuclear leukocytes continues to fall. For the first 24 hours after inoculation, when there was an apparent decrease in the number both of tumor cells and of mitotic figures, the majority of the cells appeared shrunken to the size of a small lymphocyte (Fig. 3). Between 24 and 48 hours after inoculation, the tumor cells somewhat increased in size (Fig. 4). By 48—72 hours, at the beginning of initial rapid multiplication, the average size returned to that characteristic of healthy S-37 cells (Figs. 5 and 6). Figures 7 and 8 illustrate the appearance of specimens taken for differential counts at the time of maximum cell growth and at the time of terminal necrosis and death of tumor cells, respectively, for the series inoculated with 10,000 cells.

Similar studies were made using smaller numbers of S-37 cells in the original inoculum. With 1,000 cells and, more obviously, with 100 cells inoculated, the peak of the curves for the number
of tumor cells and for the relative number of mitoses occurred later and did not reach as high levels as when 10,000 cells were inoculated. The curves for necrotic cells, on the other hand, rose earlier and reached a higher peak.

The curves of growth of similar numbers of malignant lymphoma cells (Charts 2 and 3) contrast strikingly with the curves of S-37; they show continuous increase in the number of tumor cells illustrated by stained smears showing the cellular composition of inoculated material (mashed subcutaneous implant) and of the peritoneal fluid in inoculated animals before inoculation (in a normal dba [212] mouse) and at consecutive growth stages after inoculation (Figs. 9–14).

MULTIPLICATION OF Tumor CELLS
in the PERITONEAL FLUID

Abdominal massage of mice bearing peritoneal implants of Sarcoma 37.—The possibility existed that the increase in the number of free sarcoma cells in the peritoneal fluid might result from the disintegration or sloughing of peritoneal implants induced by injection of tumor mash cells. To test this possibility, ten mice received intraperitoneal inoculations, each with approximately 1,000 sarcoma cells from the peritoneal fluid. Nine to 12 days after inoculation, a small amount of peritoneal fluid was withdrawn from each mouse. The number of sarcoma cells and of leukocytes in each specimen was counted. Immediately afterward, the abdomen of each animal was energetically massaged for 3 minutes. A small amount of fluid was again withdrawn and examined as before. At the autopsy performed on the sixteenth day after inoculation, peritoneal tumors were found in the abdominal peritoneum of nine out of ten animals (one without tumor). It appeared that in two out of ten mice the percentage of sarcoma cells was increased after the massage by 7 per cent and in only one mouse by 13 per cent. In other mice it was approximately unchanged or even decreased. In some instances, there was an increase in number of lymphocytes in the peritoneal fluid after the massage. Thus, even a severe mechanical disturbance did not induce any consistent disruption or sloughing of small peritoneal tumors.

Multiplication during rapid serial transfers.—Since Algire and Chalkley (1) have shown that inoculated tumor tissue elicited signs of tumor vascularization (capillary growth) as early as the fourth day after inoculation, we have carried out successive transfers of peritoneal fluid containing S-37 cells at intervals of 2 and 3 days. Mice received intraperitoneal inoculations of known numbers of sarcoma cells (10,000 in the first series and 1,000 in the second series). The peritoneal fluid of inoculated mice was transferred, after 3 days, into new mice; from these mice, after 2 or 3 days, into new mice, etc.

Altogether, seven successive transfers were carried out in seven groups, each of five mice, at the same intervals. Three days after the last transfer a small amount of peritoneal fluid was withdrawn from each ultimate recipient in each series, and
the number of sarcoma cells in each specimen was counted and computed per 0.1 cubic centimeter. All animals examined contained more than 0.1 cubic centimeter of fluid, but even in 0.1 cubic centimeter the number of sarcoma cells was higher than their number in the inoculum received by the first group of mice in the series. These results indicate that tumor cells increase in number in the peritoneal cavity during serial transfers of the peritoneal fluid from mouse to mouse in the absence of vascularized tumor growth.

