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Tumor Tissue Structur and Radiosensitivity

I. Tumor of simple structure

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腫 瘍 組 織 構 築 と 放 射 線 感 受 性

I.　単調な細胞構成からなる腫瘍

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腫瘍組織構築と放射線感受性との関係について ラットの吉田肉腫とマウスのメチルコラントレン で誘導せしめた肉腫を用い，人体腫瘍と対比しつ つ組織学的に検索を行ない以下の如き知見をえ た.

1.　血管および筋肉に接して増殖する腫瘍細胞 の増殖能は旺盛であるが，血管および筋肉から離 れている細胞の増殖能は低下している．この関係 は，移植後日数，腫瘍の大きさとはほとんど関係 なく認められる．ただし，腫瘍が増大すると血管 や筋肉に接しない腫瘍細胞集団が増加し，血管や 筋肉に接する腫瘍細胞集団は相対的には少なくな る.

2.　増殖能の旺盛な細胞集団の放射線感受性は 高いが，増殖能の低下した細胞集団の感受性は低 い.

3.　吉田肉腫を低温下に放置すると，分裂能の 低下が認められる．また，皮下移植早期において も分裂能の低下が認められる．いずれの場合にお いても，放射線感受性の低下が認められた.

4.　人体腫瘍においても，血管および筋肉組織 と接した細胞集団の感受性が高いことを示唆する 症例をえた.

　　Constituent cells of one tumor are not always uniform in radiosensitivity, but sometimes different. The difference can grossly be classified into two by cause—hereditary and environmental.

　　The former is exemplified firstly by chorion-carcinoma, teratoma and embryonal carcinoma, each of which comprises more than two embryologically different varieties of cells, and secondly by those in which genetic variant cells are secondatily produced by irradiation or chemotherapy. As the result of these differences, cells of one same tumor come to manifest diverse sensitivities.

　　There are various environmental factors which produce difference in sensitivity. In 1953, L. H. Gray[5] clarified that radiosensitivity is heavily dependent on oxygen concentration in tissue. And the latter, the environmental factor, has intimate relation with such morphological factor as structure of tumor tissue, especially its vascular distribution. Namely, perivascularly growing tumor cells, which receive abundant oxygen supply, have high sensitivity, where as those remote from the vessel, which are poorly

supplied with oxygen, have low sensitivity.

In perivascularly growing tumor cells, where oxygen supply is rich, various metabolisms and proliferation are vigorous, while in tumor cells remote from the vessel, metabolism is suppressed and proliferation is slow. When difference is thus caused in functional state of tumor cells by environmental factors, their sensitivity will also become different.

In order to clarify this point we made histological investigation on relation between radiosensitivity and growth rate of tumor cells as expressed by DNA synthesis and mitotic index. It was found as the result that when tumor cells were placed in unfavorable environment, their growth rate was lowered and together with it their radiosensitivity. On the other hand, they regained vigorous growth when the environment turned favorable, and at the same time their radiosensitivity was elevated.

Materials and Methods

Animals: Donryu and Wistar rats were obtained by commercial route, and C_{57} BL/6 mice were supplied by Research Laboratories of Sankyo Co. Ltd. All the animals used were male, and rats weighed about 120 g, and mice about 25 g.

Tumors: Yoshida sarcoma (Y. S.), supplied by Sasaki Institute and intraperitoneally transplanted into Donryu rats through successive generations in our department, and spindle-shaped cell sarcoma[9][10][15] induced by intracerebrally giving 20-methylcholanthrene to C_{57} BL/6 mice at the First Departmtne of Pathology, Gunma University, and subcutaneously transplanted through successive generations, were used.

Transplantation: At 4 days after intraperitoneal transplantation of Yoshida sarcoma into Donryu rats, the ascitic tumor cells were removed with a syringe, suspended in Eagle's solution at a rate of 10^6 cells 0.1 ml, and rats were injected with the suspension into the thigh muscle, or intraperitoneally or subcutaneously in the dorsum of the paw. The MC-induced tumor was transplanted as follows: The tumor bearing mice were sacrificed, the subcutaneous tumors removed asepticly, smashed on a doubllstainless net to make them pass through the meshes, and the resultant material was suspended in Eagle's solution at a rate of 10^6 cells/0.1 ml; the suspension was inoculated to C_{57} BL/6 mice into the thigh muscle.

