Effect of X-Irradiated Tumor Bed on Tumor Cells
Part 1. Effect of Tumor Bed on Tumor Growth and Host Survival

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移植前照射された腫瘍発育環境の腫瘍細胞におよぼす影響
第1報 腫瘍発育ならびに宿主生存への影響

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悪性腫瘍の放射線治療において，腫瘍発育環境の放射線による変化が，腫瘍ならびにその宿主に何らかの影響を与えるものと考えられる．著者は移植前照射の移植腫瘍に及ぼす影響を知る目的にて次の如き実験を行つた．移植腫瘍としては，滝沢肉腫を用い，腫瘍移植予定部位であるマウス右大腿部にレ線照射を行い，その後同部に移植し，移植前照射による腫瘍発育環境の変化が腫瘍に及ぼす影響（tumor bed effect, TBE）を腫瘍の発育ならびに宿主の生存の2点より観察した．

第一に線量との関係をみるべく1000Rより4500Rを右大腿部に一回照射し，その3日後に腫瘍を移植したところ，ＴＢＥは著明に認められた．即ち，腫瘍発育は一様に抑制され，宿主の生存期間は延長した．平均生存期間は対照群の14日に比べ移植前照射群では線量にはほとんど関係なく，約20日であつて，明らかに生存期間の延長がみられた．また腫瘍の発育は移植後12日目までは照射線量に関係なく一様に抑制されているが，それ以後特に logistic curve の公式を用いて計算した腫瘍の極大値は照射線量と反比例する．この傾向は3000Rまでは認められるが3000R以上では有意差は見られなかつた．

次に腫瘍発育環境に2000Rを一回照射し，その後3時間より84日の種々なる期間をおいて，レ線照射部位に移植した．腫瘍の発育ならびに宿主の生存をみると，前実験同様に発育抑制，生命延長は有意に認められたが，照射と移植との期間には，少くとも3時間より84日の間では有意の差は見出せなかつた．

第3に，移植前照射部位に発育した腫瘍細胞それ自体が，その中でどのように影響されるかを調べるため2000R移植前照射部位に発育した腫瘍をとり出し，それを正常のマウス右大腿部に移植し，その発育を観察した．その結果，片対数グラフ上に描かれた腫瘍発育曲線は，まつたく正常の発育曲線をたどつた．Logistic curve の公式を用い，移植時の健常細胞量をみると，正常の滝沢肉腫のそれに比し，はるかに少ないことがわかつた．

（この研究は昭和38年度文部省科学研究費＝悪性腫瘍の手術前照射に関する研究：班長，山下久雄＝の一つとして行つたものである．）

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In the radiotherapy of malignant tumors, alterations in the tumor bed induced by ionizing radiation in the process of treatment are believed to play an important role in addition to their direct effect on tumor cells themselves.

On the other hand, the tumor bed is also considered to be a factor in the development of radioresistant tumor cells.

It is a well known clinical observation that tumors reccuring in previously irradiated regions are more radioresistant than the original tumor.[4,32] In their explanation of this phenomenon, Conger (1957)[7] and Montgomery (1953)[26] suggested that, in addition to selection or mutation of cells towards radioresistance after initial irradiation, the tumor bed might be affected by the initial treatment to make the tumor behave as though more resistant to subsequent irradiations.

There have been many studies on the direct effect of irradiation, but only few on the effect of the tumor bed (tumor bed effect, TBE).

It is the purpose of the present series of studies to investigate the effect of the tumor bed on malignant tumor cells; that is, on the growth of the tumor, on the survival of the host (part 1), and on the radiosensitivity of tumor cells (part 2).

Materials and Methods

Animals

Male mice of the ddY strain supplied by the Kansai Animal Laboratory, Kyoto, were used in all these experiments. Their room temperature was kept constant during the experiments. The animals were housed in groups of 10 in stainless steal wire cages with wood shavings for bedding. They were provided with standard purina pellets (made by the Funahashi-Nojou Company) and water ad libitum.

Tumor Transplantation

The tumor used was the Takizawa-sarcoma induced by Takizawa (Department of Pathology, Chiba University) by daily injection of 25 per cent fructose solution.[42] A subline of this strain has been propagated in the Department of Radiology, Kyoto Prefectural University of Medicine since November, 1963. A tumor cell suspension of 0.025 to 0.10 ml. was injected into the subcutaneous tissue of the right thighs of mice weighing 13 to 15 grams.