For larger series, transfers from mouse to mouse were made alternately at 3- and 4-day intervals. Each series was initiated by intraperitoneal injection of 50,000 to 250,000 sarcoma cells, from mashed subcutaneous S-37 implants, into eight mice. Their peritoneal fluid was withdrawn after 4 days and examined. The specimen showing the highest number of sarcoma cells and of their mitoses was used for the transfer of peritoneal fluid into eight new mice, in doses of 0.1–0.25 cubic centimeter. After an interval of 3 days the peritoneal fluid of these mice was examined, and a suitable specimen was selected for use in the second transfer into a new group of six mice. This specimen contained, in 0.1 cubic centimeter, 20,000–50,000 sarcoma cells, 15 or 20 per cent of which showed mitotic figures. The "recipients" of the second transfer were used as "donors" of the fluid for the third transfer, after 4 days. This procedure was repeated regularly for 10 months. The descendants of cells issued from two different subcutaneous implants were propagated for 64 transfers as three strains of free sarcoma cells (D, F, and G) in three separate series of mice. After the first 15–20 transfers of each strain, the peritoneal fluid of most animals contained at each transfer a large number of sarcoma cells (50,000–70,000 per 0.1 cubic centimeter) with a high percentage of mitotic figures (25 per cent or more), and the selection of the "best" donor was no longer necessary. The fluid was characterized by its uniform grayish opacity, the absence of macroscopic cell clumps and blood, and by its albuminoid consistency. After each transfer intraperitoneal inoculation of the sarcoma cells provoked a rapid accumulation of an effusion in the peritoneal cavity, and the cells continued to multiply in this medium.

Similar transfers of peritoneal fluid containing malignant lymphoma cells were carried out serially from mouse to mouse at weekly intervals, until it was noticed that, after the sixteenth to nineteenth transfer, three or four out of ten mice at each transfer died within a few days. Therefore, at the twentieth transfer, the withdrawn peritoneal fluid was diluted 1:5 and transferred in doses of 0.5 cubic centimeter of this dilution. All inoculated mice survived for at least a week. After the 24th transfer the dose of transferred fluid was cut again, for the same reason, to 0.5 cubic centimeter of a 1:26 dilution of the peritoneal fluid; after the 26th transfer, to 0.5 cubic centimeter of a 1:52 dilution; at the 33rd transfer, to 0.25 cubic centimeter of a 1:52 dilution; and on the 40th transfer, to 0.1 cubic centimeter of the same dilution.

**Subcutaneous "Auto-inoculation"**

In nearly all mice that received intraperitoneal inoculations of 10,000 to 1,000 sarcoma cells, and in some mice inoculated with 100 cells, a nodule, sometimes growing into a tumor, sometimes regressing, developed subcutaneously at the site of intraperitoneal injection. This phenomenon of subcutaneous auto-inoculation of the mouse by tumor cells from the peritoneal cavity seems to be related to the initial rate of multiplication of sarcoma cells inoculated into the peritoneal fluid (Table 1). There was some question as to the origin of the tumor cells which gave rise to the subcutaneous nodules following intraperitoneal inoculation—whether they were introduced originally in a subcutaneous position as a result of leakage from the syringe on injection or whether they migrated into the wound after a period of growth in the peritoneal cavity. In an attempt to settle this question, twenty animals each received three widely spaced abdominal punctures with a sterile injection needle, followed by a single intraperitoneal injection of S-37 cells at still another site. Nodules later developed at the site of all four punctures in some cases, and in others nodules developed at several sites of puncture but not at the site of tumor cell inoculation, indicating that S-37 cells migrated into the punctured areas from the peritoneal fluid.

**Growth Cycle in Serial Intraperitoneal Transfers of Tumors**

Continuity of multiplication of injected cells.—Doses of 10,000 sarcoma cells from the peritoneal fluid were inoculated intraperitoneally into 81 mice. In 14 mice the exploratory punctures of the abdomen were repeated at short intervals (after 6, 24, 48 hours, etc.) during 7 days and in 17 mice at longer intervals (every fourth or fifth day) during 11 days. The withdrawn specimens of fluid were examined for total and differential counts of sarcoma cells and leukocytes. Since the results in all groups showed complete agreement, they were pooled and plotted as a chart (Chart 4). A similar series of changes in the number, activity, and viability of S-37 cells from the same source was studied by repeated cell counts when smaller numbers of these
cells were transferred intraperitoneally (Chart 5).