Irradiation: In carrying out in vivo irradiation, the tumor bearing animals were anesthesized with Thiopental sodium, plased in a box of lead, and only the tumor-grafted hind limb was exposed to radiation. In vitro irradiation was applied to plastic dishes in which was placed the above described Y.S. cell suspension. Specification of irradiation: 180 kv, 25 mA, 0.5 Cu + 0.5 Al, 20 cm, $10 \times 10 \ cm^2$, HVL 1 mm Cu, 319/min.

Histological examination: Tumors, transplanted either subcutaneoulsy or into thigh muscle were removed after sacrificing the host animals, fixed in a 10% buffered formalin or Carnoy solution, embedded in paraffin, and the sections were stained with H.E., PAS, Mallory, van Gieson, PTAH, PAP silver impregnation for microscopical observation. In preparing samples of the intraperitoneally inoculated tumor, ascitic fluid was taken by intraperitoneal puncture with a capillary tube, and smear were stained with Giemsa.

Micro-autoradiography: The tumor-bearing rats were intraperitoneally injected with 100 μCi/ 100 g body weight of ^{3}H-thymidine, and after 45 minutes, the tumor, grafted in the thigh muscle, was extirpated. After fixation in formalin, sections were prepared by the above mentioned routine method, and coated with Sakura NR-M_2 emulsion by the dipping method. After 14 daysexposure, the development

was performed with Konidol X at 20°C for 5 minutes.　　And methylgreen-pyronin stain and hematoxylin stain were performed.

Results

1.　Histological findings and radiosensitivity of tumor produced by transplantation of Y.S. into femoral muscle

Y.S. cells, transplanted to Donryu rats into femoral muscle were transiently found in floating state between the primary muscle bundles, partly beneath the fascia.　　Later, however, they were fixed adjacent to the primary muscle bundles showing vigorous mitosis.　　But those apart from the primary muscle bundles still remain in floating state even at 24 hours after transplantation, displaying scarcely any mitotic figure.　　Those fixed to between the primary muscle bundles made further infiltration and growth with dissolving the surrounding muscle tissue.　　Until 3 days after the transplantation, mitotic index and proportion of ^{3}H-thymidine-labeled tumor cells continued to show nearly constant values in all the specimens. At this time, argyrophile fibers of the tumor tissue showed approximately parallel array, indicating the preservation of the capillary system and myolemma.　　The tumor cells were proliferated adjacent to these argyrophile fibers.　　At 4 days, muscle bundles in the central part of the tumor were dissolved and disappeared to be replaced completely by tumor cells, though in the peripheral part vigorous mitotic figure was still noted between the primary muscle bundles; In the central part both mitotic and labeling index were lowered.　　The array of argyrophile fibers became irregular with its fragmentation and disappearance. Also necrotic foci appeared there.　　Comparison of mitoses of the tumor tissue at various times after the transplantation revealed tendency of decline with time.　　Mitotic index for cells, which grew in the adjacent area to muscle tissue, was almost constant, about 3%, independent of the lapse of time (Fig. 1).　　On the other hand, mitotic index fluctuated between 1 and 1.5% for cell group lying apart from argyrophile fibers, which were present in the central part of the tumor later than 4 days after the transplantation. ^{3}H-thymidine uptake showed nearly the same tendency.　　Namely, independent of time, cell group adjacent to argyrophile fibers gave high labeling index of about 60%, whereas it ranged 20—30% in the group apart from them.　　There was scarcely any uptake of ^{3}H-thymidine in cells floating between muscle bundles early after the transplantation.