The procedure employed for the inoculum was prepared as follows:

1) A fully grown Takizawa-sarcoma was excised from the ddY strain of mice ten to fourteen days after previous transplantation.

2) The excised tumor was minced finely with ophthalmic scinssors and mashed more finely with rough homogenizers.

3) Eight ml. of this tumor cell emulsion was filtered through three sheets of gauze into 2 ml. of physiologic salt solution containing penicillin.

4) This solution was used for transplantation.

5) All procedures were done under aseptic conditions.

X-irradiation

An X-ray machine of the Toshiba KXC-18 type was operated at 120 kv., 25 mA. with a 1.0 mm Al. filter. The HVL was 2.4 mm Al., and the focus-skin distance was 30 cm. The dose rate measured with a Shimazu Dose-Reader and No. 422 chamber was 125 R. per minute in air. The right thighs of mice were irradiated in a 10 by 10 cm. field. The rest of the body was shielded by a lead plate 2 mm. in thickness. The animals were anesthetized by ether only for their fixation.

Observation of tumor growth

The tumors were measured by calipers every two to four days at 4 to 6 p.m. The tumors were ovoid,

so their volume was estimated as that of an ellipsoid according to the formula;

$$Ve=\frac{4}{3}\pi\ abc \quad \text{.....(1)}$$

where Ve is the estimated volume of tumor, and a, b, and c were the measured radii. However, since the tumors were not exactly ellipsoid, it was necessary to correlate the actual tumor volume with the estimated one. To obtain this correlation, twenty mice with tumors were sacrificed when the inoculum grew to various sizes, and the weights of the tumors were compared with the corresponding volumes as estimated by the above formula. By the least square method, the following linear correlation between the measured weight (W) and the estimated volume (Ve) of tumor was obtained ($P \leq 0.01$);

$$W=1.23\ Ve-0.25 \quad \text{.....(2)}$$

The specific gravity of the tumor was measured by the $CuSO_4$ method and the tumor weight was multiplied by the reciprocal of the specific gravity. Then equation (2) could be rewritten as follows (Fig. 1);

$$V=1.15\ Ve-0.23 \quad \text{.....(3)}$$

(V.....actual tumor volume)

Equations (1) and (3), which are based on measurement of three radii were used to plot the normal growth of Takizawa-sarcoma on semi-logarithmic graph paper (Fig. 2). The growth curve of such tumors is usually described as exponential,[1,5,6,13,40,47] but only during the early period of growth the curve was linear, and after it the curve gradually became concave. In other words, the growth rate of this tumor is almost constant until it reaches a volume of 1 to 1.5 ml., and then, it gradually slows. These growth curves can be analyzed as logistic ones.[48,49]

$$Vt=K/(1+b.e^{-at}) \quad \text{.....Formula for logistic curve.....(4)}$$

where a, b, and K are constants to be estimated from observed data for t=1, 2,.....N. Using Yale's method of "sums of reciprocals"[29] to fit the logistic curve, the growth curve of Takizawa-sarcoma was calculated. In this formula (4), when $t=+\infty$, Vt=K, that is, "K" reveals the upper asymptote of the tumor in the host.