These data show that the inoculation was followed by a short lag period of approximately 24 hours, during which time the number of sarcoma cells remained on about the same level (or even decreased in a few mice), while the percentage of mitotic figures sharply decreased. The curves of sarcoma cells and of their mitoses rose sharply between the 24th and 48th hours, indicating that the viability of inoculated cells was not impaired by transfers. There was no shrinkage of transferred cells. On the contrary, their size was often markedly increased during the first 24 hours (Figs. 15–17). The maximum percentage of mitotic figures was reached on the fourth day, while the increase in the total number of cells continued until the sixth day, when it began to decrease. The curve of necrotic cells looked like an inverted curve of cell multiplication, thus illustrating the parallelism between the loss of activity and the loss of viability in proliferated cells. Figures 18–20 illustrate the appearance of specimens of these cells taken on the third, the sixth, and the tenth days after the transfer.

Similar experiments with malignant lymphoma cells from serial intraperitoneal transfers are illustrated by Figures 21 and 22 and by Charts 6 and 7.

The growth characteristics of mash cells and of

<table>
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<th>TABLE 1</th>
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<tr>
<td><strong>GROWTH OF SUBCUTANEOUS TUMORS IN THE ABDOMINAL WALL AT THE SITE OF INTRAPERITONEAL INOCULATION OF S-37 CELLS</strong></td>
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<tr>
<td><strong>No. of cells inoculated</strong></td>
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<tr>
<td>10,000</td>
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<td>1,000</td>
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<td>100</td>
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**Chart 4.**—Growth cycle in the peritoneal fluid of 10,000 S-37 cells from intraperitoneal serial transfers.

**Chart 5.**—Growth cycle in the peritoneal fluid of 100 S-37 cells from intraperitoneal serial transfers (see legend of Chart 4).

**Chart 6.**—Changes in the cellular composition of the peritoneal fluid following intraperitoneal inoculation of 10,000 lymphoma cells from the serial intraperitoneal transfers.

**Chart 7.**—Growth cycle in the peritoneal fluid of 100 malignant lymphoma cells from the serial intraperitoneal transfers (see legend of Chart 6).
cells from peritoneal fluid were compared as to the levels of growth and of necrosis at certain intervals after intraperitoneal inoculation of various doses (10,000, 1,000, and 100) of cells. This comparison suggested that for each group of mice inoculated with the same number of tumor cells, the level of cell multiplication was higher and the rate of cell death lower for descendants of cells from peritoneal fluid than for descendants of mash cells (Tables 2 and 3).

Growth potential of tumor cells cultured in the peritoneal fluid.—In order to investigate the significance of this observation more completely, a series of experiments with S-37 in CFW mice and with lymphoma in dba mice was carried out in which different numbers of cells were inoculated (10,000, 5,000, 1,000, 100, and 10). They were obtained either from mash tumor or from peritoneal fluid and were injected either subcutaneously or intraperitoneally. The number of mice in each group varied from 8 to 25. After the inoculations were made, the mice were examined at intervals over a period of 18 days for the appearance of tumor nodules. Notes were taken as to the time of appearance of nodules and of their changes in size. The results were clear cut and demonstrated that, when injected subcutaneously, tumor cells from the peritoneal fluid produced larger tumor nodules more quickly than were produced by the same number of cells from mashed subcutaneous

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<th>TABLE 2</th>
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<td>COMPARISON OF THE GROWTH CHARACTERISTICS OF SARCOMA 37 CELLS FROM MASHED SUBCUTANEOUS IMPLANTS AND FROM PERITONEAL FLUID*</td>
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<tr>
<td>SOURCE OF SARCOMA CELLS; NUMBER OF MICE IN EACH GROUP FROM MASHED IMPLANTS</td>
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<tr>
<td>5,000 cells</td>
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<tr>
<td>First count: Number of sarcoma cells (in 0.1 cc.)</td>
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<tr>
<td>Per cent of mitoses</td>
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<tr>
<td>Second count: Number of sarcoma cells (in 0.1 cc.)</td>
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<tr>
<td>Per cent of mitoses</td>
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<tr>
<td>Third count: Number of sarcoma cells (in 0.1 cc.)</td>
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<tr>
<td>Per cent of mitoses</td>
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<tr>
<td>Fourth count: Number of sarcoma cells (in 0.1 cc.)</td>
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<tr>
<td>Per cent of mitoses</td>
</tr>
<tr>
<td>Per cent of necrotic cells</td>
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* Similar data for mice inoculated with 10,000 cells from mashed implants and peritoneal fluid are shown on Charts 1 and 4.