Fig. 1.　Mitotic index for Yoshida-sarcoma cells which grew in the adjacent area (——) and the distant area (– – –) from argyrophile fibers.
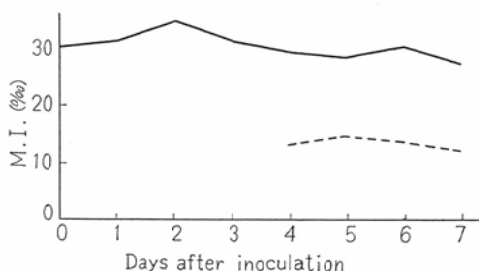
The tumors at different times after transplantation were irradiated with 500 R to examine their radio-sensitivities histologically.　　And it was found out that, as a whole, the proportion radiationdamaged cells decreased with times, but those contiguous to muscle and blood vessel was highly sensitive independent of

time.   This means that the relative number of less sensitive cells increased with time.   Below are given histological observations of tumors from animals sacrificed at 1, 3, 6, 12, 24, 48, 72 hours of irradiation with 500 R at 4 days after Y.S. transplantation:

Tumor cells, grown between the primary muscle bundles:

At 1 hour of irradiation, cells of rather deep stainability appeared, but there was not any marked morphological change.   However the mitotic figure became invisible.   At 3 hours, we could see strong damages in the nucleus such as clumping of chromatin and pyknosis.   No mitotic figure was visible.   At 6 hours, damages became more serious, and the majority of cells manifested pyknosis.   Sometimes nuclear disruption was noted.   At 12 hours disrupted cells were partly absorbed, and tissue defects were visible. At 24 hours, nuclear debris from disrupted cells were almost completely absorbed and cleared, and tissue defects became more remarkable.   On the other hand, unaffected cells began to show recuperative picture.   At 48 hours, mitotic figures were seen sporadically, and there were scarcely any pyknotic cells or nuclear debris, but giant cells with bizarre nuclei or cells with swollen cytoplasm were still present, suggesting the effect of irradiation.   At 72 hours the findings were similar to the preceding ones, but with more marked regenerative feature.

Tumor cells remote from muscle and blood vessel:

At 3 hours after irradiation with 500 R, morphological change was not distinct.   At 6 hours, deep staining tendency of cells was noted for the first time, but karyoklastic figure was scanty.   Later than 12 hours there were no findings indicative of irradiation effect.   (Photos. 1—6)

2.   Radiosensitivity of tumor on the dorsum of the paw produced by subcutaneous transplantation of Y.S.:   For Y.S. the dorsum of the paw of Wistar rat was more unfavorable environment than femoral muscle of Donryu rat, and the tumor cells transplanted there showed scarcely any growth within 1—2 days, but remained subcutaneously in floating stage.   On 3—4 days, they started to grow, and swelling began to be seen.   Mitotic index, which was 3% immediately after transplantation, became near zero on 2 days, but again increased to 1.5—2% on 4 days.

The tumor was irradiated with 125 R on 0. 2 and 4 days after transplantation, and radiosensitivity was comparatively examined by measure of cure rate.   The results are shown in Table 1.   The cure rate was lowest in group which received irradiation on 2 days after transplantation when mitotic potency was at the bottom.   It is necessary to add that in this experiment the cure can not be attributed solely to the effect of irradiation, but that immunization reported previously,[12)18)] may have some indirect bearing on it.

Tabel 1.   Effect of time interval between tumor inoculation and irradiation on cure rate of Yoshida-sarcoma bearing rat irradiated with 125R.

Days After Inoculation	No. of Rats	No. of Death	Death Rate (%)
0	6	1	17
2	6	4	67
4	6	1	17
Control	6	5	83

3.   Decline of mitotic index of Y.S. owing to low temperature and radiosensitivity

Y.S. cells, after 3 time washing with Eagle's solution, were suspended in this medium to a rate of 10^7 cells per 1 ml, and the suspension was dispensed into 8 test tubes each 1 ml.   The tubes were allowed

to stand in a water bath at 15°C, and at 0, 3, 6 and 24 hours, 1,000 R of radiation to one tube was applied. Immediately after irradiation each 0.1 ml of the suspension was intraperitoneally inoculated to healthy Donryu rats.　Control animals were inoculated non-irradiated Y.S. suspension.