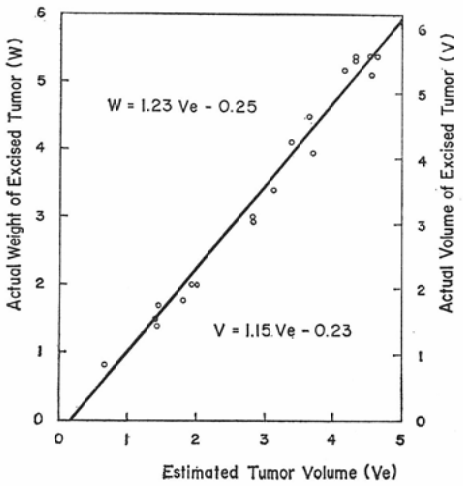

Figure 1. Correlation between Actual Tumor Weight and Estimated Volume

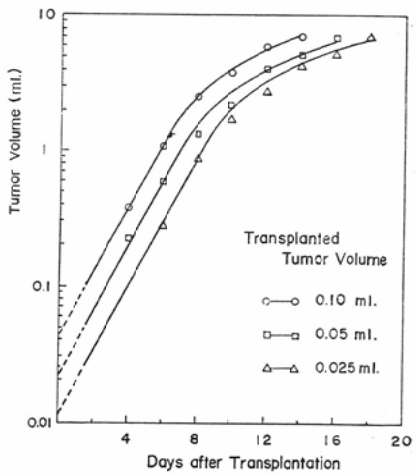

Figure 2. Growtn of Transplanted Takizawa-Sarcoma

Table 1 Theoretical, Initial and Maximum Tumor Volume computed according to Yale's Method of "Sums of Reciprocals" for Fitting the Logistic Curve.

Tumor	Volume of Inoculum	Computed Initial Volume	Computed Maximum Volume
Takizawa-Sarcoma (No. x-ray)	0. 10ml.	0.044ml.	7.607ml.
	0. 05ml.	0.020ml.	6.653ml.
	0.025ml.	0.011ml.	7.364ml.

In table 1, a difference is noted between the inoculated volume and the calculated volume of the inoculum. This difference is considered to be due to both dead cells in the inoculum and the initial delay of mitosis. If it is presume, however, that tumor cells begin to grow soon after inoculation without any delay of mitosis, $V(t=0)$ can be considered to be the volume of viable cells in the inoculum that can grow in the grafted sites.

Evaluation of TBE

In the following experiments, growth curves of the tumor were calculated according to formula (4), and $V(t=+\infty)$ were compared for the evaluation of TBE. In addition, the survival time of the host was checked every day at the same time.

Experiments and Results

Dose Response of the TBE

The first series of experiments investigated the effect on the tumor of several degrees of tumor bed alteration induced by various dosages of x-irradiation. The tumor beds in the right thighs were irradiated with 1,000—4,500R. three days before inoculation, except in twenty control animals. The survival time of the hosts after transplantation and the tumor growth were investigated as indications of TBE.

Tumors developed in all the irradiated and non-irradiated control animals. TBE was evident in the formar group, that is, pretransplant irradiation prolonged the average survival time and inhibited tumor growth.

The average survival time of the control mice was 14.9 ± 1.8 (S.D.) days. The mice irradiated with 1,000 R. before transplantation survived 18.9 ± 2.8 days, and those irradiated with 2,000, 3,000, and 4,500 R. survived 20.7, 20.2 and 21.0 days, respectively (Table 2). Tukey's statistical method indicated that the difference is significant between irradiated and non-irradiated ($P\leq0.01$). The host with tumor in their right thighs to which more than 2,000R. were irradiated could survive slightly longer than those irradiated 1,000R. The differences, however, between the different dosage groups were not statistically significant. The prolongation of survival time is considered to be a TBE influenced by pretransplant irradiation. It was difficult, however, to obtain a dose-response curve from these results.

The tumor volumes of control and irradiated mice compared 4, 8, and 12 days after transplantation. The growth of tumors in irradiated beds was found to be significantly slower ($P\leq0.01$). No significant difference was observed, however, among the various dosage groups until 12 days after inoculation. On the 16th day after inoculation the tumors in 1,000R. group had grown significantly larger than those in the higher dosage groups ($P\leq0.01$), and those in the 2,000 R. group were larger than those in the 3,000 and 4,500R. groups (Fig. 3).

These results suggest that maximum volumes of tumors may vary with X-ray dosage. The maximum volume of each tumor was computed by logistic cruve (Table 4), and it was noted that tumor volume became gradually smaller in inverse proportion to the X-ray dosage. The control tumors grew up to 6.9 ml., whereas those in thighs irradiated with 4,500R. before transplantation grew to only 3.6 ml. Following correlation between the logarithms of maximum Tumor weights (Y) and the irradiated dosages (X) was obtained by least square method ($P \leq 0.01$).

$$Y = -0.0859 \times 10^{-3} X + 0.8273 \quad (X \leq 3{,}000) \quad \text{(Fig. 4)}$$