In mice inoculated with 1,000 cells, the first count was performed after 3 or 4 days, the second after 7-8 days, the third after 10 or 11 days, and the fourth after 16-18 days; in mice inoculated with 100 cells, the first count after 6—7 days, the second after 10—14 days, the third after 15—18 days, and the fourth after 17 days. Computed data were as follows:

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<tr>
<td>COMPARISON OF THE GROWTH CHARACTERISTICS OF MALIGNANT LYMPHOMA CELLS FROM MASHED SUBCUTANEOUS IMPLANTS AND FROM PERITONEAL FLUID*</td>
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<tr>
<td>SOURCE AND NUMBER OF LYMPHOMA CELLS; NUMBER OF MICE IN EACH GROUP FROM MASHED IMPLANTS</td>
</tr>
<tr>
<td>10,000 cells</td>
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<tr>
<td>First count: Number of lymphoma cells (in 0.1 cc.)</td>
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<tr>
<td>Per cent of mitoses</td>
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<tr>
<td>Second count: Number of lymphoma cells (in 0.1 cc.)</td>
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<tr>
<td>Per cent of mitoses</td>
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<tr>
<td>Third count: Number of lymphoma cells (in 0.1 cc.)</td>
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<tr>
<td>Per cent of mitoses</td>
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<tr>
<td>Growth of subcut. tumors at site of inoc.</td>
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* In mice inoculated with 10,000 cells, the 1st count was made after 4 days, the 2nd count after 7-9 days, and the 3rd count after 10-11 days. In mice inoculated with 1,000 cells, the 1st count was made after 5-7 days, the 2nd count after 10-11 days, and the 3rd count after 15-18 days. In mice inoculated with 100 cells, the 1st count was made after 9-10 days, the 2nd after 11 days after per. fl. inoculation and 10 days after mash inoculation, the 3rd count after 14 and 15 days, respectively.
tumors. They also showed that more tumor nodules developed by "auto-inoculation" following intraperitoneal inoculation than following subcutaneous inoculation.

DISCUSSION

Our results have shown that continued multiplication of tumor cells can be obtained by serial intraperitoneal transfers of these cells from mouse to mouse, at intervals of 3-7 days. This method of tumor cell culture in the peritoneal fluid also enabled us to study the growth cycle of tumor cells on a quantitative basis. This method also provided data concerning the quantitative relationship between the number of cells inoculated either subcutaneously or intraperitoneally and the rate of growth of resulting subcutaneous implants. Finally, the intraperitoneal transfer of free tumor cells from mouse to mouse enabled us to obtain subcutaneous tumors by intraperitoneal inoculation of very small numbers (100 or 10) of tumor cells from the peritoneal fluid.

The results also show that tumor cells inoculated into the peritoneal cavity obey the same quantitative rules as cultures of free organisms—i.e., the amount and rate of cell multiplication are proportional to the size of the inoculum. However, they also demonstrate that when small numbers of cells are inoculated such cells appear to be handicapped in their proliferation. They show lower percentages of mitosis and higher percentages of necrotic cells than do descendants of larger inocula in corresponding stages of growth.

We feel that the material presented in this study serves as an adequate demonstration that quantitative as well as qualitative data may be obtained from an analysis of free tumor cells growing in the peritoneal fluid of mice. It is hoped that the data may serve as a basis for the development of an additional screening method to test the activity of physical and chemical agents against the growth of tumor cells, a method which would supplement the various screening techniques at present in use.

SUMMARY AND CONCLUSIONS

1. Requisite numbers of Sarcoma 37 or malignant lymphoma cells, obtained either from subcutaneous implants or by serial transfers in the peritoneal fluid, were inoculated intraperitoneally into several groups of CFW or dba mice. The rate and the amount of cell growth in each mouse were studied quantitatively. Specimens of peritoneal fluid were withdrawn repeatedly, at various time intervals, from the same mouse, for total and differential cell counts (number of tumor cells per 0.1 cubic centimeter, percentage of mitoses, of necrotic elements, and of leukocytes). The results from each group of mice were tabulated and plotted in graphs.

