A Study of Proton Therapy on Malignant Tumors
—Effects in Twenty-four Hours After Proton Irradiation—

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悪性腫瘍の陽子線治療に関する研究（第一報）
—特に照射後24時間の効果について—

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移植腫瘍をマウスの皮下に移植し52MeVの陽子線を照射し照射後24時間の腫瘍について標本を作製し光学顕微鏡及び電子顕微鏡を使用し腫瘍細胞の障害を研究した．先づ陽子線を家兎の耳に照射し経時的に表皮の肉眼的変化及び顕微鏡的観察を実施した．次で移植腫瘍としてHarding-passey mouse melanomaを使用し実験した．照射は皮膚より1 cm深さにBrugg曲線のPeakが位置する様に設定し5000red一回照射を行い経時的観察を行った．観察部位はBrugg曲線のPlateau, Peakの1/2，及びPeakの部位に分け腫瘍細胞の障害について検討した．その結果Peak部位の腫瘍細胞の変化は原形質よりも，むしろ核に高度の障害をみとみた．

§1 Introduction

In order to improve the percentage of cures by radiation therapy, several methods have recently been developed. However, some of them, which we used to biologically increase the radiation sensitivity of tumor cells, are not practical when hypoxic cells exist.

However, both techniques and instruments for the radiation therapy have been remarkably improved. Especially the neutron therapy has been shown to have remarkable effects in Japan and other countries. Therapy using heavy charged particles has been developed especially in U.S.A., though its study is just now in progress in Japan.

It has been well known that the dose distribution of π^- mesons in an absorber is very desirable for the radiation therapy. Indeed the π^- meson therapy has been studied in several foreign laboratories for example, the Los Alamos Scientific Laboratory. As the result of these studies it was experimentally confirmed that the dose distribuation in an absorber for π^- mesons is quite desirable for therapy.

Since the dose distribution of protons is similar to that of π^- mesons, the proton therapy should be also studied. Proton beams release selectively an amount of energy at a limited desired location in an absorber. Using this desirable aspect of the dose distribution of protons, we can irradiate the tissues of limited parts of focus only with slight damage to the surrounding normal tissues.

We carried out an experiment to study the morpholgical changes of cells in the same kind of tumors which are irradiated by protons of energies corresponding to both the plateau and peak parts of the Bragg curve.

§2 Experiment

We used 52 MeV proton beams from the INS-FM cyclotron. The beams pass through a differential type parallel plate ionization chamber which was calibrated by using a standard dose chamber, and also a multi-wire ionization chamber system which is used for monitoring the beam profile. Absolute radiation dose and the beam intensity distribution were measured. The multi-wire ionization chamber system and a Tissue Equivalent chamber (TE chamber) as a standard absolute dose chamber were designed and provided by A. Ito. The proton beams were extended and then the 3cm×3cm square of the beams was formed by a slit system within 10% homogeneity of intensity.

A Bragg curve (Fig. 1) for the 52 MeV proton beams was observed, by the TE chember placed at a target position and by several thin films of acryc resin absorbers.

Targets (ears of rabbits and mice) were fixed on a plastic holder in perpendicular to the proton beams located between the ionization chamber and a Faraday cup.

We varied the proton energy by using the acryc resin absorbers of appropriate thickness so that the energy corresponds to the part of the Bragg curve in cases (a), (b) and (c).

2-1 Ears of rabbits

Rabbits weighing three kg were prepared for the present experiment. Their ears were fixed perpendicular to the proton beams. Using appropriate absorbers, we adjusted the energy of the proton beams to be (a) at the plateau part of the Bragg curve, (b) at the half-way point of the Bragg peak, and (c) at the Bragg peak. The amount of radiation was 5000rad in each case.

(a) Irradiation at the plateau part of the Bragg curve.

In this case the proton beams pass completely through the ears. From visual observation, we noted almost no change until the 7th day after the irradiation. However, the surface then became rather dry and depilation was observed after that day.