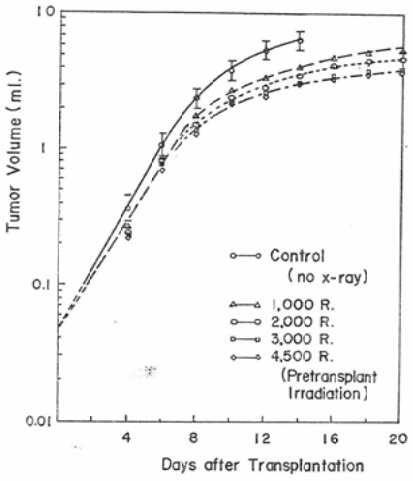

Figure 3. Growth Curve of Takizawa-Sarcoma Transplanted into Normal and Irradiated Tumor Bed (with Various Dosage)

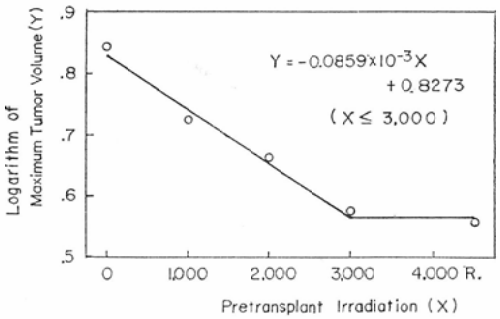

Figure 4. Correlation between Logarithm of Maximum Tumor Volume and Dosage of Pretransplant Irradiation

Duration of TBE

In the second series of experiments, the effective duration of the TBE was investigated. The tumor beds (right thighs of mice) were irradiated with 2,000R., as described above, three hours to eighty-four days before transplantation. It was expected that if the TBE is transient and reversible, it would decline with time, and that if, on the other hand, it is permanent and irreversible, it would be constant or increase. The survival time of the mice after transplantation and the tumor growth were investigated as indications of TBE.

Twenty mice were used in each group. Tumors developed in all the irradiated and non-irradiated control animals.

The TBE was demonstrated by significant prolongation of survival time and inhibition of tumor growth. Statistical analysis indicated, however, that TBE was independent of elapsed time.

While the average survival time of control mice was 14.2 ± 3.1 (S.D.) days, that of all the animals inoculated three hours to eighty-four days after local irradiation was significantly prolonged to 19.9 ± 4.7 to 23.2 ± 3.4 days ($P \leq 0.01$) (Table 3). Among the irradiated mice, however, the average survival time did not show any relation to the intervals between irradiation and transplantation.

The volume of tumors in irradiated beds, was much smaller than in the control animals (Fig. 5). This growth inhibiting effect of the tumor bed did not increase nor decrease with time. No statistically

Table 2 Average Survival of Mice Grafted with Tumors 3 days after Various Doses to the Graft Site

Dose	Number of mice	Average Snrvival ± SD
Control (No. x-ray)	20	14.9± 1.8 days
1,000R.	20	18.9± 2.8 days
2,000R.	20	20.7± 3.8 days
3,000R.	20	20.2± 4.6 days
4,000R.	20	21.0± 3.1 days

Table 3 Average Survival of Mice Grafted with Tumors at Various Intervals after 2,000R. to the Graft Site

Interval	Number of mice	Average Survival ± SD
Control (No. x-ray)	20	14.2± 3.1 days
3 hours	20	20.3± 4.6 days
3 days	20	19.8± 4.7 days
7 days	20	19.9± 5.4 days
21 days	20	21.1± 4.8 days
42 days	20	23.2± 3.4 days
84 days	20	22.3± 5.4 days
Average except control	120	21.1± 4.8 days

significant difference in tumor volumes could be observed among hosts irradiated three hours to eighty-four days before transplantation.

It was also noted that the maximum volume of tumors in irradiated tumor beds was much smaller than that of those in normal beds. Theoretically, the maximum volume was calculated according to the formula of logistic curves (Table 4). The upper asymptote of control tumors and of tumors in irradiated beds was 7.0 ml. and 4.6 ml., respectively.

TBE and Activity of Tumor Cells

It was demonstrated in the experiments described above that tumor growth was definitely inhibited in tumor beds irradiated before transplantation. The next problem was to determine whether an irradiated tumor bed inhibites tumor growth with or without any effect on viable cells. Tumor cells grown in beds previously irradiated with 2,000R, were transplanted to normal non-treated tumor beds by the following procedure; 1) The right thighs of mice irradiated with 2,000R. three days before transplantation. 2) Thirteen days after Takizawa-sarcoma cells had been inoculated into these irradiated beds, the devleoping tumors were excised and suspensions of cells (0.1 ml.) were retransplanted into normal nonirradiated tumor beds. 3) The volumes of these tumors were determined to obtain their growth curves every other days. (Twenty mice were used.)

