Stimulated by the clinical impression that some patients show rapid development of distant metastases for the first time shortly following surgical or irradiation treatment of their primary tumor, the surgical service of the National Cancer Institute has undertaken a series of experiments dealing with the effect of surgical excision or local irradiation of a transplantable mouse tumor on the development of distant metastases arising from this tumor.

An experimental study by Schatten (5) revealed that surgical excision of two different transplantable mouse tumors results in, or is attended by, an increased number of pulmonary metastases or an increase in average size of metastases when compared with a control group.

A similar experiment undertaken to study the effect of local irradiation likewise demonstrated changes in the pattern of pulmonary metastases (2). In a study involving 131 irradiated mice and 89 control mice, the irradiated animals received 3000 r locally to the "primary" tumor. When one tabulated the number of spontaneous pulmonary metastases found 3 weeks following irradiation of an intramuscular melanoma and compared this with the number of pulmonary metastases in an untreated group of mice, two effects were noted. A large group of treated animals averaged fewer metastases than did the controls, and a smaller group averaged many more metastases than did the controls. Forty-five per cent of the irradiated group had less than three pulmonary metastases, as compared with 28 per cent in the controls; this included 19 per cent of the irradiated mice completely free of pulmonary metastases as compared with only 6 per cent of the controls. Also of great interest was the finding in eleven of 131 irradiated mice of more metastases than were found in any of their respective control animals. Three of these had more than twice the number of metastases found in any control mouse.

The large group of irradiated animals with fewer metastases was perhaps the result to be expected. It was felt that, in this group, irradiation of the "primary" tumor had in some way altered the growth potential of the tumor cells, so that when these cells embolized to the lungs they were not able to grow and were destroyed by the host. The metastases which were found could already have existed at the time of irradiation and so were not affected. No explanation was found for the development or occurrence of more prolific growth of metastases seen in a small group of irradiated animals. There was no apparent correlation between numbers of metastases and the size of the tumor at the time of irradiation or the growth pattern of the "primary" tumor following irradiation. Irradiation of the contralateral thigh of the mouse with no irradiation of the "primary" tumor had no measurable effect on the number of pulmonary metastases.

Kaplan (1, 7) found an increased number of mice with pulmonary metastases following irradiation of a different transplantable mouse tumor which, under normal circumstances, rarely metastasized to the lungs. His explanation for this phenomenon was that local tumor irradiation caused a local transient disturbance in the tumor-host relationship, in which disruption of the blood vessel walls permitted an increased embolization of tumor cells. For this hypothesis to be true, it is necessary not only that radiation have the postulated effect on tumor embolization but that the radiation be ineffective against the tumor cells, so that they can develop into metastatic tumors. To test in our system the viability of irradiated cells the following experiment was done.

**MATERIALS AND METHODS**

The plan was to study the capacity of irradiated Cloudman S91 melanoma to grow as artificial
pulmonary metastases in untreated adult CDBA mice (BALB/c × DBA/2 F₁ hybrids) beginning several hours following irradiation with 3000 r in vivo and at varying intervals thereafter, up to and including 3 weeks. (Artificial pulmonary metastases is the term used for lung tumors resulting from the intravenous inoculation of a tumor cell suspension.) The donor tumor in all cases was the intramuscular S91 melanoma of the same lot and age at the onset of the experiment. Three weeks following inoculation a group of intramuscular tumors was irradiated in vivo with 3000 r. This irradiation was delivered from a 275 kv x-ray machine with physical factors enabling one to deliver an estimated tumor dose of 3000 r in approximately 8 minutes. At varying intervals thereafter a tumor cell suspension was prepared by cytosieving (6) 1 gm. of this irradiated tumor in 10 ml. of heparinized physiological saline.

Three individual irradiated tumors were used each time. Five hundredths of a ml. of the intravenous inoculum from each of these donor tumors was injected into the tail vein of four recipient mice. Three weeks later all recipient mice were sacrificed and the lungs examined (Chart 1). The sizes of the individual lung tumors were measured and their total number tabulated.

The intervals between irradiation in vivo and intravenous inoculation were as follows: 3 hours, 12 hours, 24 hours, 36 hours, 2 days, 3 days, 4 days, 6 days, 8 days, 11 days, 17 days, and 20 days. This particular time schedule was chosen because of the results of a pilot study which indicated that the number of lung tumors resulting from the intravenous inoculation of irradiated tumor depended greatly on the time interval between irradiation and inoculation. As controls, untreated tumors were cytosieved and injected intravenously into groups of mice on the 1st, 11th, and 20th days of the experiment.