In the second or third week after the irradiation, almost complete depilation was observed at the entry and exit points of the beams on the surface of the ears. In particular the skin at the entry were moist and showed some erosion. As time passed, scabs formed on part of the skin. (Fig. 2-3)

(b) Irradiation at the half-way point of the Bragg peak.

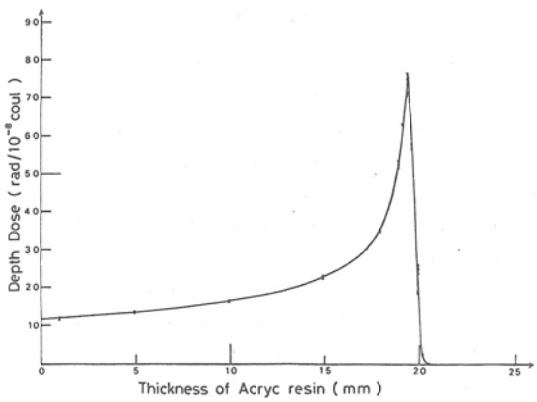

Fig. 1 A Bragg curve of 52 MeV proton from INS-FM cyclotron in acryc resin.

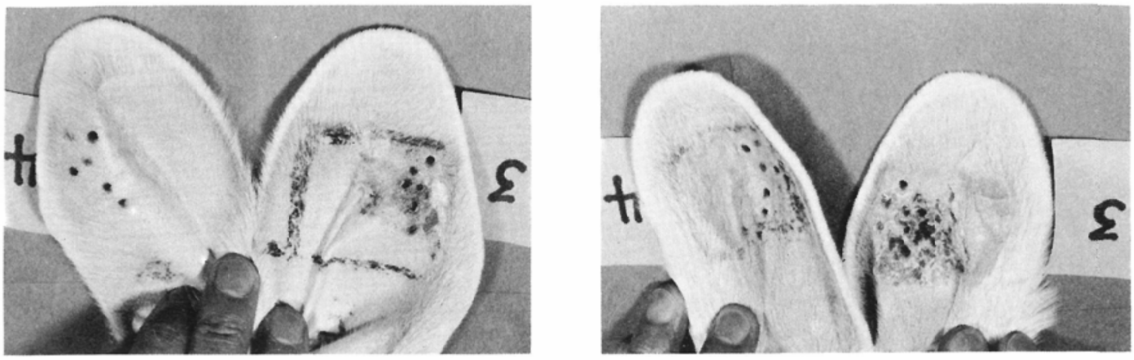

Fig. 2-3 Rabbit's ears in 28 days after 5000 rad proton irradiation at the energy corresponds to the plateau part of the Bragg curve.

A similar change to case (a) was usually observed, but part of the exposed skin showed a greater reaction. The exposed skin showed almost complete depilation which was not recovered by the fourth week following the irradiation.

(c) Irradiation with the Bragg peak located at a depth of 1mm in ears of rabbits.

We observed depilation on skin at the point of entry of the beams in one to two weeks after the irradiation. The skin, however, recovered more quickly than in case (a). The epidermis behind the ears did not show complete depilation and hairs falling out were quickly reproduced. (Fig. 4-5)

2-2 Observation by using an electronmicroscope.

Using an electronmicroscope, we observed the cells of the epidermis in the neighbor the basilar membranes. In most cells, the nuclei showed little damage and the structure of the nuclear double membranes was still clearly visible. However, mitochondria in the protoplasma were severly damaged. As a result, though the double membranes structure still remained, the crista of the mitochondria tended to swell.

Furthermore in the protoplasmas lisozom swelled, while desmosome did not change very much. Some cells, however, were severely damaged. Their boundaries and a part of their doule membranes structure became unclear. Their inner structure was depopulated and their double membrane structure had partly disappeared.