The retransplanted tumors grew in the same way as normal Takizawa-sarcoma, and their growth curves plotted on semi-logarithmic graph paper were identical with that of the original tumor (Fig. 6). A slight difference was observed, however, when the initial value of this growth curve was computed according to the formula of logistic curves, that is, the initial volume of the retransplanted tumor was approximately 0.02 ml., and in the case of the original Takizawa-sarcoma the value was usually about

Table 4 Theoretical, Initial and Maximum Tumor Volume computed according to Yale's Method of "Sums of Reciprocals" for Fitting the Logistic Curve

Tumor	Volume of Inoculum	Computed Initial Volume	Computed Maximum Volume
Pretransplant Irradiation with Various Dosage			
Control (No. x-ray)	0.10ml.	0.047ml.	6.928ml.
1,000R.	0.10ml.	0.046ml.	5.227ml.
2,000R.	0.10ml.	0.049ml.	4.594ml.
3,000R.	0.10ml.	0.037ml.	3.742ml.
4,500R.	0.10ml.	0.034ml.	3.577ml.
Pretransplant Irradiation at various Intervals before Transplantation			
Control (No. x-ray)	0.10ml.	0.049ml.	7.032ml.
2,000R.	0.10ml.	0.045ml.	4.556ml.

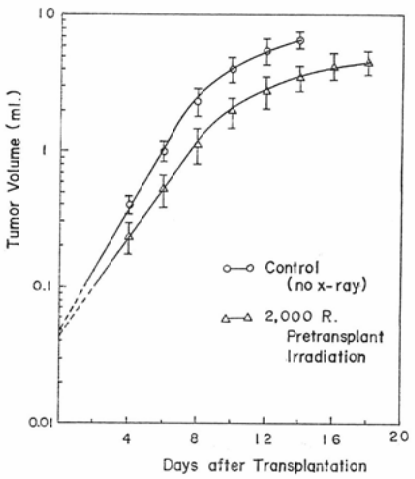

Figure 5. Growth Curve of Takizawa-Sarcoma Transplanted into Normal and Irradiated Tumor Bed (at Various Intervals before Transplantation)

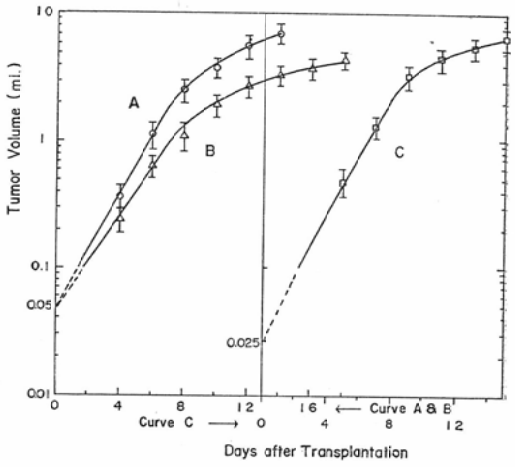

Figure 6. Growth Curve of Takizawa-Sarcoma,
(A) grown in Normal Bed.
(B) grown in 2,000R. Irradiated Bed.
(C) grown in 2,000R. Irradiated Bed and Re-transplanted into Normal Bed.

0.04—0.05 ml., i.e., about half the inoculated volume. These results suggest that tumors in irradiated beds may contain more necrotic tumor cells. The fact that the growth curve of this tumor is identical with that of the original one is considered to suggest that its growth activity was not affected by irradiation of tumor bed.

Discussion

In much of the literature on the TBE, the prolongation of the host survival time and the reduction of tumor growth have been described as effects of tumor beds which have been irradiated before transplantation. In addition to these findings, it has been demonstrated in the present experiments that the

maximum volume of tumors grown in irradiated tumor beds was smaller than that of those grown in normal beds, when tumor growth was calculated as a logistic curve; and that the growth curve of tumors grown in irradiated beds, then retransplanted to normal beds was essentially the same as that of the original tumor.

In the first quarter of this century, the tumor bed was considered to have a great effect on cancer cells in X-ray therapy.