2. The growth in the peritoneal fluid of tumor cells from both strains presented the following essential features: (a) Intraperitoneally inoculated tumor cells multiplied in the peritoneal fluid independently of peritoneal implants, i.e., before and sometimes without the growth of tumor tissue in the peritoneum; thus, the multiplication of free tumor cells appeared to be a primary phenomenon. (b) Free tumor cells multiplied continuously in the peritoneal fluid in serial intraperitoneal transfer of this fluid from mouse to mouse, thus appearing as a culture of the inoculated tumor strain. (c) Serial intraperitoneal transfers increased the growth potential of tumor cells in the peritoneal fluid and in the subcutaneous tissue. (d) For the cells of the same source, i.e., of the same growth potency, the amount and the rate of growth were proportionate to the number of inoculated cells. It is suggested that these phenomena are probably not specific for the tumor strains used in our work, but may be reproduced with other tumor strains.

3. The growth of free malignant lymphoma cells in the peritoneal fluid, as compared with the growth of S-37 under the same conditions of experiment, presented the following special features: (a) The growth cycle of lymphoma cells in the peritoneal fluid lacked the final stage of inactivity and of partial regression which terminated the growth cycle of S-37 free cells. (b) The percentage of lymphoma cells in the cellular content of the peritoneal fluid increased in each mouse steadily from the end of the lag period until the death of the animal. This phenomenon contrasts sharply with the intense leukocytic reaction to S-37 cell multiplication in CFW mice. These differences in growth characteristics of free lymphoma cells in the peritoneal fluid would appear to be attributable to the essential difference between the "strain specific" quality of lymphoma cells for dba mice and the "foreign" nature of S-37 cells for CFW mice.

REFERENCES


Figures 1–8.—Appearance of S-37 cells from subcutaneous implants in various stages of their growth cycle in the peritoneal fluid. Wright, ×800.

Fig. 1.—Peritoneal fluid from a CFW mouse before inoculation. Wright, ×800.

Fig. 2.—S-37 Tumor mash used for inoculation. Aceto-orcein, ×800.

Fig. 3.—Appearance of inoculated S-37 cells in the peritoneal fluid 6 hours after inoculation of 10,000 cells. Aceto-orcein, ×800.

Fig. 4.—Appearance of inoculated S-37 cells in the peritoneal fluid after 24 hours. Aceto-orcein, ×800.

Fig. 5.—Appearance of inoculated S-37 cells in the peritoneal fluid after 48 hours. Aceto-orcein, ×800.

Fig. 6.—Appearance of inoculated S-37 cells in the peritoneal fluid after 72 hours. Aceto-orcein, ×800.

Fig. 7.—Appearance of inoculated S-37 cells in the peritoneal fluid after 5 days. Aceto-orcein, ×800.

Fig. 8.—Appearance of inoculated S-37 cells in the peritoneal fluid after 8 days. Aceto-orcein, ×800.
FIGURES 9-14.—Appearance of malignant lymphoma cells from subcutaneous implants in various stages of their growth cycle in the peritoneal fluid.

Fig. 9.—Peritoneal fluid from a dba mouse before inoculation. Wright, ×800.

Fig. 10.—Malignant lymphoma mash (from 14-day-old subcutaneous implant) used for inoculation. Wright, ×800.

Fig. 11.—Same. Aceto-orcein, ×800.

Fig. 12.—Malignant lymphoma cells in the peritoneal fluid of a dba mouse 8 days after inoculation of 10,000 cells.

Fig. 13.—Malignant lymphoma cells in the peritoneal fluid of a dba mouse 12 days after inoculation of 10,000 cells.

Fig. 14.—Malignant lymphoma cells in the peritoneal fluid of a dba mouse 14 days after inoculation of 10,000 cells.
FIGURES 15-20.—Growth cycle of S-37 cells in the peritoneal fluid of a CFW mouse after the 40th intraperitoneal transfer of 10,000 cells. Aceto-orcein, ×800.

Fig. 15.—Appearance of the cells after 6 hours.
Fig. 16.—Appearance of the cells after 24 hours.
Fig. 17.—Appearance of the cells after 48 hours.
Fig. 18.—Appearance of the cells after 72 hours.
Fig. 19.—Appearance of the cells after 6 days.
Fig. 20.—Appearance of the cells after 10 days.

Fig. 21.—Lymphoma cells in the peritoneal fluid of a dba mouse 10 days after the 19th serial transfer of 1,000 cells. Aceto-orcein, ×800.

Fig. 22.—Lymphoma cells in the peritoneal fluid of a dba mouse 14 days after the 19th transfer of 100 cells. Aceto-orcein, ×800.
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