Nucleoli appeared to be enriched and their boundaries became unclear. However, the basal

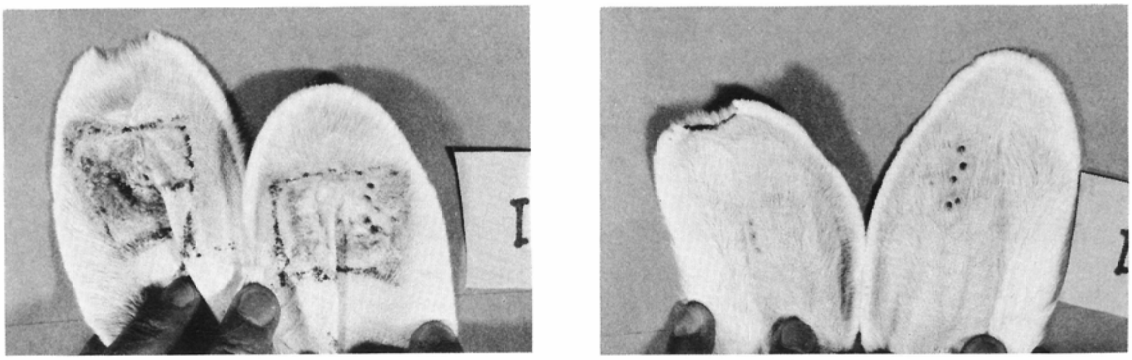

Fig. 4-5 Rabbt's ears in 28 days after 5000 rad proton irradiation at the energy corresponds to the peak point of the Bragg curve.