RESULTS

Changes in number of artificial metastases.—The controls had approximately 600 lung tumors per mouse. Irradiated tumors injected intravenously 3 hours following irradiation in vivo resulted in an average of 14.5 lung tumors per mouse. Tumor injected 12 hours following irradiation resulted in an average of 19.7 lung tumors per mouse, etc. (see Chart 2). There was a definite trend toward fewer lung tumors for 3 days, then a gradual increase. By the 20th day the average was almost back to the control level. Note that the untreated tumor injected on the 1st, 11th, and 20th day of the experiment resulted in from 437 to 751 tumors per mouse; the age of the tumor alone had no appreciable effect on the number of artificial pulmonary metastases.

Changes in size of artificial metastases.—A similar picture was found when one measured the size of artificial metastases in the various time categories. The tumor sizes were measured by comparing the tumor diameter to a chart on which were reproduced a series of black circles with diameters varying from 0.023 mm. to 2.8 mm. Each circle was 50 per cent larger than the preceding one. These sizes were graded from −2 to +11 and are so classified on the graph (Chart 3). The largest individual tumor seen was +7 or 0.55 mm. in diameter, but the large number of smaller tumors always brought the average size down to the +2 category or lower.
Control (untreated) tumor cell suspension injected intravenously on the day of irradiation resulted in an average size of 2.0 on our scale, or 0.074 cm. Irradiated tumor injected 3 hours following irradiation resulted in an average tumor size of 0.12 or 0.035 cm., etc. (Chart 3). There was a definite trend toward decreasing sizes, so that by the 8th day following irradiation the average tumor diameter was one quarter that of the control. However, by the 11th day following irradiation the tumor size began to increase and in some cases approach the range of sizes found in the control animals.

DISCUSSION

These data are interpreted to mean that this particular tumor is affected in some way by irradiation either immediately or within hours, resulting in an inhibition of the tumor cells' capacity to become established as pulmonary implants. The decrease in numbers of pulmonary tumors may be an indication that the host is able to overcome a greater number of these irradiated cells, or possibly that many of the irradiated cells are nonviable. The smaller size of the lung tumors following irradiation, however, would seem to suggest that there is truly an inhibiting effect on the growth potential of those tumor cells that were not rendered completely nonviable by the irradiation. As the length of time between irradiation and implantation in the lung increases, the number of lung tumors increases and approaches the number found when untreated tumor is injected intravenously. It would appear, therefore, that either the suggested inhibition of growth potential is gradually lost and the irradiated cells regain their ability to become established in the lung, or the increase in lung tumors is a manifestation of growth of cells within the "primary" tumor which escaped the effects of irradiation and eventually became numerous enough to be a significant fraction of the inoculum and would grow as nonirradiated tumor.

It is interesting to speculate that this aforementioned inhibition of growth as seen in this experiment might be similar to the inhibition of reproduction of single cells following irradiation in vitro as described by Puck and his associates (3, 4). These investigators found that irradiation of isolated HeLa cells in culture kills part of the population, the proportion depending upon the dose of irradiation, and inhibits the growth of the survivors (3). The inhibition or slowing of reproduction may be transient and reversible, i.e., reflecting physiologic action, reparable under favorable conditions. Also, the inhibition may be permanent, i.e., a genetic change of chromosomal origin such that the affected cells may undergo a limited number of divisions, forming "abortive colonies" but not regaining their normal abilities of continued reproduction (4).

Whatever the explanation for this phenomenon might be, it would appear that Kaplan's hypothesis, that irradiation permits an increased embolization of tumor cells which results in an increased number of pulmonary metastases, does not apply to our previous experiments (2). As a result of this experiment and the aforementioned work of Puck et al., there is support of our inhibition hypothesis to account for the large number of animals with fewer spontaneous pulmonary metastases following local irradiation of the tumor. According to this interpretation those lung tumors found in this group are mostly due to cells embolized prior to irradiation, the irradiated tumor being practically incapable of growing following pulmonary implantation intravascularly. Those factors responsible for the small number of animals with greatly increased numbers of metastases following irradiation are still unknown.

The authors are aware that, although such a study as described here with one transplantable tumor is of interest, it must be determined whether the results are repeatable and applicable to other tumors. Such investigations are already under way in this laboratory.

SUMMARY

1. This study was initiated by the clinical impression that surgery or irradiation of a primary tumor in some cases may promote the development of distant metastases.
2. This experiment was conducted to study
the viability of irradiated cells as manifested by their growth as artificial pulmonary metastases following intravenous inoculation at varying intervals following irradiation in vivo.

3. The differences in number and size of artificial pulmonary metastases in mice receiving irradiated tumor intravenously at varying intervals following irradiation were tabulated and compared with the number and size of artificial pulmonary metastases in mice receiving untreated tumor intravenously. Irradiation of the "primary" tumor greatly reduced the number and size of these artificial metastases. This effect increased for several days, after which the apparent viability of the artificial metastases from the irradiated tumors returned toward normal.

4. These results support our interpretations of the results of previous experiments with spontaneous metastases from irradiated tumors.

REFERENCES


An Experimental Study of the Effect of Irradiation on the Dissemination of Cancer

Peter D. Olch, Richard V. Eck and Robert R. Smith