　　Survival days of all the groups are given in Table 2.　The cells kept at the low temperature for 3, 6 and 24 hours all gave mitotic index of about 1.5%, which is evidently lower than 3% for the non-incubated cells.　But when intraperitoneally transplanted into healthy rats, the former showed marked growth, which was scarcely different from that of the latter.　After 1,000 R irradiation, however, non-incubated cells were damaged so much that survival days were remarkably prolonged in those transplanted them, whereas the incubated cells were not affected so much, and the animals transplanted them had survival days only slightly longer than those of the controls, non-irradiated groups.

　　Fig. 2 compares days needed for the intraperitoneally transplanted cells to come to occupy more than 95% of intraperitoneal cells, that is, to attain the state of "pure culture".　Therein can be seen the same as with survival days.　(Irradiation was made at 15°C to all groups.)

Table 2.　Survival time (Days)

Incubation Time	Irradiated Group	Non-Irradiated Group
0 hr	18.0 ± 1.5	7.5 ± 0.9
3	12.3 ± 0.4	9.0 ± 1.2
6	11.3 ± 0.4	8.3 ± 0.8
24	13.3 ± 1.5	8.5 ± 0.9

Fig. 2.　Days needed for the intraperitoneally transplanted tumor cells to come to occupy more than 95% of intraperitoneal cells.
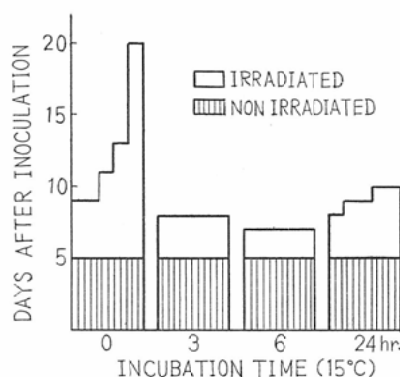
4.　Radiosensitivity of MC-induced tumor

　　A suspension, containing 10^6 cells of the MC-induced tumor, which was transplanted through successive generations subcutaneously on the flank of $C_{57}BL$ mouse, was transplanted into femoral muscle of mice of the same strain.　And when the diameter of the tumor attained 10—15 mm, 500—3500 R radiation was applied to the tumor.

　　The tumor tissue comprised plentiful proliferation of spindleshaped tumor cells, and exhibited a simple structure.　But as seen in Y.S., mitotic index was higher in cells which grew in the peripheral part, adja-

(Yoshida-sarcoma in rats)

Periphery part of the tumor   Central part of the tumor
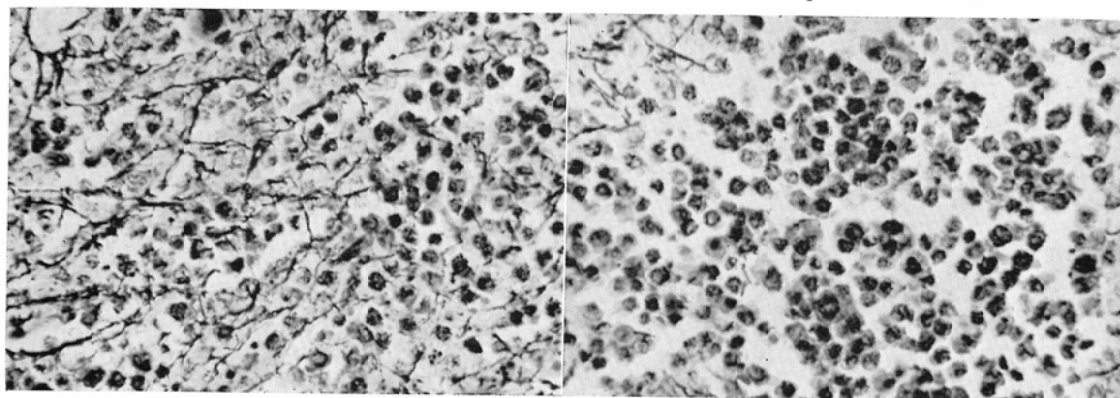