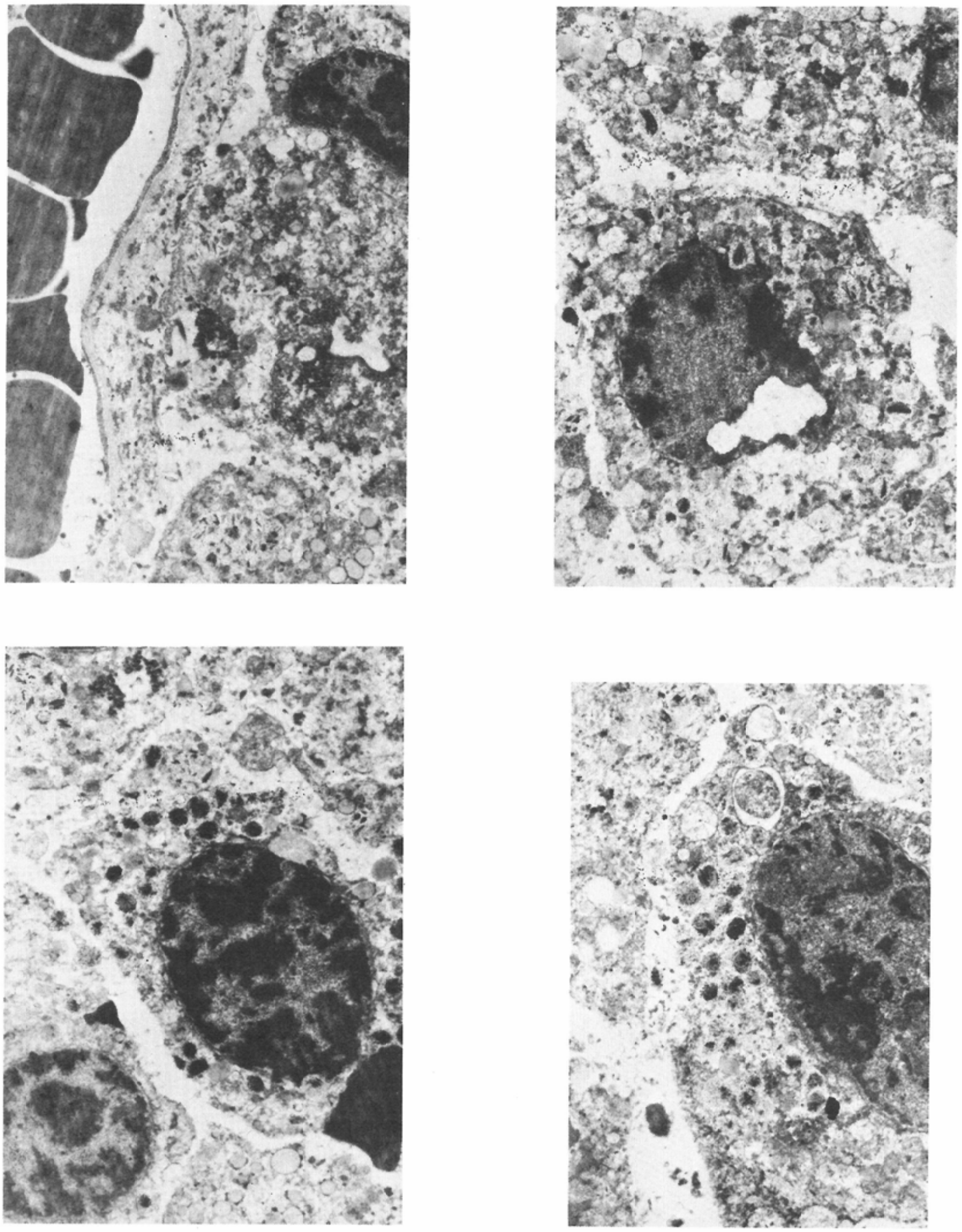

Fig. 6-9 Melanoma cells in 24 hours after 5000 rad proton irradiation at the energy corresponds to the plateau part of the Bragg curve.

membranes of the epidermis changed very little and their double-membrane structure was still clearly visible.

2-3 Experiment by using transplanted tumors.

We carried out another experiment concerning melanoma which were believed to be insensitive to radiations.

The Harding-Passey mouse melanoma was transplanted into the subcutan of a three week old mouse.

In this experiment, we used tumors, the diameter of which grew up to 1.5-2cm in 2-3 weeks after the transplantation.

2-3-1 Observation of the melanoma 24 hours after irradiation with 5000 rad

(a) Irradiation at the plateau part of the Bragg curve.

Using the electromicroscope, we observed both lightly and severely damaged cells. In the latter, the mitochondria tended to swell and the crista of the mitochondria disappeared in almost all of them. However, the double membrane structure was still clearly visible in a very small number of mitochondria.

In their nuclei, the double membrane structure was still visible and nucleolus showed little damage. In the nuclei of some cells, however, the double membrane structure was visible only in a part of the membranes. Vacuoles were created in the inferior of the nuclei and nucleoli were not clearly visible.

In some cells, the nucleus double membrane structure remained clearly, but the membranes themselves lost tension and became plait-like. Furthermore, the space between the membranes was widened.

A substance, which was presumed to be chromatin with high density in the nuclei, was irregularly formed and concentrated. (Fig. 6-9)

(b) Irradiation at the peak of the Bragg curve.

In damaged cells of the melanoma which were located at the peak, they, as a whole, appeared to

