Effect of Intravenously Injected Radioisotopes on Metastatic Tumor Cells in Organs of the Mouse.* (20707)

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Several authors(1) have observed that inoculation of cell suspensions (brei) of apparently healthy organs from tumor-bearing mice induced occasionally tumor growth at the site of injection. However, these results were not used for systematic screening of viable tumor cells in various tissues partly because they were attributed by some investigators to "cancer virus" spread from the primary tumor, and mainly because injection of organ brei often provoked intense local reaction inhibiting growth of few tumor cells. We have found(1) that these difficulties can be overcome by using the intraperitoneal route for assay inoculation (graft). Moreover we have noted previously(2) that after introduction of a mixture of normal cells and tumor cells into the peritoneal cavity, only tumor cells grew as free cells in the peritoneal fluid, while normal tissue cells rapidly disintegrated. This method of using the peritoneal cavity as a location that selectively promotes growth of tumor cells has demonstrated consistently that for each tumor strain the frequency and the distribution in organs of metastatic cells followed a certain pattern(1).

It occurred to us that the growth of organized tumors from metastatic cells could be prevented by introduction into the organs of radioactive or chemical agents inhibiting, in well tolerated doses, the viability of malignant cells. We have shown previously(3-6) that direct contact with radioactive gold or iodine in sufficient concentration inhibited, in many instances completely, the growth of tumor cells. This effect was demonstrated by considerable decrease in their number per cmm of peritoneal or pleural fluid, by disappearance or abnormal appearance of their mitoses and by their inability to initiate growth after transfer into normal mice (biological test of viability). Moreover, it is known that colloidal Au^{198} is stored mainly in the liver and spleen(7), while the large insoluble particles of $CrPO_4$ suspension may be filtered from the blood by the capillary net in the lung(8). Therefore these radioisotopes were selected for trial of their effect on the viability of metastatic cells in the liver, the spleen and the lung.

Materials and methods. A. *Tumor and mouse strains.* Sarcoma strain S-37 was carried in CFW mice in serial intraperitoneal transfers as free cells growing in the peritoneal fluid(2). Technics of intraperitoneal and intrapleural inoculation of requisite numbers of tumor cells were described elsewhere(2). Ten to 50 millions of cells were inoculated in each series of experimental mice. B. *Treatment.* Radioactive material† was diluted with 0.85% NaCl solution to the concentration of 1 mc per ml. Doses of 0.3 to 0.35 mc (shown previously (4) to be well tolerated by mice) were injected by the i.v. or i.p. route. Diluted suspensions of chromic phosphate were shaken before each injection. C. *Pattern of the experiment.* Mice ("donors of organs") were inoculated with the same dose of tumor cells of the same strain and by the same route. In each series one group was left untreated (controls) and another group was treated on the day of inoculation (graft) or at various intervals before or after inoculation. Mice of both groups were sacrificed 4 or 5 days after inoculation. Their livers, spleens, kidneys and lungs were mashed separately, and the brei of each organ was injected intraperitoneally into normal mice. These mice ("recipients of organ brei") were examined after 10 days for the presence of tumor cells in their peritoneal fluid and for gross evidence of tumor growth in the peri-

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TABLE I. Assay Organ Grafts (Assay for Presence of Tumor Cells in Organs) from CFW Mice Bearing Primary Intraperitoneal Sarcoma 37 Grafts and Treated with Radioisotopes.*

Series	Days between primary tumor graft and treatment	Organs examined — Liver	Spleen	Kidney	Lung
1†	5	8/2	6/4	9/1	8/2
	2	6/4	8/2	10/0	6/4
	0	5/5	5/5	8/2	6/4
	−2	7/3	6/4	8/2	8/2
	Controls	10/0	8/2	10/0	7/3
2	5	8/2	6/4	9/1	7/3
	2	1/9	3/7	10/0	4/6
	1	2/8	5/5	10/0	5/5
	0	1/9	4/6	9/1	4/6
	−2	1/9	4/6	10/0	4/6
	−5	10/0	5/5	10/0	7/3
	Controls	10/0	7/3	9/1	6/4
3	5	7/3	2/8	10/0	8/2
	2	2/8	1/9	8/2	9/1
	1	2/8	2/8	9/1	10/0
	0	1/9	1/9	10/0	8/2
	−2	2/8	2/8	8/2	9/1
	Controls	9/1	3/7	9/1	9/1
4	5	7/3	6/4	10/0	3/7
	2	6/4	4/6	9/1	1/9
	1	5/5	4/6	9/1	0/10
	0	6/4	3/7	10/0	0/10
	−2	5/5	4/6	10/0	1/9
	Controls	10/0	7/3	9/1	7/3

* For each group of 10 mice, results are presented as a fraction, the numerator indicating No. of takes and the denominator, No. of no takes.

† Primary tumor graft: 1st, 2nd and 4th series intraperitoneal, 3rd-intrapleural. Treatment with radiogold: 1st series intraperitoneal, 2nd and 3rd intravenous. Treatment with radioactive chromic phosphate—4th series intravenous.