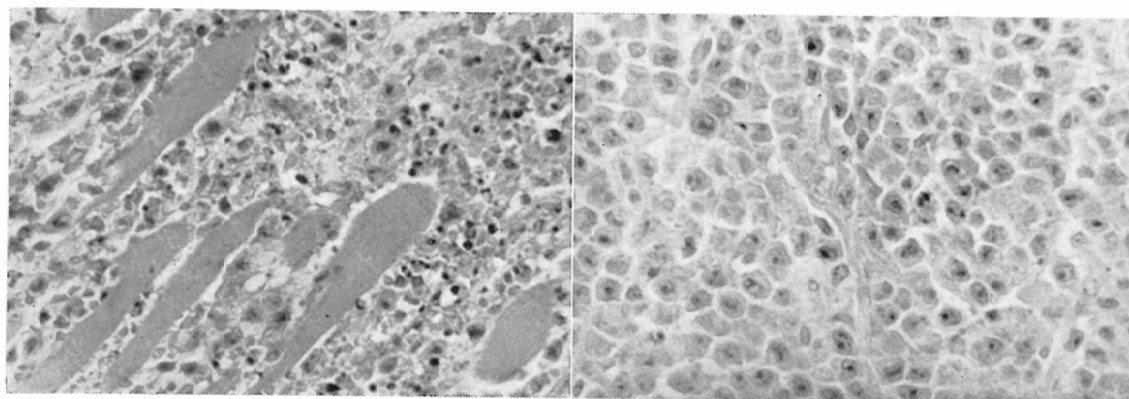Photo. 1 & 2.   Non-irradiated. PAP. silver impregnation.

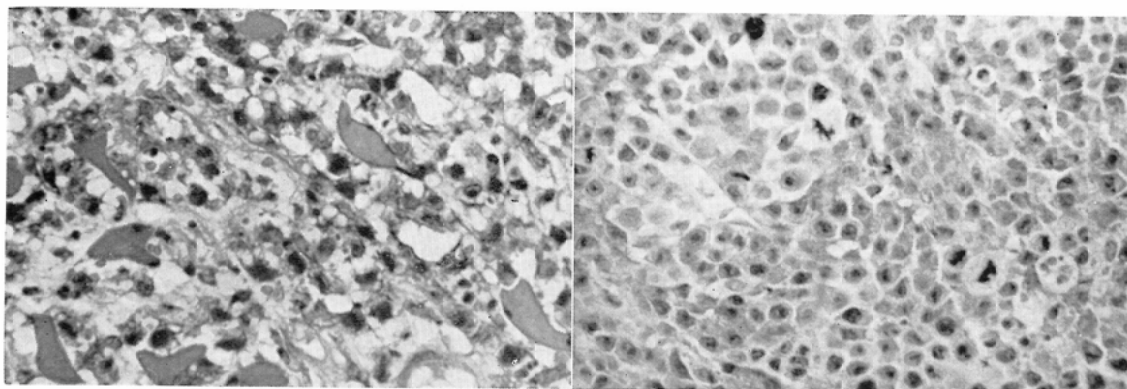Photo. 3 & 4.   At 6 hours after irradiation with 500 R.

Photo. 5 & 6.   At 12 hours after irradiation with 500 R.

(MC-induced sarcoma in mice)

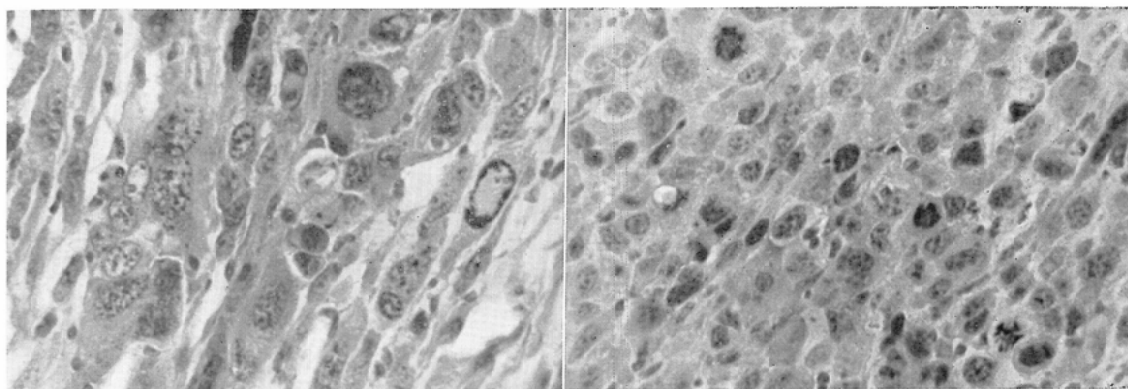Periphery part of the tumor									Central part of the tumor