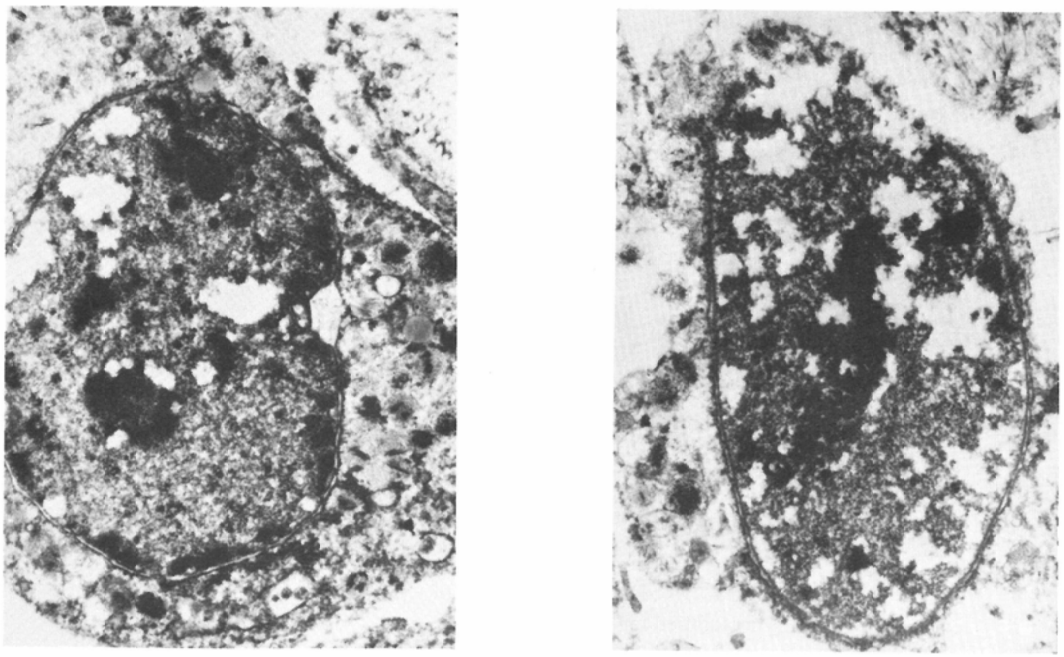

Fig. 10-11 Melanoma cells in 24 hours after 5000 rad proton irradiation at the energy corresponds to the peak point of the Bragg curve.