Note: (1) second column, − before numbers indicates that treatment was given before primary tumor graft; 0 indicates simultaneous graft and treatment. (2) 3rd column, actually 15 to 20 mice were used in each group but for easier reading and interpretation of results they were calculated in table as per 10 mice. (3) Assay organ grafts were done 5 days after primary intraperitoneal grafts and the results recorded 10 days later.

toneal wall.

Results. The results are reviewed in Table I. The first series of experiments shows the results of the treatment with colloidal Au^{198} given at the site of inoculation in a closed cavity and thus directed in the first line locally against the primary growth and only to a lesser extent (since the absorption of colloidal material from the cavity in the blood is relatively low) against tumor cells disseminated into organs. Inverse experimental conditions were present in the second series of the table illustrating the effect of radioactive agents given intravenously and taken in large amounts from the blood by some organs, but only in very small amounts by the cavity containing primary growth. In the third series the growth was initiated in another cavity—the pleural cavity. Thus, the cell migration from the primary tumor into the abdominal organs and the lungs followed different paths as compared with the second series. The fourth series provided data obtained with non-colloidal but particulate radioisotope-chromic phosphate.

The records of Table I show that in several groups of each series the incidence of growth from inoculated organs was invariably lower for treated animals than for controls. In experiments with colloidal radiogold which is known to be stored mainly in the liver(7), clear cut results were observed in animals inoculated either into the peritoneal or the pleural cavity and treated intravenously. Similarly in experiments with intravenously injected suspensions of chromic phosphate, lower frequency of tumor growth was recorded mainly in animals injected with lungs which previously were found to intercept large particles of $CrPO_4$(8). Local treatment of primary tumor growth with colloidal radiogold did not reduce significantly the frequency of tumor growth induced by the liver, (first series). All these data indicate that the storage of a radioisotope in the organ of a tumor bearing mouse was a prerequisite for the several instances of failure of growth from this organ after intravenous treatment. The most marked results were obtained by giving the treatment on the day of primary inoculation or shortly (1 or 2 days) before or after. The independence of this effect from the route of tumor cells spread into organs is illustrated by similarity of results in the second and the third series.

The 10-days period of observation was accepted because previous work(1) has shown that this interval provided most consistent results. However some of mice negative on examination after 10 days developed peritoneal growth 20 to 40 days later. This was attributed to the presence in injected material of tumor cells in very small numbers of very low viability, etc.

Discussion. Since only traces of radioactive material (considering its dilution in the body

and its decay after 3- to 5-day interval between inoculation and autopsy) were introduced in the course of assay implantation with the organ brei of treated mice into mice recipients, the delay in tumor growth can be attributed to damage by intravenously injected radioisotopes to metastatic tumor cells in these organs. Whether this damage consisted in inhibition of mitoses or advanced disintegration of the cell structure, it implied, according to our results, loss or attenuation of viability of a certain number of tumor cells.

Previous work has shown(3,4,9,10) that free tumor cells are more vulnerable to radiation and to some physical and chemical agents than organized tumor structure. This may account for the deep damage inflicted in our experiments to free metastatic cells by radioisotopes in doses well tolerated by animals. It may also explain the importance of early treatment (not more than 2 or 3 days before or after inoculation) *i.e.* before metastatic cells intercepted by organs had sufficient time to elicit vascularization and organized growth. This interpretation may suggest the use of intravenous injections of suitable radioisotopes immediately before or after surgical intervention on certain tumors in order to induce at once damage to metastatic cells spread into the liver or the lungs from manipulation during operation.

Summary and conclusion. 1. Intraperitoneal inoculation of liver brei from sarcoma-37 (i.p. growth, 5-day-old) induced tumor growth in nearly all of mice. This was attributed to the presence in the liver of metastatic cells spread from the primary tumor. Similar results were obtained only in rare instances with liver brei from the mice of the same series injected intravenously with colloidal Au^{198} on the date of primary tumor inoculation (graft) or 2 days earlier or later. Analogous difference in results was recorded in experiments with i.p. inoculation of lung brei from tumor-bearing mice untreated and treated intravenously with radioactive chromic phosphate. It was concluded that injected radioisotopes reduced the number and the viability of metastatic tumor cells in the liver or in the lung. 2. Tumor growth from the organs which retain only small amounts of particulate isotopes (spleen) or none was but slightly inhibited or not at all. It was concluded that retention of radioisotopes by the organ was a prerequisite for their effect on metastatic tumor cells in this organ. 3. The possibility to induce damage to metastatic tumor cells without inflicting similar damage to normal tissues of the host was attributed to high vulnerability of metastatic cells as free cells (*i.e.* before their organized growth). 4. It is suggested that the method reported above may be useful for screening the activity of radioactive and chemical agents against metastatic tumor cells in organs of the mouse, and it is presumed that in certain clinical conditions it may be attempted to attack selectively metastatic tumor cells by suitable radioisotopes.

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