Using the spontaneous mammary carcinoma of mice, Murphy, Maison and Sturm (1923)[28] reported that (1) in flanks irradiated with an erythema dose before transplantation, the transplantability rate was much lower than in non-irradiated normal flanks, and tumor growth was inhibited; (2) when a piece of tumor was irradiated with an erythema dose "in vitro" and transplanted into a normal flank, no inhibition of tumor growth was observed. They concluded that "the beneficial result from X-ray therapy is due to the reaction in normal tissue, not to any direct effect on the cancer cells".

Nakahara (1923)[30] studied tumors growing in X-rayed areas histologically, and showed a series of degenerative changes which was in every way comparable to cancer cell degeneration following X-ray treatment.

Recent studies have shown that more than an erythema dose is necessary to kill such tumor cells, and that a tumor lethal dose is related to the number of tumor cells in addition to an extrapolation number and thirty-seven per cent dose.[12,14,34,35,43] It is also well known that the transplantability rate depends on the number of viable cells inoculated.[2,36,52] These facts make it doutful that the killing effect of the irradiated tumor bed on cancer cells is a TBE.

In the present experiments tumor cells were not killed when the site of graft had been irradiated with less than 2,000R. The volume of the inoculum, when computed by the formula of logistic curve presuming that it began to grow without any delay of mitosis, was slightly smaller in sites irradiated with 3,000R. or more. However, this difference was not statistically significant.

Russ and Scott (1927)[38] exposed the tissue surrounding Jensen's rat sarcoma to a square radium capsule (30 mg.) for 30 to 50 minutes and found that the tumors were always smaller than control tumors in non-irradiated tissue. In 1940 they[39] reported the rate of growth was considerably slowed when thesurrounding tissue was irradiated approximately 1,200R.

Stenstrom et al. (1955)[41] and Vermund et al. (1956,[50] 1958[51]) showed that pretransplant irradiation of the tumor bed prolonged host survival time and reduce the growth rate of the tumor. Using spontaneous mammary carcinoma in female Z mice, Stenstrom[41] showed in 1955 that the growth rate for pretreated cancers was the same over a radiation dose range of 1,000 to 6,000R., and that dose of 3,000R. was most efficient in prolonging the lives of the inoculated animals, while raising the dose to 4,500 and 6,000 R. did not yield higher survival. Summers et al. (1964)[44] demonstrated TBE as the prolongation of host survival time which varied with the dose of X-ray, with a maximum of 3,000R. in various histological types of mouse tumors.

The results of the present experiments are essentially similar. The prolongation of host survival time and reduction of tumor growth were also evident in these studies. A slight difference between these and other studies is that it was difficult to obtain the dose-response curve in present studies from the average survival time of mice irradiated with various doses. This difficulty may have been due to the remarkably rapid growth of Takizawa-sarcoma and the large volume of the inoculum. It can be easily obtained, however, from the asymptote of the tumor growth curve.

These two manifestations are considered to be due to the same mechanism. An elucidation of the exact mechanism of TBE should include radiation effects on the skin, connective tissue and vessels, because they compose the tumor bed and their radiation-induced alterations presumably lead to under-nourishment of the tumor bed.

Functional radiation-induced changes of vessels are described as incresed capillary permeability[3,24,27,53] and fragility,[15,37,45] and radiaiton is also stated to decrease the formation of new capillaries.[16,25,46]

Bigelow et al. (1951)[3] made fistulae in thoracic ducts of dogs and showed radiation-induced "increased capillary permeability" by comparing the rate of flow through the fistulae of normal and X-rayed dogs (with an LD-50 dose). The rate of flow and number of erythrocytes were increased in X-rayed dogs soon after irradiation, and these findings were maximum seven to fourteen days after irradiation. Wish et al. (1952),[53] using labeled homologous plasma, erythrocytes and Evans blue dye, demonstrated increased capillary permeability in rabbits and mice. More recently Mount and Bruce (1964)[27] investigated autoradiographs and tissue radioactivity in irradiated rabbit ears after the injection of rabbit serum albumin labeled with iodine-125. In the irradiated ear plasma volume increased within 24 hours after irradiation, and seven days later the rate of flow from the intravascular to the extravascular space increased greatly. These papers all indicate that capillary permeability was increased by X-ray irradiation, and as a result blood plasma and erythrocytes moved more rapidly from the intravascular to the extravascular space.