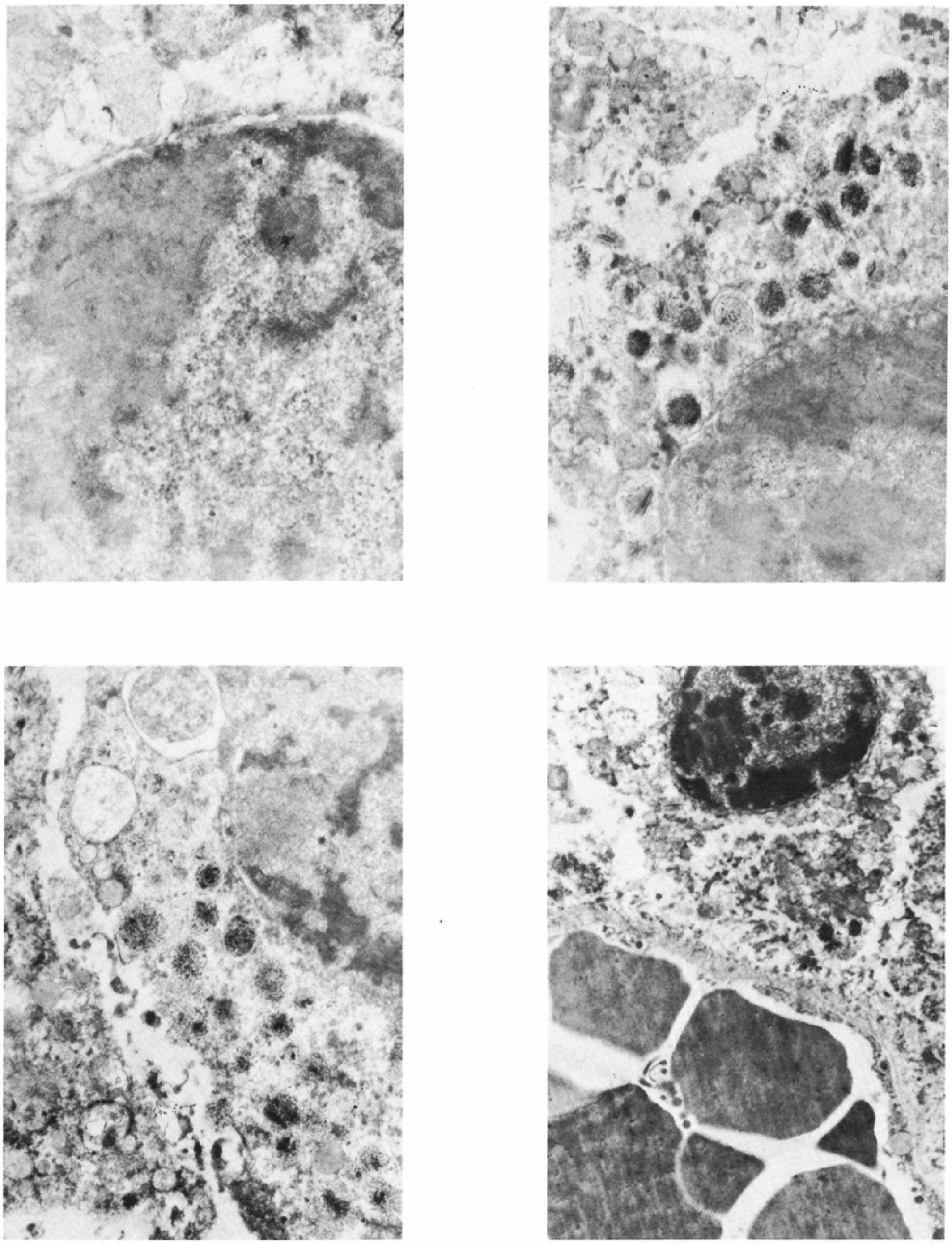

Fig. 12-15 Fine structures of the proton irradiatied melanoma cells.

shrink. Part of their protoplasma became irregular in density and was slightly defomed, while their nuclei were badly damgaed. That is to say, we could recognize that the nuclei had the double-membrane structure but part of the nucleus membranes had disappeared. The space between the membranes had widered. The interior of the nuclei consisted of a mixture of two parts, in one part small granules were formed and in the other part no structure was seen. The density of these small granules increased.

In the nuclei we saw vacuoles similar to those in the protoplasma generated generaly by irradiation. The nucleoli appeared to consist of rather larger granules. In those comparatively undamaged cells which were located in the same area as the cells discussed above, the space between their membranes had widened and small granules were observed in the interior of the nuclei, although their protoplasma was only slightly damaged and the double membrane structure was recognized.

We noted parts of nuclei with granules of high and low densities and many vacuoles both large and small.

In the area of the nucleolus, furthermore, we observed vascuoles large and small granules with high density and similar granules along the nucleus mambranes. (Fig. 10-11)

In the growing process of melanin we observed that the ribosome and Golgi body vesicle showed little changed and a substance without structure had accumulated round vacuoles in the Golgi body region.

The amount of granules in intermediate vesicles increased between the Golgi vacuoles and premelanosome.

It was observed that the linear structure inside melanosome was destroyed and the linear substance was rearranged at random without a filament-like arrangement. (Fig. 12-15)

§3 Discussion

Using proton irradiation with the Bragg peak located at depth of 1mm in the rabbit's ears, we studied the change in the tissue. Irradiated epidermis fell off and was eroded because of radiation damage, but the rear surface of the rabbit's ears was only slightly damaged. This shows that the reduction in proton intensity in the Bragg curve is very steep and that tissue deeper than that at a certain depth is not damaged. This means that the proton dose distribution is very suitable for radiation therapy.