Photo. 7 & **8**.　At 2 days after irradiation with 3500 R/2 weeks.
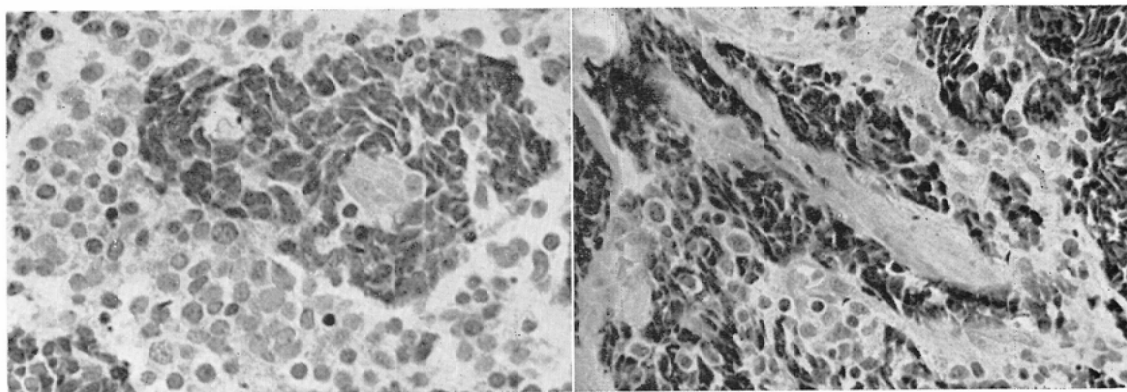**(Skin metastatic cancer in human)**

Photo. **9** & 10.　At a day after irradiation with 50 R.　The tumor cells adjacent
to blood vessel and muscle show marked pyknosis.
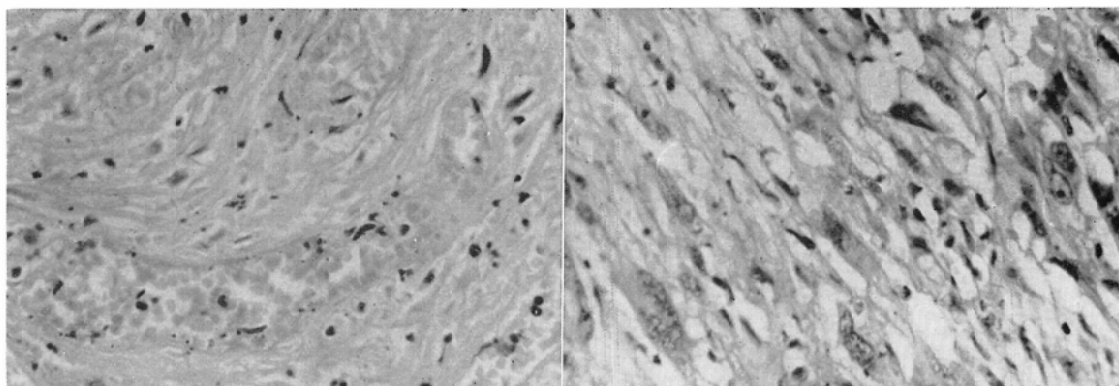**(Spindel cell sarcoma in human)**.

Photo. 11 & 12.　At 10 days after irradiation with 5400 R.　The tumor cells
adjacent to blood vessel are damaged remarkably, but in those
remote from vessel, morphological change is not distinct.

cent to remaining muscle there, but lower in those of the central part. Further more, necrotic foci were observed in the central part.