Szabó et al. (1958)[45] stated that tendencies to bleeding and diffuse hemorrhage (after X-irradiation) might be produced not by increased permeability, but by increased capillary fragility. Griffith et al. (1947)[15] demonstrated hemorrhage in the distal part of ligated veins as a result of increased fragility. Rieser (1955)[37] also showed that irradiation of the mesenteric capillaries of frogs with 3,600R. caused increased fragility.

In addition to these functional changes, X-irradiation is said to induce poorer formation of new capillaries. Takahashi(1930)[46] demonstrated fewer new capillaries in healing skin sutured soon after radium-irradiation, and these new capillaries were swollen and contained minute vacoles. Merwin and Hill (1955)[25]studied the effect of a single dose of 400 to 1,500R. on the capacity of the endothelium. Unvascularized areas following burning were larger when the area was irradiated before burning. In areas irradiated with more than 1,200R. poor vascularization continued for 39 days after burning. Grillo (1963)[16] showed, with autoradiography of wounds after the injection of tritiated thimidine, that more endothelial cells that might produce new capillaries were present in non-irradiated than in irradiated wounds.

While X-irradiation induces less fibroblasts and collagen in the process of wound healing,[8,9,10,23] it induces more fibrous changes in normal connective tissue. Kitagawa (1961)[21] measured quantitatively the content of phydroxyproline in collagen in irradiated tissue. It gradually rose up to fifty weeks after 3,000 rads of irradiation.

In addition to these effects on tissue, many authors have reported on the abscopal effect of radiation. X-irradiation on human thighs causes capillary permeability to increase in the oposite thigh (Nuemayr and Thurnher, 1952)[31]; local irradiation of the hypophysis reduces the number of lymphocytes (Langendorff and Lorenz, 1952)[22] and increases capillary permeability (Hecht et al., 1953)[17]; local irradiation of some parts of rats induced destructive changes in the spleen (Pape and Pringer-Kuchinka, 1956)[33]

and liver (Eger et al., 1960)[11], and inhibits tumor growth (Hoffman et al., 1954[18] and 1955[19]). Jolles et al. (1961)[20] studied the interplay between normal and radiation-injured tissue that could be observed with radiotherapy through a sieve or grid, and demonstrated that skin perfusates obtained 30 to 90 minutes after irradiation inhibited the mitosis of cultured chick fibroblasts.

Summers et al. (1964)[44] summarized the factors considered to inhibite tumor growth in the irradiated tumor bed as: (1) diffusible factors or toxin, (2) connective tissue changes, (3) retarded capillary formation and (4) changes in vascular function.

These factors might make the tumor cells under-nourished. New formation of capillaries cannot overtake tumor growth, while fibrous changes in connective tissue inhibit tumor growth mechanically. It is doutful, however, that the retardation of growth which is seen in tumor growth curves is caused by delay of the doubling time, death of many tumor cells or both factors.

In these studies it was of interest that locally irradiated host animals succumbed with smaller tumors. Tumors growing in tumor beds irradiated before transplantation contained many more necrotic cells than tumors of the same size in normal beds, because of the delay in new capillary formation and fibrous changes in the connective tissue. Functional change in the blood vessels is considered to be one of the factors that hasten the death of tumor bearing animals, by increasing the loss of blood cells and proteins.

Summary

1) The effect of pretransplant irradiation of the tumor bed on mouse tumor growth and host survival time was investigated with mouse Takizawa-sarcoma.

2) TBE was demonstrated in these experiments as (1) prolongation of host survival time, (2) reduction of tumor growth and (3) smaller maximum volume of tumor; i.e., the final size of tumors causing death of animals was smaller in X-irradiated than in non-irradiated tumor beds.

3) TBE was observed when the tumor bed was irradiated with more than 1,000R. three days before transplantation. Prolongation of host survival time was almost constant without any relation to X-ray dosage up to 4,500R. A negative corelation with X-ray dosage was noted, however, with maximum tumor volumes and up to 3,000R. of irradiation.

4) When the tumor bed was irradiated with 2,000R. at various periods (3 hours to 84 days) before transplantation, the TBE was constant regardless of the interval between X-irradiation and transplantation. That is, TBE persisted for at least eighty-four days.

5) When tumor cells grown slowly in tumor beds irradiated before transplantation were reinoculated to normal beds, their growth curve was essentially similar that of normal tumor cells.

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