In the experiment using the Harding passey melanoma, very few cells were damaged by γ-ray irradiation. On the other hand, the melanoma cells were serverly damaged with the proton beam with a dose of 5000 rad when they were located at the peak of the Bragg curve. Unlike the γ-ray irradiation, the proton beam caused severe damage of the nuclei in a short time in comparison with the damage to the protoplasma. We next irradiated the melanoma of mice at the plateau of the Bragg curve. Studying the melanin granules in melanoma with an electromicroscope, we found no essential change in the process of producing melanin in melanoma. At the same time, the inner structure of the Golgi vacuoles and large granuloma, the melanoma in the protoplasma remained almost normal, without any marked change. In the generation process, melanin granules are organically produced from several kinds of proteins including enzyme tyrosinase and from the products of biosythesis from phospholipid. The granules are called premelanoma. Aminoacid and tyrosine in protoplasma are oxidised by tyrosinase on the surface of the granules. They become the polymer indole-5,6-dihydroquinone. The polymers (melanin) are deposited on the granules which in turn blacken, while the tyrosinase lessens its activity. When a certain amount of melanin is deposited, the tyrosinase ceases its activity and the production of melanin stops. Regarding the effect on the formation of melanin by the irradiation of protons at the peak of th Bragg curve, melanosomes were the most affected. We found that the filament-like structure in the melanosomes was destroyed and the regularity of its arrangement disappeared. It would be interesting to learn what is the effect on biochemical change of such morphological change.

We studied morphological changes in tumor cells irradiating of proton beams corresponding to the plateau part. As the result, only slight damage was found. On the other hand, the tumor cells irradiated proton beams corresponding to the peak part showed serious damage. As the radiation therapy for human body, the proton therapy has a rather desirable condition, because the plateau part of proton beams can be located at normal tissues in most cases. However, we confined ourselves to the morphological study in the present report. Unlike π^-meson, proton beams do not have much effect on hypoxic cells.

It is however, quite interesting to learn from the present experiment that the proton beams at the peak of the Bragg curve have a marked effect on tumor cells and particularly on their nuclei.

It is, however, not yet confirmed if thrombosis is found in tissues at the peak position or not.

Following this basic study we are planning a further experimental work using the Ridge fillter to flatten the peak part of the proton Bragg curve.

§4 Conclusion

We irradiated proton beams on the ears of rabbits and the Harding-passey mouse melanoma and observed their morphological change.

In particular the nuclei of the Harding-passey mouse melanoma were damaged by the proton beams.

Literatur

1) Trott, K,R., Szczepanski, L.V.,Kummermehr, J. and Hug, O.: Tumour control probability and tumour regression rate after fractionated radiotherapy of two mouse tumours. In Radiobiological Research and Radiotherapy. Vienna, International Atomic Energy Agency, 1977, p. 29—42
2) Rohde, B. and Wiskemann, A.: Über das Wachstum röntgenbestrahlter Melanomalignome in der Gewebekultur und im Transplantationsversuch. I. Versuche am Melano-malignom des Menschen und am Tiemelanom nach Einzeitbestrahlung. Strahlentherapie, 123: 534—544, 1964
3) Barranco, S.C., Romsdahl, M.M. and Humphrey, R.M.: The radiation response of human malignant melanoma cells grown in vitro Cancer Res., 31: 830, 1971
4) Fowler, J.F. and Sheldon, P.W., Denekamp, J., and Field S.B.: Optimum fractionation of the C3H mouse mammary carcinoma using X-rays, the hypoxic-cell radiosensitizer Ro-07-0582, or fast neutrons. Int. J. Radat, Oncol. Biol. Phys, 1: 579, 1976
5) Dewey, D.L.: The radiosensitivity of melanoma cells in culture. Brit. J. Radiol., 44: 816, 1971
6) Fischer, J.J. and Moulder, J.E.: The steepness of the dose-response curve in radiation therapy. Radiology, 117: 179, 1975
7) Lieven, H.V. and Skopal, D: Zur Strahlenempfindlichkeit des malignen Melanoma. Strahlentherapy, 152: 1, 1976
8) Howes, A.E.: An estimation of changes in the proportions and absolute numbers of hypoxic cells after irradiation of transplanted C3H mouse mammary tumours. Brit. J. Radiol., 42: 441, 1969
9) Hulse, E.V., Mole, R.H. and Papworth, D.G.: Radiosensitivites of cells from which radiation-induced skin tumours are derived. Int. J. Radiat. Biol., 14: 437, 1968