This tumor had lower sensitivity than Y.S., and 500 R irradiation exerted only slight inhibitory effect on its growth, producing no conspicuous alteration histologically. When, however, the tumor was irradiated with 500 R every other day, 3,500 R in total dose, marked degeneration and lysis appeared in the periphery, and pleomorphism became prominent in the surviving cells, and especially giant cells with bizarre nuclei were remarkable. Also in the stroma, fibrosis became prominent. On the other hand, the central part almost completely underwent necrosis, but tumor cells which remained like islet among nectotic foci manifested scarcely any evident morphological changes indicative of irradiation's effect. Extensive necrotic foci in the central part were coagulation necrosis, and not different from those in non-irradiated cases, and therefore can not be assumed to present the effect of irradiation. (Photos. 7, 8)

Discussion

Tumors, produced in femoral muscle by transplantation therein of Y.S. or MC-induced sarcoma from $C_{57}BL$ mouse had simple cell composition. But they showed difference in radiosensitivity dependent on the distance from the vessel and muscle tissue. As the primary cause of high sensitivity of perivascularly growing tumor cell, effect of oxygen is considered. Since L.H.Gray[5][6][7] gave theoratical basis to oxygen effect on tumor, it has widely been applied in clinical practice, and Churchil-Davidson[3][4] introduced a technic of irradiation under high oxygen pressure. According to Thomlinson and Gray[16], oxygen tension in tumor RIB-5 become 0 when it is 180 μ apart from the vessel. Hewitt,[8] Powers et al[13][14] and Belli et al[1][2] experimentally demonstrated that radiosensitivity of tumor was determined by the number of anaerobic cell aggregates.

As the second cause, can be considered high mitotic potency of perivascularly growing tumor cells. It is known as the Bergonie-Tribondeau's law that in general the more vigorous the mitosis, the more sensitive the cell. According to Kligerman et al,[11] ^3H-thymidine labeled cells were found within a range of 30—45 μ from the sinusoid in mamma cancer of C_3H strain mouse. In our experiments with tumors produced by transplantation of Y.S. into femoral muscle, remarkable mitotic figure was observed as far as 4th—5th layer of cells from a capillary. There is intimate relation between oxygen supply and mitotic potency, and therefore we can not say that mitotic rate is the only determinant of the sensitivity, although *in vivo* the former is closely associated with the latter. It is, however, certain that mitotic potency of the tumor cell is an important factor to determine its radiosensitivity, since in our present study, close relation between these two was confirmed in in vitro experiments as well, and since in vivo both mitotic potency and radiosensitivity are found reduced in the early stage after transplantation or after metastases.

Cell group in the central part of the swelling of Y.S. or of MC-induced sarcoma manifested low mitotic potency and resistance to radiation. This is considered to be a cause of relapse in radiotherapy. The majority of these cells are assumed to have destiny of spontaneous death even if they may evade destruction by radiation. But if a part of them may survive, they may remain in a dormant state neither being proliferated nor being destroyed but with possibility of becoming a source of relapse when the environment turns favorable.

It was confirmed in the present experiments that muscle of offered a favorable enviromental to the growth of the tumor. The reason is assumedly that muscle tissue is well vascularized and that between the

primary muscle bundles, flow of tissue fluid is smooth.

Recently we have found with human tumors that those with simple tissue compsoition manifested, like Y.S. and MC-induced sarcoma, difference in radiosensitivity depending on special relation with blood vessel and muscle. (Photos. 9—12)

Summary

Relation between tumor tissue structure and radiosensitivity was investigated with Yoshida sarcoma in rats and MC-induced sarcoma in mice, with the following results:

1.　Mitotic potency was higher in tumor cells which grew adjacent to blood vessel and muscle, but low in those which were remote from them.　This was true almost regardless of days after transplantation and size of tumor.　However, when tumor is large, cells not adjacent to muscle and blood vessels are increased, reducing as the result the relative number of those adjacent to them.

2.　Radiosensitivity was high in cell group with vigorous mitosis, and low in group with suppressed mitosis.　In general, the part showing vigorous mitosis was well supplied with oxygen.　In this way, vigorous mitosis would not be the only determinant of radiosensitivity.

3.　Mitotic potency of Yoshida sarcoma was reduced when it was kept in low temperature.　It was also suppressed in the early period after subcutaneous transplantation.　In both cases, radiosensitivity was also lowered.

4.　There were clinical cases which showed that cell group adjacent to blood vessel and muscle were high in sensitivity.

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